N.M.R. STUDIES OF THE PROTONATION OF PYRIMIDINES

A thesis submitted to the University of Keele in part fulfilment of the requirement for the Degree of Doctor of Philosophy.

by

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The work in this thesis was carried out by the author under the supervision of Dr. D. V. Griffiths.

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LIST OF CONTENTS

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Abstract

This dissertation is concerned with the development of an n.m.r. method for assessing the N-1:N-3 protonation ratio in a number of monoprotonated pyrimidines based on a consideration of the ^{1}H - ^{13}C coupling constants in these protonated systems.

The vicinal through-nitrogen coupling $^{3}JC2H6$ was found to be particularly sensitive to the N-1:N-3 protonation ratio and was found to provide the best method for assessing this ratio. N-Methylpyrimidinium iodides were used as model systems to provide JCH values for the effects of protonation at N-1 and N-3. Evidence is presented to support the use of the methiodides as model systems.

A number of 4-substituted 2-aminopyrimidines and 4-alkyl/aryl-substituted pyrimidines have been synthesised together with the corresponding methiodides. The N-1:N-3 protonation ratios in these pyrimidines have been investigated to determine the relative importance of the steric and electronic factors associated with the 4-substituent. Steric factors were found to play an important role in affecting the N-1:N-3 protonation ratios.

The possible use of ^{13}C protonation shifts as a means of assessing the protonation ratios in these pyrimidines has been investigated. It was concluded that the results obtained in this way were less reliable than those protonation ratios determined from a consideration of the value of $^{3}JC2H6$ in the monoprotonated systems.

INTRODUCTION

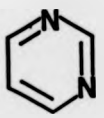
THE SYNTHESIS OF PYRIMIDINES

The Synthesis of Pyrimidine (1)

(1)

Although substitued pyrimidines have attracted much interest over the past few decades, the parent ring system, 1,3-diazine, has found very limited use in organic chemistry.

Pyrimidine was first prepared by Gabriel [1] in 1900 by the dehalogenation of 2,4,6-trichloropyrimidine. However, although this is still a convenient route to pyrimidine a number of other routes have since been developed based on acyclic precursors. Thus, for example, 1,1,3,3- tetramethoxypropane reacts with formamide to form pyrimidine (1) (proposed mechanism discussed later).

$$HCONH_2 + (OCH_3)_2CHCH_2CH(OCH_3)_2 \longrightarrow$$ 

(1)

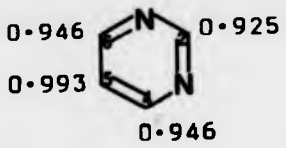
The Synthesis of Substituted Pyrimidines

(a) Substitution of the parent ring system

There are few examples of direct substitution into the unsubstituted pyrimidine ring system. This is in

marked contrast with benzene chemistry where electrophilic substitution provides a convenient means of introducing substituents onto the benzene ring. This reluctance of the pyrimidine ring to undergo electrophilic substitution is due to the presence of the two nitrogen atoms in the ring. Since nitrogen is more electronegative than carbon the remaining carbon atoms in the pyrimidine ring are somewhat more electron deficient than in benzene. This is termed a π-deficient nitrogen heterocycle. Figure 1 shows the relative π-electron densities of sites on the pyrimidine ring in comparison to those in benzene.

Fig. 1.

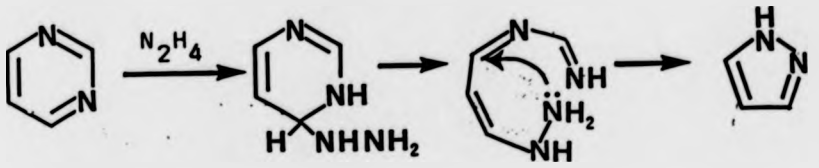


This clearly shows that the pyrimidine ring is particularly electron deficient at carbons 2,4 and 6. If electrophilic attack was**to succeed** this would seem most likely at carbon 5 although even here the site of attack is deactivated with respect to benzene. This is supported by the observation that only one electrophilic substitution reaction on pyrimidine itself has been reported, that is the bromination of pyrimidinium chloride on the C-5 position. [2]

Although the reduced π-electron density at the C-2, 4 and 6 positions strongly deactivates these sites towards electrophilic attack it does, however, make them susceptible to nucleophilic attack. Thus, for example the destruction

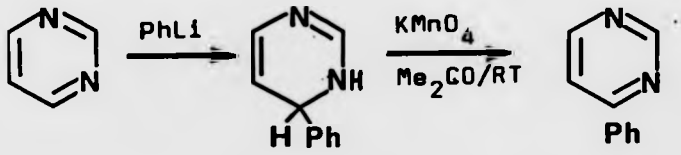
of pyrimidine when heated with aqueous base almost certainly involves an initial nucleophilic attack by the hydroxyl group on the ring. Similarly the conversion of pyrimidine to pyrazole by heating with aqueous hydrazine hydrate[3] (Fig. 2) can be explained in terms of an initial nucleophilic attack on the ring.

Fig. 2.



Nucleophilic attack by alkyl and aryl lithium reagents can also occur to give non-aromatic compounds. However, in these cases it is possible to achieve a re-aromatisation of the ring system under appropriate oxidising conditions[4] (Fig. 3).

Fig. 3.



This approach has been used successfully to prepare a number of 4-alkyl or aryl-substituted pyrimidines.

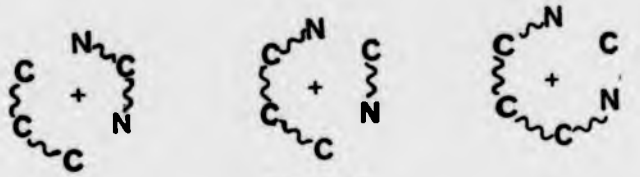
(b) Direct Synthesis from acyclic precursors

In the synthesis of substituted pyrimidines it is therefore usual to establish the substitution pattern when the heteroaromatic ring is formed from the acyclic precursors rather than by substitution into the parent system. The

substituents present after formation of the pyrimidine ring can then, if necessary, be modified to give the desired compound.

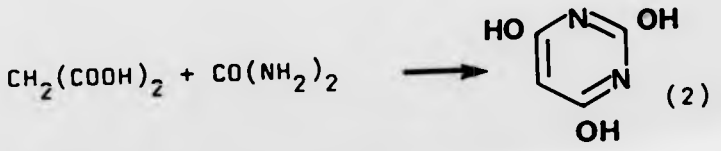
Although a wide variety of pyrimidine syntheses have been reported these invariably fall into one of three main types depending on the nature of the acyclic precursors (Fig. 4).

Fig. 4.



(i) Syntheses based on the condensation of two "3-atom" units. (Type I)

One of the most commonly used routes to substituted pyrimidines involves the condensation of a 1,3-bifunctional C~C~C fragment with an N~C~N fragment, such as a urea or amidine. Interestingly, the earliest recorded pyrimidine synthesis was a reaction of this type. In 1879 Grimaux [5] obtained barbituric acid (2) by condensing urea with malonic acid in the presence of phosphorus oxychloride.



By varying the nature of the N~C~N fragment, pyrimidines with a wide variety of substituents on C-2 can be produced.

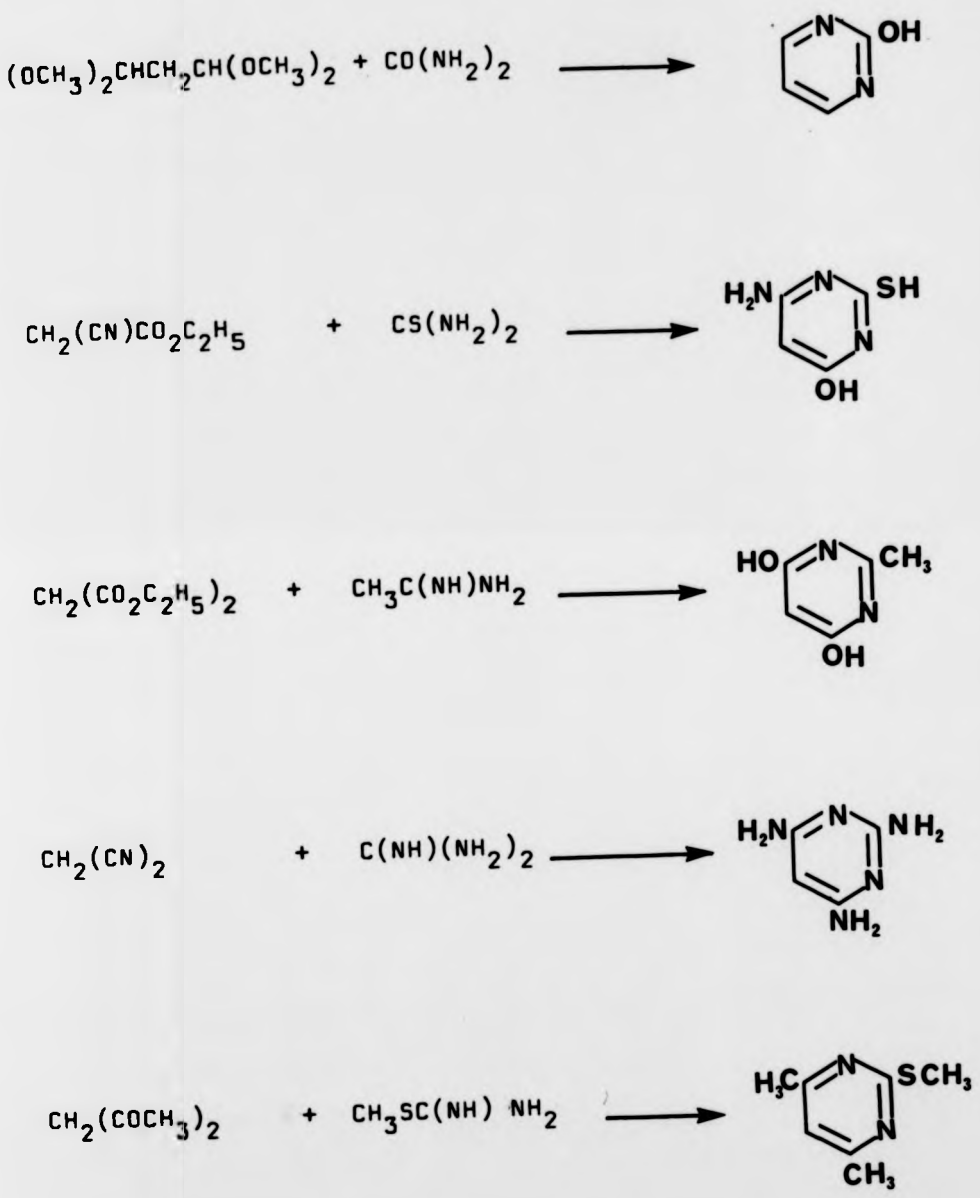
Thus, for example, while urea yields pyrimidines with a 2-hydroxy substituent, thiourea yields 2-mercaptopyrimidines and guanidine yields 2-aminopyrimidines.[6] Alkyl groups can be introduced at C-2 by using amidines such as acetamidine [7] whereas formamidine produces pyrimidines which are unsubstitued at C-2[8]. In this latter case, however, the yields are often very poor because of the ease of hydrolysis of the formamidine.

The nature of the 1,3-bifunctional C~C~C fragment can also be varied to produce a range of substituents on C-4 and C-6 in the pyrimidine ring. An aldehyde group at one end of the fragment (often initially present as its acetal) condenses to produce an unsubstituted position on the ring while the ketone group gives an alkyl or aryl substituent. A carboxylic acid, ester or amide group, on the other hand, gives rise to a hydroxyl substituent. The cyano group can also be used, which provides a means of directly introducing an amino substituent into the ring.

It can therefore be seen that the "Type I" approach offers much flexibility and a large number of substituted pyrimidines have been synthesised by this method. Some examples are given in Fig. 5.

The reaction conditions used in any particular case depend on the nature of the acyclic precursor. The basicity of the N~C~N fragment is an important factor in determining the ease of reaction. Thus, for example, a strong base such as guanidine readily attacks pentan-2,4-dione in the absence of a catalyst or solvent to give 2-amino-4,6-dimethylpyrimidine [9]. Urea on the other hand

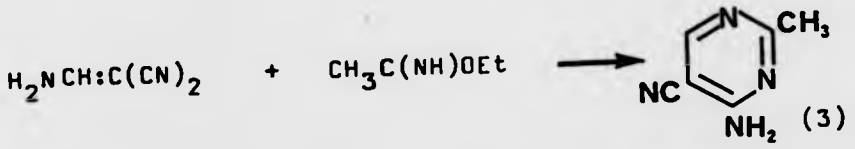
Fig. 5.


(OCH3)2CHCH2CH(OCH3)2 + CO(NH2)2
OH
N
N
CH2(CN)CO2C2H5 + CS(NH2)2
H2N
SH
OH
CH2(CO2C2H5)2 + CH3C(NH)NH2
HO
CH3
OH
CH2(CN)2 + C(NH)(NH2)2
H2N
NH2
NH2
CH2(COCH3)2 + CH3SC(NH) NH2
H3C
SCH3
CH3

is a weaker base and requires a catalyst for this reaction. For example the reaction of urea with pentan-2-4-dione [10] is carried out in a mixture of ethanol and concentrated hydrochloric acid. Most reactions are carried out however in the presence of organic or inorganic bases. For example acetamidine reacts with diethylmalonate in the presence of sodium ethoxide to give 2-methyl-4,6-dihydroxy-pyrimidine.[7]

(ii) Syntheses based on the condensation of a "4-atom" unit with a "2-atom" unit (Type II)

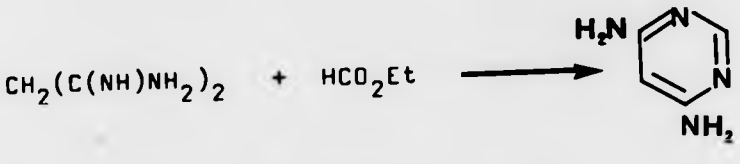
Syntheses of this type are less common than those of "Type I" and usually involve the addition of a C~C~C~N fragment to a C~N fragment. An example of this is the reaction of aminomethylene malononitrile with ethyl acetamidate to give 4-amino-5-cyano-2-methyl pyrimidine (3).[11]



(3)

(iii) Syntheses based on the condensation of a "5-atom" unit with a "1-atom" unit (Type III)

Malondiamidine provides a useful N~C~C~C~N fragment for "Type III" syntheses. It condenses with esters to give 4,6 diaminopyrimidine which is difficult to prepare by other methods. For example the reaction of this with ethyl formate produces 2,4 diaminopyrimidine (4).[12]



(4)

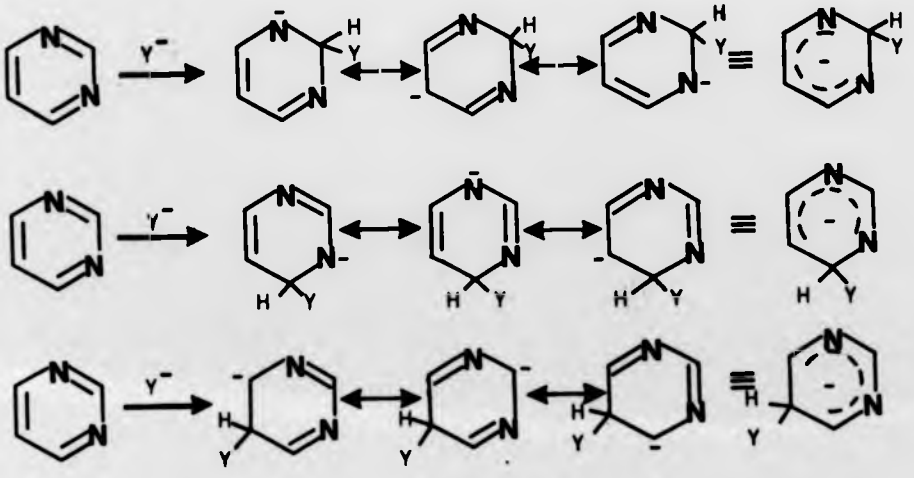
(c) By Modification of Substituted Pyrimidines

(i) Nucleophilic Substitution Reactions

We have already seen (Fig 1) that the 2,4 and 6 positions on the pyrimidine ring are somewhat electron deficient in comparison to benzene and that they are susceptible to nucleophilic attack. For this reason labile groups such as a chlorine atom at these sites can be readily displaced in a nucleophilic substitution reaction.

Consideration of the resonance structures of the intermediates resulting from nucleophilic attack at C-2, 5 and 4/6 (Fig. 6) clearly show that attack at C-2 and 4/6 is favoured over C-5. With attack on C-5 no stabilisation of the resulting intermediate anion by the ring nitrogens can occur.

Fig 6.



Although attack at C-2 might be expected to be thermodynamically favoured over attack at C-4/6 due to the symmetry of the 2-substituted anion, in practice it is found that C-4 is most susceptible to nucleophilic attack. Thus, the reaction of 2,4-dichloropyrimidine with sodium methoxide gives 2-chloro-4-methoxypyrimidine.[13]

An apparent exception to this rule has been reported[14] in that it is claimed that 2,4-dichloropyrimidine reacts with methanolic ammonia at room temperature to give 2-amino-4-chloropyrimidine as the major product (60%) together with 4-amino-2-chloropyrimidine (40%). However, we now have evidence to suggest that this is not the case (see later discussion).

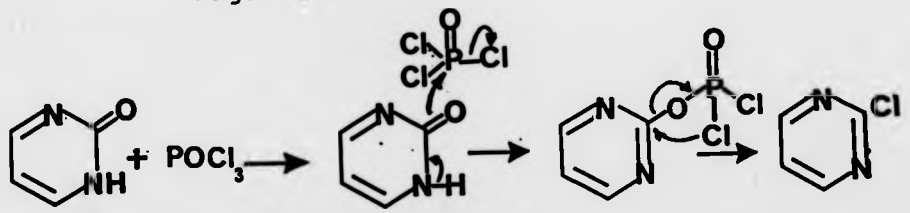
It would thus appear that reactions of this type are under kinetic rather than thermodynamic control. It has been suggested that the electrostatic repulsion between the lone pairs of electrons of the ring nitrogens and the incoming nucleophile reduces the rate of attack on C-2 more effectively than at C-4/6.

Additional substitution on the pyrimidine ring will also affect the ease with which nucleophilic substitution can occur. Electron-withdrawing groups particularly at C-5 (see fig. 6) will help stabilise the intermediates resulting from nucleophilic attack at C-2 or 4/6 and thus favour substitution. Similarly electron-donating groups will deactivate the ring towards nucleophilic attack. Thus for example the reaction of 2,4,6-trichloropyrimidine with alcoholic ammonia at room temperature yields a

mixture of 2 amino-4,6- dichloropyrimidine and 4-amino -2,6-dichloropyrimidine. Replacement of the second amino group to give 2,4 - diamino-6-chloropyrimidine only occurs at 160° and replacement of the third halogen atom only occurs at temperatures above 200°C.[15]

Finally, it is worth noting that while the chloropyrimidines are the most extensively used compounds for nucleophilic substitution reactions,there are a number of other groups which can be readily displaced in this type of reaction.[16] These groups include MeO, MeS, $MeSO_2$ and Me_3N. The reaction of phosphorus oxychloride on hydroxy pyrimidines can also be regarded as a nucleophilic substitution reaction which occurs intramolecularly (Fig. 7).

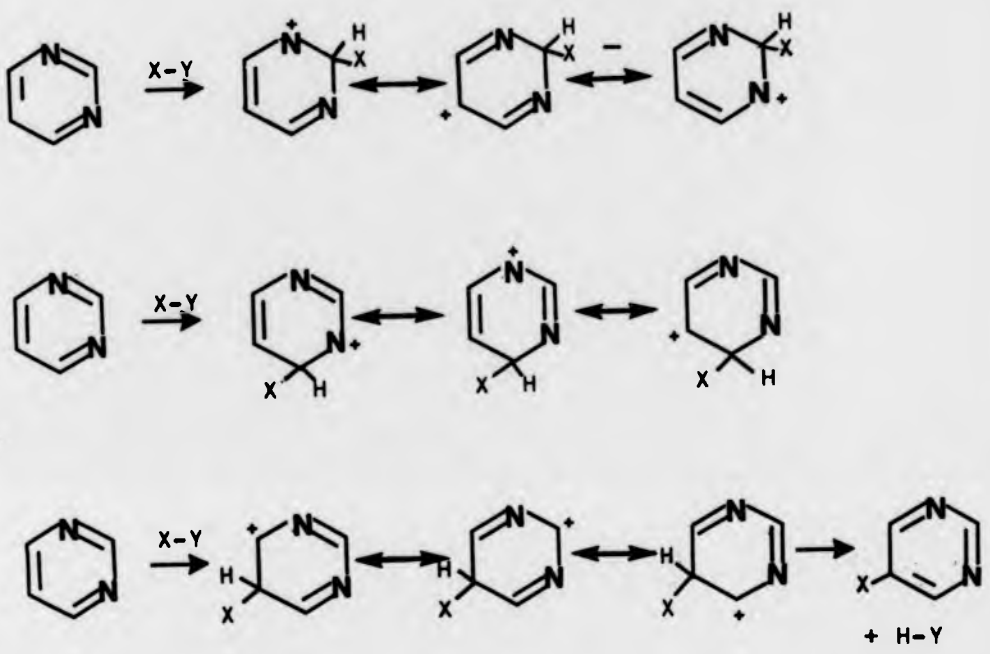
Fig. 7.



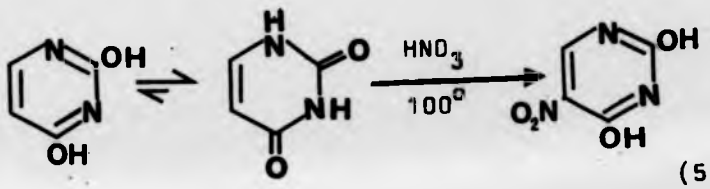
(ii) Electrophilic Substitution Reactions

Electrophilic substitution reactions are less useful in pyrimidine chemistry than is the case for benzene chemistry since only C-5, which is the least π-electron deficient carbon (Fig. 1), undergoes this type of reaction. A consideration of the resonance structures of the intermediates for electrophilic substitution at all possible sites on the ring (Fig.8) confirms that electrophilic attack at C-2or 4/6 would produce unfavourable resonance structures with the electron deficiency residing on the ring nitrogens.

Fig. 8.



However, as has been previously noted, the pyrimidine ring is a π-electron deficient system and therefore electrophilic attack at C-5 is difficult without activating groups at C2, 4 or 6. For example uracil is readily nitrated using fumic nitric acid at 100°C to give 2,4-dihydroxy-5-nitropyrimidine [17] (5).



(5)

(iii) Miscellaneous reactions

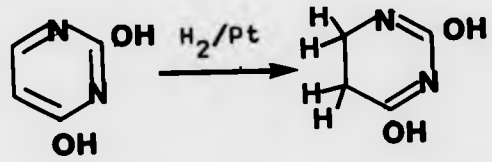
(a) Reactions of Methylpyrimidines

Like the 2 and 4-methylpyridines a methyl group in the 2 or 4 position of the pyrimidine ring is activated. Such groups are readily oxidised to the carboxylic acids and also react with benzaldehyde to give styryl pyrimidines.

(b) Reduction of pyrimidines

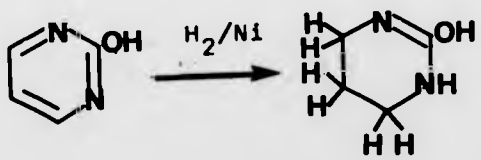
The subject of reduced pyrimidines has not been extensively studied. It has been reported that uracil when hydrogenated over platinum or palladium is reduced to dihydroderivative [18] (Fig. 9).

Fig. 9.



The reduction of 2-hydroxypyrimidine with Raney Nickel however has been reported to give the tetrahydroderivative [19] (Fig. 10).

Fig. 10.



The reduction of chloropyrimidines has been widely used in pyrimidine synthesis and this can be achieved either by the reaction with zinc and hydrochloric acid [20] or by hydrogenation with a palladium catalyst in a basic

medium which results in replacement of the chlorine by hydrogen without reduction of the ring [21].

Catalytic desulphurisation of mercaptopyrimidines with Raney Nickel has been demonstrated as a useful synthetic way of replacing the mercapto group by an hydrogen [22].

(c) N-alkylation

Pyrimidines readily react with alkyl halides to give monoquaternary salts. There are two possible sites of alkylation in asymmetrically substituted pyrimidines and while only one isomer is usually formed, both isomers can sometimes be produced. The site of alkylation is controlled by inductive, mesomeric and steric effects.

MEASUREMENT OF BASICITY

(a) Ionisation Constants

The basicity of a compound is now universally expressed in terms of the acidic ionisation constant, Ka, which is a measure of the compounds affinity for hydrogen ions. Although at first this may seem perverse it is advantageous to be able to express both acidity and basicity on the same scale.

Both acidity and basicity involve an equilibrium with hydrogen ions.

For an acid the Ka refers to the equilibrium

$$HA \rightleftharpoons H^+ \quad A^-$$

and is given by the expression (1)

$$K_a = \frac{[H^+]\,[A^-]}{[HA]} \qquad (1)$$

and similarly for a base Ka refers to the equilibrium

$$BH^+ \rightleftharpoons B \quad H^+$$

and is given by the expression (2)

$$K_a = \frac{[B]\,[H^+]}{[BH^+]} \qquad (2)$$

Since the value of Ka is often very small it is usual to express acidity and basicity in terms of the negative logarithm (to base 10) of Ka, this is known as the pKa value. Strong acids have low pKa values while strong bases have high pKa values.

Although the basic ionisation constant, pK_b, is now no longer used, previously reported pK_b values can be

easily converted to the corresponding pKa values by use of Equation (3). At temperatures other than 25°C a small correction is necessary.[23]

$$pK_a + pK_b = 14{\cdot}0 \qquad (3)$$

It should be noted that the Ka expressions previously given in Equations (1) and (2) use the concentration rather than the activity of the species involved. The Ka values derived in this way are therefore "concentration ionisation constants". In order to obtain the "thermodynamic ionisation constant" it is necessary to use the activities of the species involved. However, since at infinite dilution the two ionisation constants become equal, and since pKa determinations are typically carried out at concentrations at or below 0.01 mol dm^{-3}, most pKa values can be considered to be "thermodynamic ionisation constants".

It should be noted that pKa values are sensitive to temperature and that the change in value over a given temperature change depends on the pKa value. Fig (11) shows the temperature coefficients at a number of pKa values. It can be seen that the stronger the base the more sensitive is the value of pKa to temperatures[23].

Fig. 11.

pKa	coefficient/°C rise
3.3	-0.011
5.0	-0.015
7.5	-0.018
10.0	-0.021

(b) Methods for the Determination of Ionisation Constants

There are a number of methods available for determining ionisation constants but the two most convenient methods involve potentiometry and ultra-violet spectroscopy[23]. The basis of the calculation of the pKa by these methods is the Henderson equation (4) which is derived by taking negative logarithms to base 10 of the Ka expression (2)

$$pK_a = pH + \log_{10} \frac{[BH]}{[B]} \qquad (4)$$

Obviously, at the half neutralisation point pKa = pH but values so derived are not considered to be acceptable. The potentiometric method involves the neutralisation of the base with one equivalent of acid added in ten equal portions. The pH is measured after each addition and the pKa of the base calculated from each of the results using equation (4). If the scatter of results is within acceptable limits the average of the pKa values derived is considered to be correct. The concentration of the base used in the titration is usually 0.01 mol dm^{-3}, but the acid added is usually 10x the strength of the base so that the small increase in volume after each addition can be ignored.

Ultra-violet/visible spectroscopy is the other main technique used to determine pKa values. It is more time consuming than the potentiometric method but is ideal for sparingly soluble substances. A pKa determination by spectroscopy can be carried out in the 10^{-5} mol dm^{-3} range if the substance has a strong enough

extinction coefficient. Determinations can also be carried out in highly acidic solutions by this method by use of the Hammett acidity function. The only requirement of the spectroscopic method is that the molecule and the ion have different U.V./visible spectra. The relative proportions of the molecule and the cation can therefore be measured directly at a number of chosen pH values and the pKa values calculated from equation (4).

There are other methods other than the two already discussed for determining acidic ionisation constants. Conductimetry was the major technique before the advent of potentiometry but is a more complicated and more time consuming process so it is rarely used today. More recently nuclear magnetic resonance spectroscopy and Raman spectroscopy have been used to determine pKa values which cannot be determined by potentiometry or spectroscopy. For example some molecules do not have a useful U.V./visible spectra.

MONOPROTONATION OF PYRIMIDINES

(a) The Basicity of substituted pyrimidines

Although aromatic nitrogen heterocycles where the ring nitrogen possesses an unshared pair of electrons are basic, they are generally less basic than aliphatic amines. Thus, for example, while pyridine has a pKa value of 5.2, piperidine has a pKa value of 11.2. It has been suggested that this is due to the greater "S" character in the Sp^2 hybridised lone pair in aromatic nitrogen heterocycles relative to the Sp^3 hybridisation found in aliphatic amines.[24]

Although pyrimidines have two nitrogen atoms in the ring they can be regarded essentially as monobasic compounds. Diprotonation of the ring system can be achieved but only in very strong acid (pKa_2 -6.9). [24] The presence of the second nitrogen atom is, however, significant since it causes destabilisation of the initially formed monoprotonated system by inductive electron withdrawal. This is believed to explain the weaker basicity of the pyrimidine ring system (pKa 1.3)[24] relative to that of pyridine.

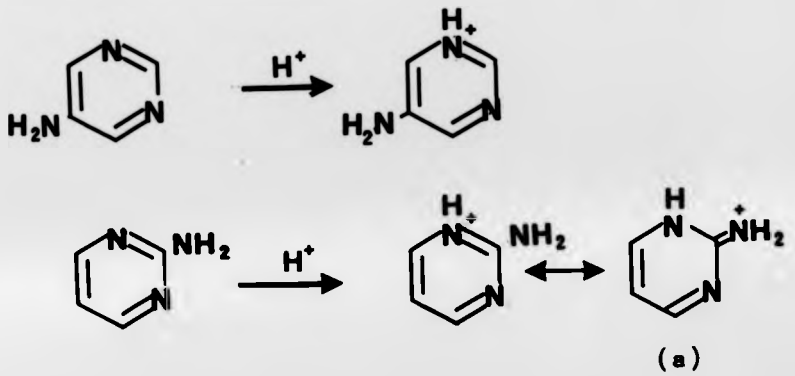
As expected electron withdrawing substituents on the pyrimidine ring reduce the pKa value for the ring still further. Thus, 2-chloropyrimidine (pKa <1)[24] is a weaker base than pyrimidine due to the -I effect of the chloro group.

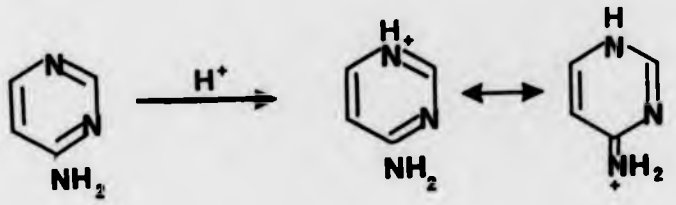
Substituents exhibiting a strong mesomeric effect (+M) can also have a considerable effect on the basicity

of the ring system. When these substituents are placed on the C-2, 4 or 6 positions then delocalisation of the positive charge can be extended over the substituent leading to a stabilisation of the monoprotonated system. 4-Methoxypyrimidine for example, is a stronger base (pKa 2.5)[24] than pyrimidine due to the +M effect of the methoxy group which more than offsets the -I effect of this substituent.

Perhaps the most basic of the pyrimidines are the amino-substituted pyrimidines. While the amino group has a strong +M effect its -I effect is weaker than that of the methoxy group. The position of the amino group on the pyrimidine ring is important. Thus while 5-aminopyrimidine has a pKa value of 2.6, 2-amino and 4-aminopyrimidine have pKa values of 3.54 and 5.71 respectively.[24] The higher basicities in these latter cases is because amino groups at C-2 and 4 can delocalise the positive charge resulting from monoprotonation more effectively (Fig. 12).

Fig. 12.

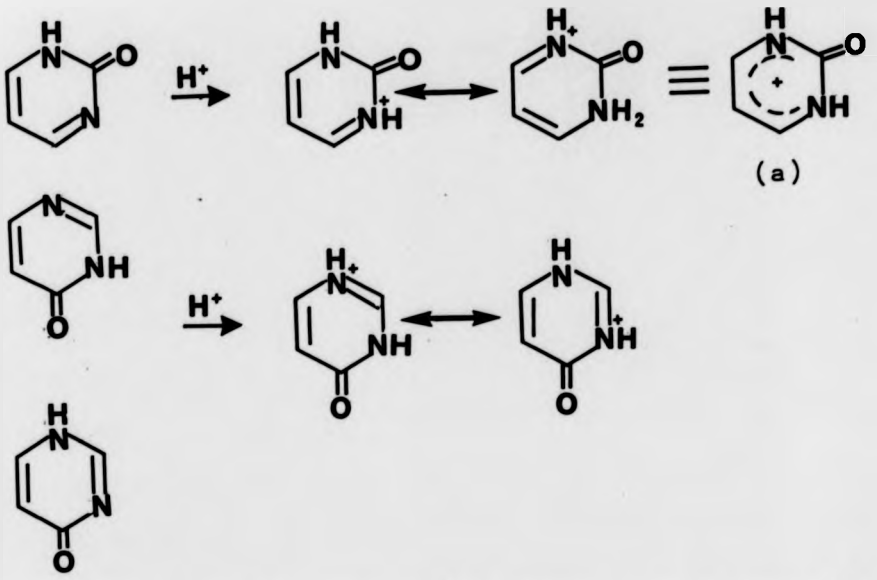




(b)

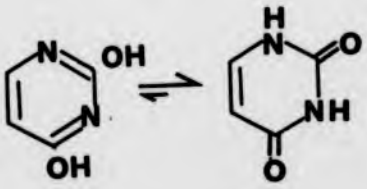
The greater basicity of 4-aminopyrimidine compared to 2-aminopyrimidine has been attributed to the greater stability of the para-quinoid resonance form (b) relative to the ortho-quinoid form (a).[25] The introduction of additional amino groups onto the pyrimidine ring further enhances its basicity, for example 2,4-diamino-pyrimidine (pKa 7.40).[24]

Hydroxy substituted pyrimidines are of interest since the 2 and 4 isomers have been shown to exist as pyrimidone tautomers (Fig. 13). 5-Hydroxypyrimidine (pKa 1.85) is a slightly stronger base than pyrimidine because of the electron releasing effect of the hydroxy group as are 2-(1H)pyrimidinone (pKa 2.44) and 4-(3H)pyrimidinone (pKa 1.87). The higher basicity of the 2 isomer is considered to be due to the delocalisation of the positive charge over both nitrogen atoms resulting in the symmetrical cation (a).



(a)

Pyrimidines having hydroxy substituents in both the 2 and 4 positions, for example uracil (pKa - 3.38)[24], are very weak bases because both nitrogen atoms are involved in lactam tautomerism (6).



(6)

(b) The Site of Protonation

Although pKa values have been reported for a large number of pyrimidines, very little work has been published on the site of protonation on the pyrimidine ring. In symmetrically substituted pyrimidines or in pyrimidine itself, where the two ring nitrogen atoms are equivalent there is no problem. When the ring is monoprotonated rapid proton exchange occurs between the two equivalent protonation sites so that each can be considered to be effectively 50% protonated. In these circumstances the pKa value of these pyrimidines, as measured by conventional means, reflects the pKa of the molecule as a whole rather than that of the individual sites of protonation. However, it can be shown (see later discussion) that the individual pKa values can be readily obtained from the observed pKa value by use of Equation (5).

$$pK_aN\text{-}1 = pK_aN\text{-}3 = pK_{obs} - \log_{10}2 \qquad (5)$$

In asymmetrically substituted pyrimidines, however, where the ring nitrogens have different basicities the situation becomes more complex and more difficult to study. While it is still a straightforward matter to determine the pKa value of the whole molecule it is necessary to know the relative proportions of the N-1 and N-3 mono-protonated forms before the individual pKa values of the ring nitrogens can be calculated. This type of information is of interest not only from a theoretical point of view but it may also

prove to be valuable in the design of certain enzyme inhibitors. When considering enzyme-inhibitor interactions involving the nitrogen atoms in the pyrimidine ring it is clear that it is the basicity of an individual site rather than of the molecule as a whole which is of importance. The inhibitor-enzyme interaction may dictate protonation at one site while precluding it at the other.

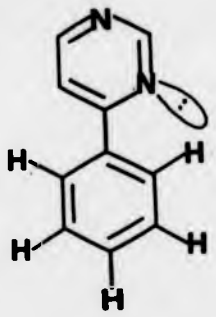
It is not possible to determine the N-1:N-3 protonation ratio by conventional analytical techniques but nuclear magnetic resonance spectroscopy is a promising technique in this area since protonation is known to have a marked effect on some n.m.r. parameters. ^{1}H n.m.r. spectroscopy has been used to study the protonation of pyrimidines and other nitrogen heterocycles. An early paper by Jardetzky, Pappas and Wade [26] categorically showed that amino substituted pyrimidines protonate on the ring nitrogens rather than on the amino group. By recording the ^{1}H n.m.r. spectra of aminopyrimidines in anhydrous trifluoroacetic acid they were able to observe broad resonances for hydrogen atoms bound to nitrogen. Separate NH resonances were observed for the amino group and the ring nitrogen which integrated in the ratio of 2:1 hence confirming that protonation was occuring on the ring nitrogen.

Wagner and Von Philipsborn [27] have used ^{1}H n.m.r. spectroscopy to study pyrimidines in a number of acidic solvents. By studying the changes in chemical shifts and coupling constants which occured on protonation they were able to establish that pyrimidines were monoprotonated in trifluoroacetic acid and that no diprotonation was evident under these conditions. Diprotonation could

be achieved, however, in very strong acids such as sulphuric acid and fluorosulphonic acid.

An early study to determine the site of protonation in 4-phenylpyrimidine by ^{1}H n.m.r. spectroscopy concluded that protonation by trifluoracetic acid [28] occured essentially at N-1 although no precise quantitative ratio was given (see later discussion). The reason postulated to explain this extreme ratio was that the ortho protons of the phenyl ring sterically hindered protonation at the N-3 nitrogen. (Fig. 14).

Fig. 14.



The usefulness of ^{1}H n.m.r. spectroscopy for determining the N-1:N-3 protonation ratio in pyrimidines is however, rather limited. The example of 4-phenylpyrimidine serves to illustrate the rather qualitative nature of the information obtained. Because the changes in chemical shifts and coupling constants in ^{1}H n.m.r. spectra are relatively small the conclusions drawn from this data is inevitably imprecise. Furthermore, the approach used for 4-phenylpyrimidine cannot be extended to polysubstituted pyrimidines where there are few, if any, proton resonances to observe.

The advent of Fourier transform n.m.r. spectroscopy meant it was possible to study the carbon nuclei. This stimulated increased interest in the study of protonation of pyrimidines and other nitrogen heterocycles and many papers have been devoted to this subject. Pugmire and Grant[29] have carried out a systematic study of pyridine, pyrimidine, pyridazine, pyrazine and their cations by ^{1}H and ^{13}C n.m.r. spectroscopy. Monoprotonation of the bases was studied in trifluoroacetic acid and aqueous solutions of sulphuric acid. No evidence for the diprotonation of pyrimidines in trifluoracetic acid was found and diprotonation was only achieved in 100% sulphuric acid.

The effect of monoprotonation on the ^{13}C chemical shifts of the ring carbon atoms α, β and γ to the site of protonation were determined for pyridine and found to be -7.78, +5.04 and +12.42 p.p.m. respectively. The chemical shift changes observed for pyrimidine were rationalised using simple additivity rules of the α, β and γ effects of protonation knowing that each nitrogen was effectively 50% protonated. The α, β and γ values on protonation of pyrimidine were found to be -7.28, +3.04 and +9.94 p.p.m. respectively which compare favourably with the sign and magnitude of the pyridine values. These parameters however deviate from additivity significantly for the diprotonated diazines, for example for pyrimidine the α, β and γ effects are found to be -1.23, +3.05 and +2.80 respectively on addition of the second proton.

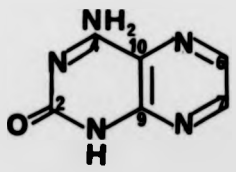
One of the few detailed studies of the site of protonation of monoprotonated pyrimidines was carried out by Riand, Chenon, and Lumbroso-Bader [30] who studied a number of methyl and amino substituted pyrimidines. A set of protonation parameters were determined which were then used to determine N-1:N-3 protonation ratios in a number of asymmetrically substituted pyrimidines. The protonation parameters were also found to be dependant on the substituent on the carbon under consideration and were denoted α_R, β_R and γ_R for the ortho, meta and para carbon atoms bearing a substituent R relative to the protonation site. These parameters were derived from the ^{13}C spectra of a number of symmetrical pyrimidines recorded in water and D.M.S.O for the free bases and for the monoprotonated forms in TFA or as the hydrochlorides in water and D.M.S.O. (corrected for deprotonation). Dioxan was chosen as the reference compound throughout this study because its chemical shift is not very sensitive to changes in medium. It was also established that the anion had little effect on the chemical shifts. It was also necessary to introduce correction terms ΔS_R etc. for the change in solvent in the calculation of protonation ratios when different solvents were used for the free base and the monoprotonated form. It was also found that the nature of the substituent in the 2 position had a marked effect on the protonation parameters. Thus a new set of parameters were required for pyrimidines bearing a 2-methyl or 2-amino substituent.

The conclusions of this study were that a methyl

in the 4 or 6 position favouredprotonation at the nitrogen para to it in the ratio of 7:3 and an amino group favoured protonation at the nitrogen atom para to it in the ratio of ~9:1. The effect of an amino group was shown to dominate over the effect of a methyl group by the determination of the protonation ratio of 4-amino-6-methylpyrimidine which was found to be approximately 95% N1 protonation. The use of ^{13}C chemical shift data to determine protonation ratios is a difficult process because of the variety of factors which can influence the chemical shifts. The work of Riand has attempted to allow for these factors, such as concentration and solvent effects but the method becomes quite involved and hence may be liable to give erroneous results.

This method has only been concerned with chemical shifts to calculate the protonation ratio but a considerable quantity of ^{13}C - ^{1}H coupling constant data can also be derived from the ^{13}C n.m.r. spectrum. The work of Guenther [31] has been concerned with the effect of protonation of nitrogen heterocycles on ^{13}C - ^{1}H coupling constants. An important observation was that the vicinal ^{13}C - ^{1}H through nitrogen coupling constant in pyridine decreased by 4.7 Hz on protonation. An example of the application of this observation was a study of the diprotonation of iso-pterin (fig. 15) which is initially protonated at N-3.

Fig. 15.



The addition of the second proton is expected to occur at N-8 (maximum charge separation). The changes observed in $^{3}JC9H7$ and $^{3}JC10H6$ supported this view. $^{3}JC9H7$ was observed to decrease by 3.1 Hz on diprotonation whereas $^{3}JC10H6$ decreased only by approximately 1Hz.

This dissertation is concerned with a systematic study of monoprotonation of pyrimidines with a view to determining the N-1:N-3 protonation ratio quantitatively by consideration of changes in the $^{13}C - {}^{1}H$ coupling constants, particularly vicinal through nitrogen $^{13}C - {}^{1}H$ couplings.

This method would have the advantage over the chemical shift approach because coupling constants are much less susceptible to solvent and concentration effects. However, as with the chemical shift method the problem of establishing protonation parameters is difficult because for only one special case of symmetrical pyrimidines is the protonation ratio known without doubt. A study by van de Weijer and Mohan[32] on the effects of protonation and methylation of pyrimidine concluded that both reactions had similar effects on the ^{13}C chemical shifts (fig. 16). The important difference between protonation and methylation however is that unlike a proton where a rapid exchange occurs between the two nitrogen atoms the methyl group does not.

Fig. 16.

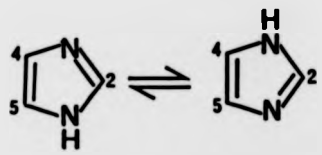
PROTONATION		METHYLATION	
α	-5·46	α	-2·99,-3·97
β	+1·64	β	+1·28
γ	+7·29	γ	+7·18

N-methyl pyrimidinum iodides were hence used extensively in this dissertation as potential model compounds for the extreme monoprotonated species.

Since commencing this work Riand has published ^{13}C - ^{1}H coupling constant data[33] for amino and methyl substituted pyrimidines. It was noted that all the one bond couplings (^{1}JCH) increased on protonation with the largest effect occurring at the carbon α to the site of protonation which could be used as an approximate measure of the N-1:N-3 protonation ratio of some amino and methyl substituted pyrimidines. It was also noted that the through nitrogen vicinal couplings (^{3}JCH) were more sensitive to the site of protonation and could provide an indication of the protonation ratio. It was assumed that the protonation ratios determined by the previous chemical shift method were correct and consideration of the changes in ^{3}JCH values observed on protonation supported these ratios, although no quantitive data was presented.

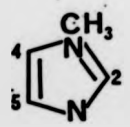
Also very recently Guenther [34] published a method of determining the site of protonation of imidazole (7) and purine (8). Imidazole exists in a tautomeric equilibrium whereby each nitrogen is effectively 50% protonated (fig.17)

Fig. 17.



(7a) (7b)

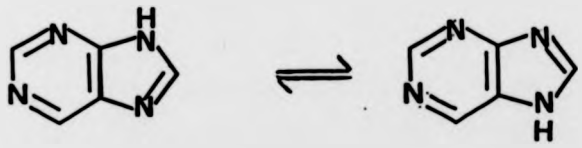
The $^{3}JC2H4$ and $^{3}JC2H5$ couplings are therefore averaged and are equivalent. For N-methylimidazole (9) the couplings are non-equivalent.



(9)

Comparison of the ^{3}JCH values for imidazole and N-methyl imidazole enabled a set of parameters based on $^{3}JC2H4$ and $^{3}JC2H5$ to be developed for the two extreme tautomeric forms (7a) and (7b). It was noted that a small correction factor was required for the protonation parameters because the sum of the $^{3}JC2H5$ and $^{3}JC2H4$ values was slightly greater for imidazole compared with the N-methylimidazole. (9).

This approach was extended to purine (8). The tautomeric ratio between N-7 (8a) and N-9 (8b) was found to be 50:50, thus essentially unchanged from imidazole.



(8a) (8b)

^{13}C NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

Although n.m.r. spectroscopy has proved to be an invaluable technique for chemists over the last 30 years, it was not until the early 1970s with the advent of Fourier Transform n.m.r. spectrometers that natural abundance carbon-13 n.m.r. spectra became routinely available. Since the natural abundance of the ^{13}C nucleus (spin $\frac{1}{2}$) is only 1.1% and its relative sensitivity compared to the ^{1}H nucleus is 0.016 the early continuous wave n.m.r. spectrometers, which had been designed primarily to study the ^{1}H nucleus, were not sufficiently sensitive to study the ^{13}C nucleus. Some spectra were obtained by repeatedly scanning the ^{13}C n.m.r. spectrum and averaging the accumulated spectra but this process was extremely time consuming and not practical for general use. The weakness of the continuous wave spectrometer lay in the fact that at any instant in time only information about a given frequency was being obtained.

The Fourier Transform n.m.r. spectrometer was able to overcome this weakness by obtaining information about all the resonating nuclei in the sample at the same time. To do this the sample is placed in a homogeneous magnetic field and then exposed to a single short pulse of radiofrequency radiation at a frequency appropriate to the nucleus being studied. The pulse generates a range of radiofrequencies the spread of which is governed by the duration of the pulse. Such a pulse is therefore capable, for example, of exciting all the

^{13}C nuclei in the sample. As these nuclei relax characteristic emissions of radiofrequency occur which rapidly decay away (the free induction decay, f.i.d.). The computer in a Fourier Transform spectrometer is capable of storing and analysing this data to give the resonant frequencies of the relaxing nuclei. This information is usually displayed in the same manner as that from the continuous wave spectrometer. Since the process of acquiring the ^{13}C data following a single pulse takes often less than a second the sample can be repeatedly pulsed and the decays stored much more quickly than with a continuous wave spectrometer. By averaging the accumulated free induction decays a good signal to noise ratio can be achieved in a relatively short time. Since the "noise" is of a random nature this will average itself out over a large number of scans whereas any signals present, however weak, will be reinforced. The quality of the resulting spectrum is expressed as the signal to noise ratio and this is directly proportional to the square root of the number of accumulated scans. Thus, for example, if one hundred accumulations are averaged this will improve the signal to noise ratio by a factor of 10.

<u>The chemical shift</u>

The equation which determines the resonant frequency of a particular nucleus is given by the expression

$$\nu = \frac{\gamma B}{2\pi}$$

where ν = resonant frequency, B = magnetic field

γ = magnetogyric ratio of the nucleus under investigation.

Clearly if the magnetic field experienced by the ^{13}C nucleus was the same in every case then only one resonant frequency would be observed for all ^{13}C nuclei. Fortunately, however, when a molecule is placed in a magnetic field the electrons within the molecule produce a magnetic field in opposition to the applied field, effectively shielding the nuclei to a small extent from the magnetic field. The degree of shielding of a particular nucleus depends on the nature of the electron distribution around the nuclei which is related to the chemical environment. The resonant frequency of a nucleus will therefore be sensitive to its chemical environment. In practice it is difficult to measure the high magnetic field in an n.m.r. spectrometer to a high degree of accuracy. It is therefore usual to measure the resonance condition relative to a chosen standard. The position of a resonant nuclei relative to a standard is termed the chemical shift (δ) and this is defined by the expression

$$\delta = \frac{\nu \text{ sample} - \nu \text{ reference}}{\text{oscillator frequency}} \times 10^6 \text{ p.p.m.}$$

The factor of 10^6 is included since without this the values of δ would be very small. Chemical shifts are thus quoted in units of p.p.m. Chemical shift data determined on spectrometers operating at different field strengths are directly comparable.

In ^{13}C n.m.r. spectra the range of chemical shifts usually observed is approximately 0 to 240 p.p.m. relative to TMS and as with ^{1}H n.m.r. spectroscopy there are discrete regions of the spectrum where certain ^{13}C nuclei usually occur.

Electron-coupled Nuclear Spin-Spin interactions

Although we might expect nuclei in a given chemical environment to resonate at one particular frequency we often find that the signal from such nuclei occurs as a set of signals (multiplets) rather than a single resonance line. This effect occurs because the nuclei under observation can often interact with other magnetic nuclei in the molecule. This interaction is known as nuclear spin-spin coupling and occurs because the bonding electrons in the molecule are able to transmit to the nuclei under observation information about the orientation of the magnetic moments of other nuclei in the molecule. Coupling information can be transmitted over several bonds but it rapidly diminishes and no couplings are usually observed when the magnetic nuclei are separated by more than 4 or 5 bonds.

First and second order spectra

The number of lines observed for a particular resonance is given by the expression $(2nI + 1)$, where n is the number of equally coupled nuclei and I is the spin quantum number of thosenuclei. For nuclei for which

$I = \frac{1}{2}$, such as protons, this expression simplifies to n + 1 so that, for example, if a ^{13}C nucleus is coupled equally to 3 protons as in a methyl group the ^{13}C signal would appear as a four line signal, a quartet. For coupling to nuclei for which $I = \frac{1}{2}$ the relative intensities of the lines is given by the coefficients of the binomial expression $(a + 1)^n$, where n is the number of observed lines. For four lines this would be 1:3:3:1. The separation between the lines, the coupling constant, is given the symbol J and is measured in units of Hz. As its name suggests this value is independent of the magnetic field used in the n.m.r. spectrometer and depends on the nature of the bonding between the coupled nuclei.

In order for the coupling between nuclei to be first order there are, however, certain conditions which must be met. Firstly, the chemical shift difference between the coupled nuclei ($\Delta\delta$) must be large compared with the coupling between them (i.e. $\Delta\delta/J > 10$). Secondly, chemically equivalent nuclei must also be magnetically equivalent. Chemically equivalent nuclei can be defined as being those which can be interchanged by a symmetry operation of the molecule. The 3 protons on a methyl group could thus be considered to be chemically equivalent. To be magnetically equivalent the nuclei must have the same resonance frequency and moreover they have to be equally coupled to the nucleus under observation.

If these conditions are not met the signals resulting from the coupling will be said to be

second order and in these cases the rules for first order coupling no longer apply.

Fortunately, ^{13}C - ^{13}C couplings are not observed in non-enriched ^{13}C n.m.r. spectra since the natural abundance of the ^{13}C nucleus is 1% , the probability of finding two such nuclei in the same molecule is only about 1 in 10,000. ^{13}C resonances, however, are usually observed as multiplets due to coupling with several protons in the molecule. Analysis of these multiplets provides invaluable information about the structure of the molecule. Often this analysis can be achieved by assuming that the simple rules for first order spectra can be used. Unfortunately, however, this is not always the case and sometimes the multiplets appear far more complex than one would expect from a first order point of view. This is due to second order effects. However, perhaps of greater concern is the fact that it is possible to have second order spectra which appear to be first order, the so-called deceptively simple spectra. In these circumstances the first order analysis will give incorrect parameters because the separation between the lines in the spectra are not the true coupling constants.

One has to be particularly vigilant for second order effects in ^{13}C n.m.r. spectroscopy where the carbon nucleus is often coupled to the protons in its vicinity to varying extents. Second order effects can arise here as a result of these protons coupling with each other in addition to coupling to the ^{13}C nucleus. Since ^{1}JCH values are of the order of 100-200 Hz the

^{1}H spectra relating to that particular ^{13}C containing molecule is not as is observed in the routine ^{1}H spectrum. Instead the proton coupled directly to the ^{13}C atom would appear as a widely spaced doublet which would in some instances lead to overlap with other coupled ^{1}H resonances thus causing second order effects in the ^{13}C n.m.r. spectrum. One way of reducing these second order effects would be to determine the spectrum on a spectrometer operating at much higher field strengths While the coupling constant remains independent of the magnetic field the separation between the resonances in Hz will increase thus increasing the ratio $\Delta\delta/J$.

Alternatively, if second order effects are observed or suspected then most modern F.T. n.m.r. spectrometers have associated software which can be used to simulate the observed spectrum and hence determine the true values of the couplings constants.

Quadrupolar relaxed nuclei

Nuclei for which the spin quantum number is greater than $\frac{1}{2}$ possess a quadrupole moment, Q. This moment arises due to an asymmetrical charge distribution in the nucleus. When an electric field gradient also exists at the nucleus, due to an asymmetrical electron distribution around the quadrupole nucleus, molecular tumbling provides a very efficient relaxation mechanism for the quadrupole nucleus. The resulting short relaxation time leads to the associated energy levels becoming less clearly defined and this results in broad lines being observed in the n.m.r. spectrum of such nuclei. On

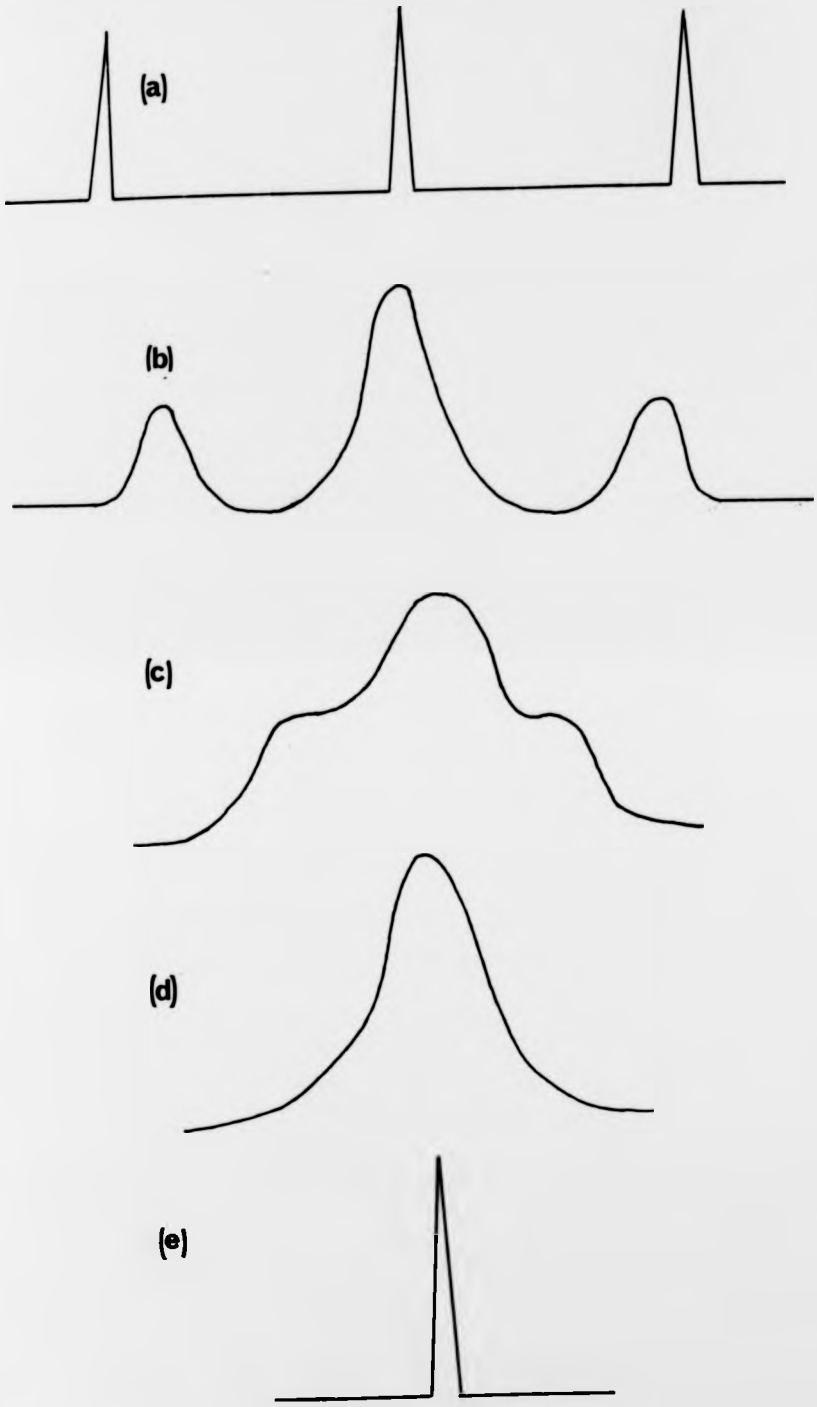
the other hand, a uniform spherical electronic distribution around the nucleus produces a zero electric field gradient at the nucleus. In these circumstances relaxation times are long and sharp lines are observed.

The symmetry of the electric field around the nucleus is therefore important in determining the degree of line broadening. The overall symmetry of the molecule is often related to the electronic symmetry at the nucleus so that, for example, the symmetrical $^{+}NMe_4$ ion has been observed to give a sharp ^{14}N resonance. However, the $C_6H_5CH_2\overset{+}{N}Me_3$ ion although not spherically symmetrical also gives a sharp ^{14}N resonance indicating that it is the electronic symmetry around the quadrupole nucleus which is important not the symmetry of the molecule as a whole.

Coupling to quadrupolar relaxed nuclei

The coupling of a ^{13}C atom to a quadrupolar relaxed nucleus, for example $^{14}N(I = 1)$, is greatly affected by the relaxation time of the quadrupole relaxed nucleus. At one extreme the relaxation time of the quadrupolar nucleus may be long and in this case the ^{13}C resonance will be observed as a sharp triplet of intensity 1:1:1 (fig.18a). At the other extreme where the relaxation time of the quadrupole nucleus is very short the nuclear spins interchange between energy states so rapidly that a "coupled" nucleus interacts with all possible spin states in a very short time. In these circumstances an averaged value of all these interactions is observed and the resonance of the "coupled" nucleus occurs as a sharp singlet since it has been effectively decoupled

Fig. 18.



from the quadrupole nucleus (fig. 18e). As is often the case an intermediate situation can exist where the coupled nucleus is broadened by the quadrupole relaxed nucleus (fig. 18 d and c).

Two factors which can markedly affect the line broadening of nuclei coupled to a quadrupole relaxed nucleus are the temperature and the viscosity of the solvent. An increase in temperature or a reduction in the viscosity of the solvent causes the rate of tumbling of the molecules to increase and this has the effect of reducing the efficiency of the quadrupole relaxation mechanism. Relaxation times increase and this usually has the effect of broadening the resonances (e.g. fig. 18 e → d) coupled to the quadrupole-relaxed nuclei. Conversely, if the viscosity of the solvent is increased or the temperature decreased then the rate of tumbling of the molecules slows down and the efficiency of the quadrupole relaxation mechanism increases. Shorter times lead to the effective decoupling of the quadrupolar nucleus and a sharper line may be observed (e.g. Fig. 18 d → e).

Heteronuclear decoupling techniques in ^{13}C n.m.r. spectroscopy

Proton noise decoupling

^{13}C n.m.r. spectra when acquired in the absence of proton decoupling contain a wealth of information about the coupling of the ^{13}C nuclei to protons in the molecule. Such spectra may be quite complex to interpret and because the resonances are split into

multiplets the spectra also often take a considerable period of time to accumulate even with a pulsed F.T. n.m.r. spectrometer. Furthermore, this type of information is often not required initially since in the early stages of spectral analysis we are usually more concerned with the number and relative chemical shifts of the carbon atoms in the molecule. Fortunately this removal of the ^{13}C - ^{1}H couplings can be readily achieved by the technique of proton broad band decoupling. To do this a broad band of decoupling power is applied across the whole of the proton region of the spectrum. This has the effect of saturating all the ^{1}H nuclei in the molecule, resulting in the loss of the ^{13}C - ^{1}H couplings. In addition to producing a simpler spectrum, the collapse of the ^{13}C multiplets to singlets increases the signal to noise ratio thus reducing the period of spectra accumulation. The irradiation of the protons also leads to a nuclear Overhauser enhancement, which further increases the signal to noise ratio of some ^{13}C resonances by as much as a factor of 3.

Off resonance decoupling

This heteronuclear decoupling technique is very useful since it enables the number of directly bonded protons on each carbon resonance to be ascertained without the very substantial loss in signal to noise ratio which would result from switching off the decoupler. In the off-resonance decoupling experiment the decoupling frequency is set outside the normal range of the proton

spectrum and the noise modulation used in broad band decoupling is turned off. In this way a partially decoupled spectrum is obtained in which only the couplings arising from directly bonded atoms are observed. These residual couplings are however reduced to only a fraction of their true values. In off-resonance spectra methyl carbon atoms occur as quartets, methylene carbons as triplets, methine carbons as doublets and quaternary carbons as singlets.

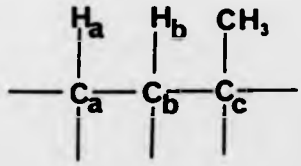
Selective proton irradiation

This technique allows us to investigate the effects on the ^{13}C n.m.r. spectrum of irradiating individual proton resonances. This usually allows us to associate the n.m.r. signals from the ^{13}C nuclei with those of their directly bonded protons.

However, in this dissertation we have used selective proton decoupling at low power levels to remove long range couplings rather than those between directly bonded atoms.

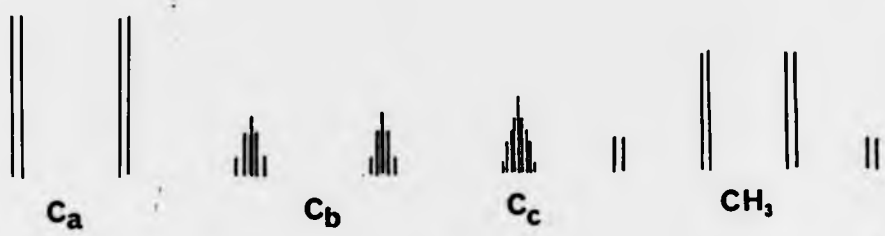
Consider the molecular fragment shown below (Fig. 19)

Fig. 19.



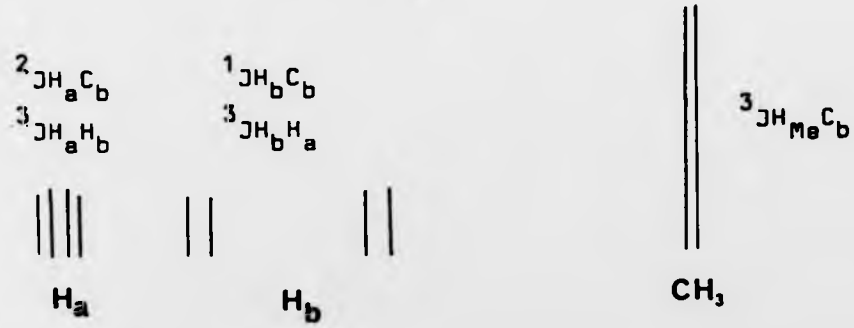
if we assume that coupling over more than 3 bonds can be ignored, the ^{13}C n.m.r. spectrum of this fragment can be represented diagrammatically (Fig. 20).

Fig. 20.



It can be seen that the resonance of C_b is complicated by coupling to the methyl protons. However, in theory it should be possible to irradiate the methyl protons and remove those coupling to C_b without substantially affecting other couplings in the system. To understand this it is necessary to consider the proton spectrum of the molecules with a ^{13}C nucleus at C_b. A simplified spectrum is shown below (Fig. 21)

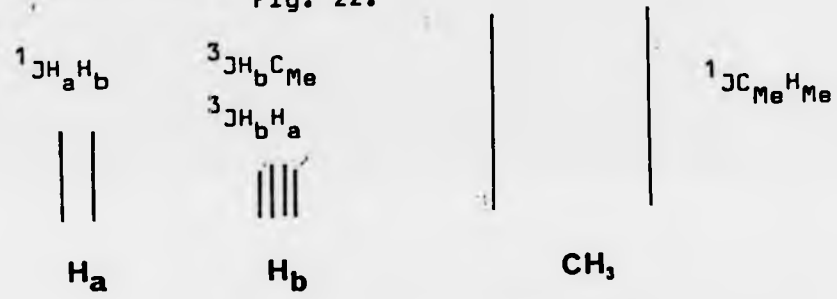
Fig. 21.



Irradiation at the frequency of the methyl resonance would remove couplings to the methyl protons in this molecule. Since the coupling to C_b is small only low power irradiation would be necessary to remove this coupling.

Now let us consider the proton spectra of the molecule with a ^{13}C atom in the methyl group. A simplified ^1H n.m.r. spectrum of this molecule is shown below (fig. 22).

Fig. 22.



It can now be seen that the methyl signal is now a large doublet. In order to irradiate this signal efficiently it would be necessary to irradiate the sample at high power levels since no nuclei are actually resonating at the frequency of the centre of the doublet. Low power irradiation would have only a modest impact on the ^1JCH coupling of the methyl group in the ^{13}C n.m.r. spectrum.

Gated decoupling techniques

Irradiation of the protons in the molecule has two effects on the ^{13}C n.m.r. spectra. Firstly, it causes the signals to collapse to singlets and secondly it increases the intensity of certain signals due to the nuclear Overhauser effect. (n.O.e.) [35]

Fortunately, the nuclear Overhauser enhancement is an equilibium effect which builds up or decays over a period of time while spin-spin decoupling is effectively instantaneous.

This means it is possible to select to have decoupling without the n.O.e. or alternatively to have the n.O.e. applied to an undecoupled spectrum. This is achieved by "Gating" the decoupler, that is, by switching the decoupler on or off at specific times during the pulse/data acquisition cycle. If the decoupler is only switched on during data acquisition then data will be obtained about the decoupled spectrum. However during the brief period of data acquisition the nuclear Overhauser effect will not have had an opportunity to build up and no significant enhancement will therefore be observed.

If the gating is operated in reverse the converse is found. The n.O.e. effect is maintained by leaving the decoupler on for most of the time but coupling information is obtained by switching off the decoupler during data acquisition. This latter approach has been used extensively in this dissertation since the nuclear Overhauser enhancement can substantially reduce the length of time required to obtain an acceptable signal to noise ratio in the spectrum.

CHAPTER 1

Development of a method of assessing N-1:N-3 protonation ratio of aminopyrimidines.

Introduction

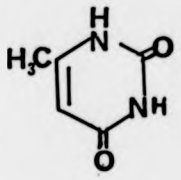
Pyrimidines have attracted considerable interest over the past two decades because of the biological importance of a number of compounds containing the pyrimidine ring system.

The pyrimidines thymine (10) and cytosine (11), for example, are two of the four base units found in DNA and RNA. The vitamin thiamin (12) also contains a pyrimidine ring as does the antibacterial drugs sulphadiazine (13) and sulphamethoxine (14).

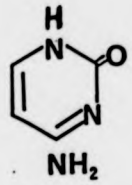
Although the pKa values of a large number of pyrimidines have been determined, relatively little attention has been given to evaluating the effects of a particular substituent on the basicities of the individual ring nitrogens. To do this we need to be able to measure the individual basicities of the N-1 and N-3 sites in the pyrimidine. This would require knowledge not only of the pKa value of the molecule as a whole but also the relative proportions of the N-1 and N-3 protonated forms of the molecule when the system is monoprotonated.

Theory

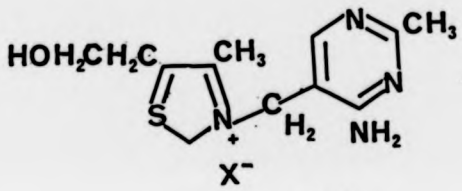
Consider the substituted pyrimidine (15). When the system is completely monoprotonated two rapidly interconverting forms (16) and (17) will be present corresponding to protonation at N-1 and N-3 respectively.



(10)



(11)



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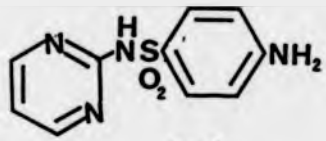

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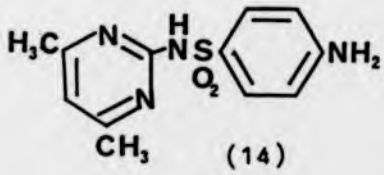

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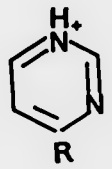
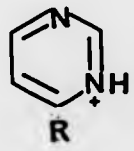


(16)



(17)

Clearly the relative proportions of (16) and (17), the N-1:N-3 protonation ratio, will depend on the relative basicities of the two nitrogens which will in turn depend on the substituent R. This can be represented by the expressions:

$$B + H^+ \overset{K_1}{\rightleftharpoons} P_1$$

and

$$B + H^+ \overset{K_3}{\rightleftharpoons} P_3$$

Where B = (15), P_1 = (16), P_3 = (17) and K_1 and K_3 are the equilibrium constants for the N-1 and N-3 nitrogens respectively.

Since $K_1 = \dfrac{[B][H^+]}{[P_1]}$ and $K_3 = \dfrac{[B][H^+]}{[P_3]}$

then $$\frac{K_1}{K_3} = \frac{[P_3]}{[P_1]}$$

hence $$pK_1 - pK_3 = -\log_{10} \frac{[P_3]}{[P_1]} \qquad (6)$$

also $$K_1K_3 = \frac{[B]^2[H^+]^2}{[P_1][P_3]}$$

and hence $$pK_1 + pK_3 = -\log_{10} \frac{[B]^2}{[P_1][P_3]} + 2pH$$

since $$pK_3 = pK_1 + \log_{10} \frac{[P_3]}{[P_1]}$$

then $$pK_1 + pK_1 + \log_{10} \frac{[P_3]}{[P_1]} = -\log_{10} \frac{[B]^2}{[P_1][P_3]} + 2pH$$

and therefore,

$$2pK_1 = 2pH - \log_{10}\frac{[P_3]}{[P_1]} - \log_{10}\frac{[B]^2}{[P_1][P_3]}$$

However, when the molecule is 50% protonated

$pH = pK_{obs}$ and $[P_1] + [P_3] = [B]$

We then obtain the expression

$$pK_1 = pK_{obs} - \tfrac{1}{2}\log_{10}\frac{[P_3]}{[P_1]} - \tfrac{1}{2}\log_{10}\frac{[P_1]}{[P_3]} + \frac{[P_3]}{[P_1]} + 2$$

This can be simplified

$$pK_1 = pK_{obs} - \tfrac{1}{2}\log_{10}\frac{[P_3]}{[P_1]}^2 + 2\frac{[P_3]}{[P_1]} + 1$$

$$\text{therefore, } pK_1 = pK_{obs} - \log_{10}\left(\frac{[P_3]}{[P_1]} + 1\right)$$

This enables us to calculate the pK_1 value from a knowledge of pK_{obs} and the N-1:N-3 protonation ratio, $[P_1] : [P_3]$. A similar calculation can be used to calculate pK_3

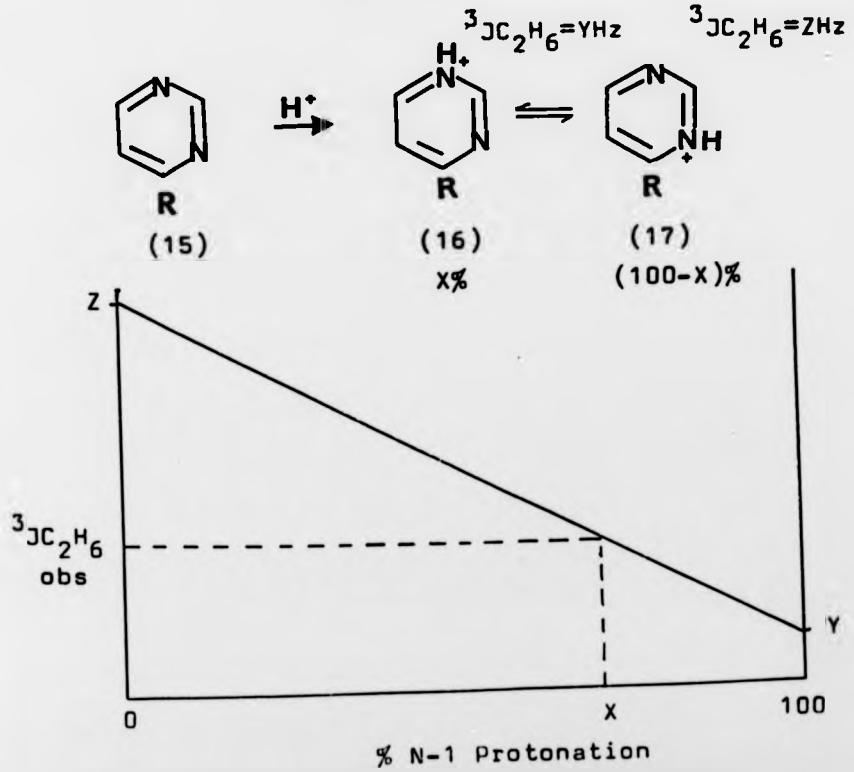
Determination of the N-1:N-3 protonation ratio

Since in general we would not expect the difference in basicities between N-1 and N-3 in the pyrimidine ring to be large it can be seen from Equation 6 that we can always expect some protonation at each site. We are therefore faced with the problem of how the N-1:N-3 protonation ratio can be determined in a given pyrimidine. Although n.m.r. spectroscopy has been used successfully to measure the pKa values

of individual ionisable groups in molecules containing several such groups [36] there are problems in applying this approach to pyrimidines. Unfortunately, the rate of exchange between the two protonated forms of the pyrimidine for example (16) and (17) is so rapid that the n.m.r. parameters obtained for the monoprotonated system represent the weighted average of the parameters from the two exchanging species (16) and (17).

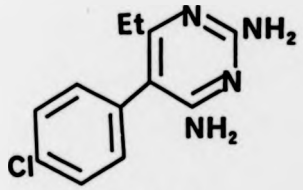
On the other hand if one were able to determine the parameters for the two extreme situations then it would be possible to determine the N-1:N-3 protonation ratio from a knowledge of these averaged parameters. Fig. 24 indicates diagrammatically the basic approach based on a consideration of the through nitrogen vicinal coupling constant $^3JC2H6$.

Fig. 24.

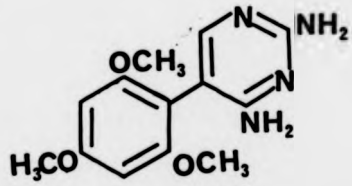


The aim of the work described in this dissertation was to find a suitable model system which would enable n.m.r. parameters to be determined for the N-1 and N-3 protonated forms such as (16) and (17) respectively. It was hoped that these parameters could then be used to establish the N-1:N-3 protonation ratio in a number of substituted pyrimidines. This in turn would enable us to determine the effect of a particular substituent on the basicities of the N-1 and N-3 nitrogens and hence investigate the relative importance of the electronic and steric effects of the substituent.

Since the 2,4 diaminopyrimidine system is found in a number of important drug molecules such as Pyrimethamine (18) and Trimethoprim (19) our initial aim was to establish a method for determining the N-1:N-3 protonation ratio and hence the basicity of the ring nitrogens in this system.



(18)



(19)

One might expect the value of the vicinal coupling constant $^3JC_2H_6$ to be sensitive to the site of protonation hence, our initial investigations were concerned with evaluating the suitability of this parameter for monitoring changes in the N-1:N-3 protonation ratio.

AN EVALUATION OF THE USE OF N-METHYLPYRIMIDINIUM IODIDES AS MODELS FOR MONO-PROTONATION

Since it has been reported that the spectroscopic

effects of N-methylation and N-protonation of pyrimidines are similar,[32] N-methylpyrimidinium iodides were the obvious compounds to study initially as potential models for monoprotonation in pyrimidines. Furthermore since in these methylated systems the methyl group was unable to undergo exchange it was hoped that separate parameters for both the N-1 and N-3 methiodides might be obtained corresponding to the N-1 and N-3 protonated molecules.

In order to assess the suitability of the parameters obtained from the N-methyl pyrimidinium iodides for determining protonation ratios it was, however, necessary to have some system in which the N-1:N-3 protonation ratio was already known. Only then could a comparison be made between this known value and that based on the parameters from the methiodides. The only pyrimidines wherethe N-1:N-3 protonation ratio is known with certainty are those symmetrically substituted pyrimidines where protonation occurs equally at both N-1 and N-3. The initial investigations into the suitability of the N-methyl pyrimidinium iodides therefore concerned the symmetrically substituted system 2-aminopyrimidine.

The 2-aminopyrimidine system

The ^{13}C n.m.r. spectra of 2-aminopyrimidine were determined indeuterochloroform ($CDCl_3$), trifluoracetic acid (T.F.A.) and aqueous hydrochloric acid (pH 0.3). This data is given in Table 1.

The assignment of the three resonances observed in the proton decoupled ^{13}C n.m.r. spectrum of 2-amino-

TABLE 1

25MHz ^{13}C n.m.r. spectral data
2-Aminopyrimidine

Chemical shifts/p.p.m.

Solvent:	$CDCl_3$	T.F.A.	H_2O/HCl[a]
C2	96.02	89.02	88.62
C4,6	90.92	90.75	90.50
C5	44.04	44.25	44.01

Coupling constants/Hz

Solvent:	$CDCl_3$	T.F.A.	H_2O/HCl[a]
$^3J_{C2H4/C2H6}$	11.7	9.9	10.0
$^1J_{C4H4/C6H6}$	177.9	190.1	188.9
$^2J_{C4H5/C6H5}$	3.8	4.1	4.0
$^3J_{C4H6/C6H4}$	5.3.	5.4	5.7
$^1J_{C5H5}$	169.7	181.9	180.1
$^2J_{C5H4/C5H6}$	7.0	5.2	5.5

(a) pH of solution adjusted to 3 pH units below pka of 2-aminopyrimidine

pyrimidine in $CDCl_3$ was readily achieved by consideration of the proton-coupled spectrum (Plate 1). C-2 was readily identified as the lowest field signal at 96.0 p.p.m. (shifts relative to 1,4-dioxan) since this lacks the large ^1JCH coupling due to a directly bonded hydrogen. The triplet character of this signal is due to coupling with H-4 and H-6. The resonances due to C-4 and C-6 are coincident and are observed as a doublet of doublet of doublets at 90.9 p.p.m. while that due to C-5 is observed at highest field, 44.0 p.p.m., as a doublet of triplets.

The ^{13}C n.m.r. spectra of the monoprotonated system were similar in both T.F.A. and aqueous hydrochloric acid (pH 0.3) although the spectrum in the hydrochloric acid was better resolved (Plate 2). Interestingly, monoprotonation appeared to produce a marked upfield shift of about 7 p.p.m. on the C-2 signal and have little effect on the other resonances. This, however, can be explained by consideration of the spectra of N-methylpyrimidinium iodide in water and deuterated dimethyl sulphoxide (D.M.S.O.), this data is given in Table 2. Although these spectra were more complex than those for the protonated pyrimidine in that separate resonances could be seen for every carbon, (Plate 3), the assignment of the signals was nevertheless straightforward. C-2 was again easily assigned since it lacked the large ^1JCH coupling. C-5 was assigned to the highest field aromatic signal at 43.84 p.p.m., and C-4 and C-6 were distinguishable since C-6 exhibited a

PLATE 1

^{13}C N.M.R. SPECTRUM OF 2-AMINOPYRIMIDINE

IN DEUTEROCHLOROFORM (PROTON COUPLED)

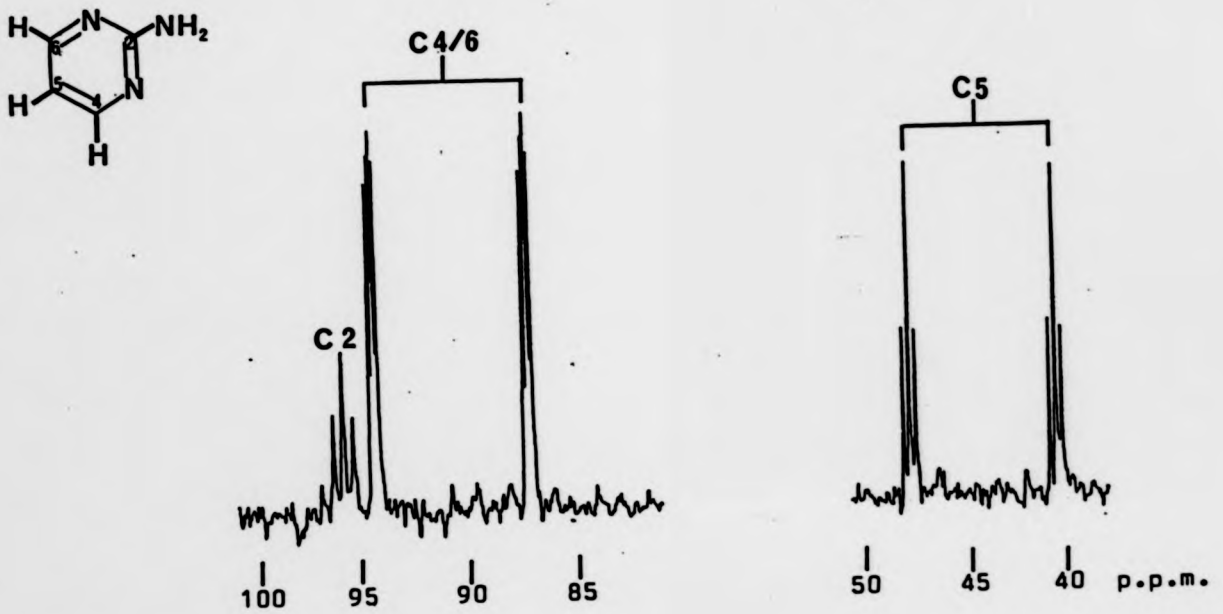


PLATE 2

^{13}C N.M.R. SPECTRUM OF 2-AMINOPYRIMIDINE

IN WATER OF PH 0·3 (PROTON COUPLED)

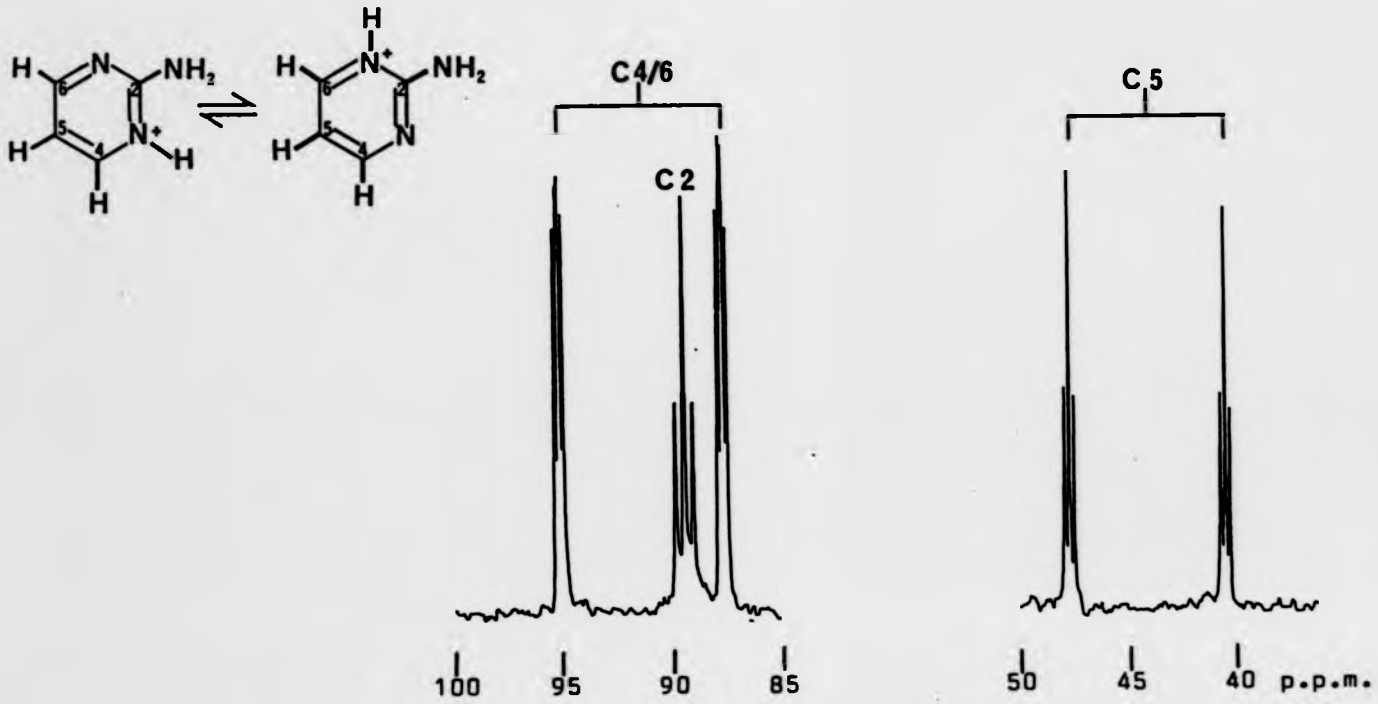


TABLE 2

25MHz ^{13}C n.m.r. spectral data
2-Amino-1-methylpyrimidinium iodide

Chemical shifts/p.p.m.

Solvent:	D_2O	D.M.S.O.
C2	89.24	89.36
C4	99.16	99.02
C5	44.15	43.84
C6	84.12	84.24
N-CH_3	-24.32	-24.17

Coupling constants/Hz

Solvent:	D_2O		D.M.S.O.	
^3JC2H4	} a	(13.8)	} a	(13.9)
^3JC2H6		(5.9)		(5.8)
^1JC4H4	187.4	(186.2)	186.8	(185.5)
^2JC4H5	2.7	(2.7)	2.7	(2.7)
^3JC4H6	6.4	(6.4)	6.4	(6.4)
^1JC5H5	180.0	(177.5)	178.8	(175.8)
^2JC5H4	7.9	(7.9)	7.9	(7.9)
^2JC5H6	3.3	(3.3)	3.3	(3.3)
^1JC6H6	191.0	(189.9)	190.4	(189.2)
^2JC6H5	} a	(5.3)	} a	(5.3)
^3JC6H4		(5.3)		(5.3)
^1JCH(N-CH_3)	144.6	(-)	143.7	(-)
^3JCH(N-CH_3H6)	3.9	(-)	3.9	(-)

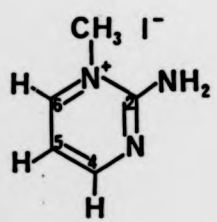
Values in parenthesises are those obtained when selectively decoupling N-methyl protons with low power irradiation.

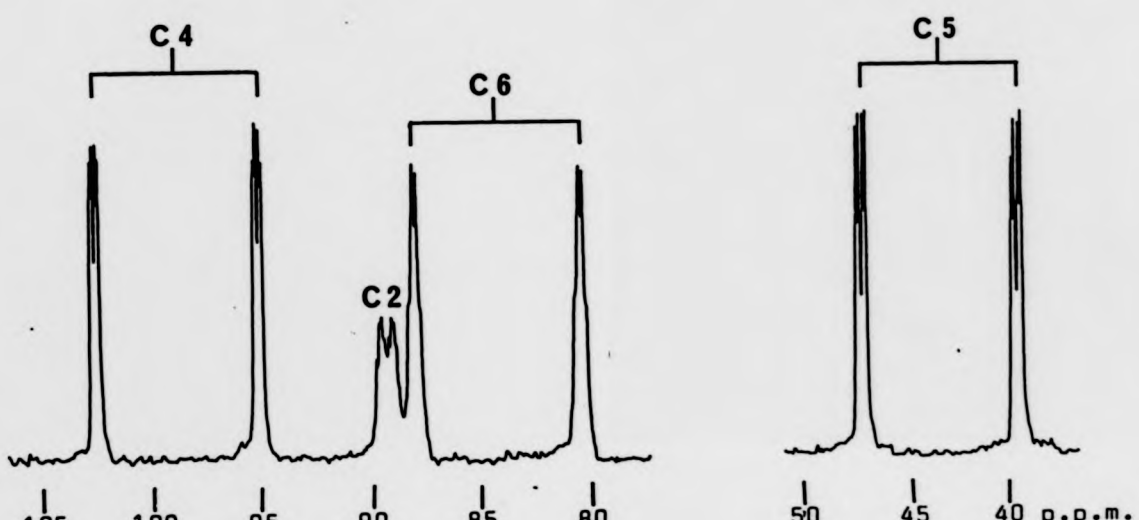
(a) Complex multiplet, couplings not resolved.

PLATE 3

^{13}C N.M.R. SPECTRUM OF 2-AMINO-1-METHYLPYRIMIDINIUM IODIDE

IN D6 D.M.S.O. (PROTON COUPLED)





long-range coupling to the N-methyl protons in addition to those from the other ring protons. A consideration of these chemical shifts shows that methylation produces a significant shift on both C-6 and C-4 but that the shifts are in opposite directions. Assuming that similar effects occur on protonation we can see that the average shift of the C-4 and C-6 carbons would appear essentially unchanged as observed.

The proton-carbon coupling constants for 2-amino-pyrimidine, its monoprotonated form and 2-amino-1-methylpyrimidinium iodide were determined assuming the spectra to be first order and the analysis confirmed by spectrum simulation.

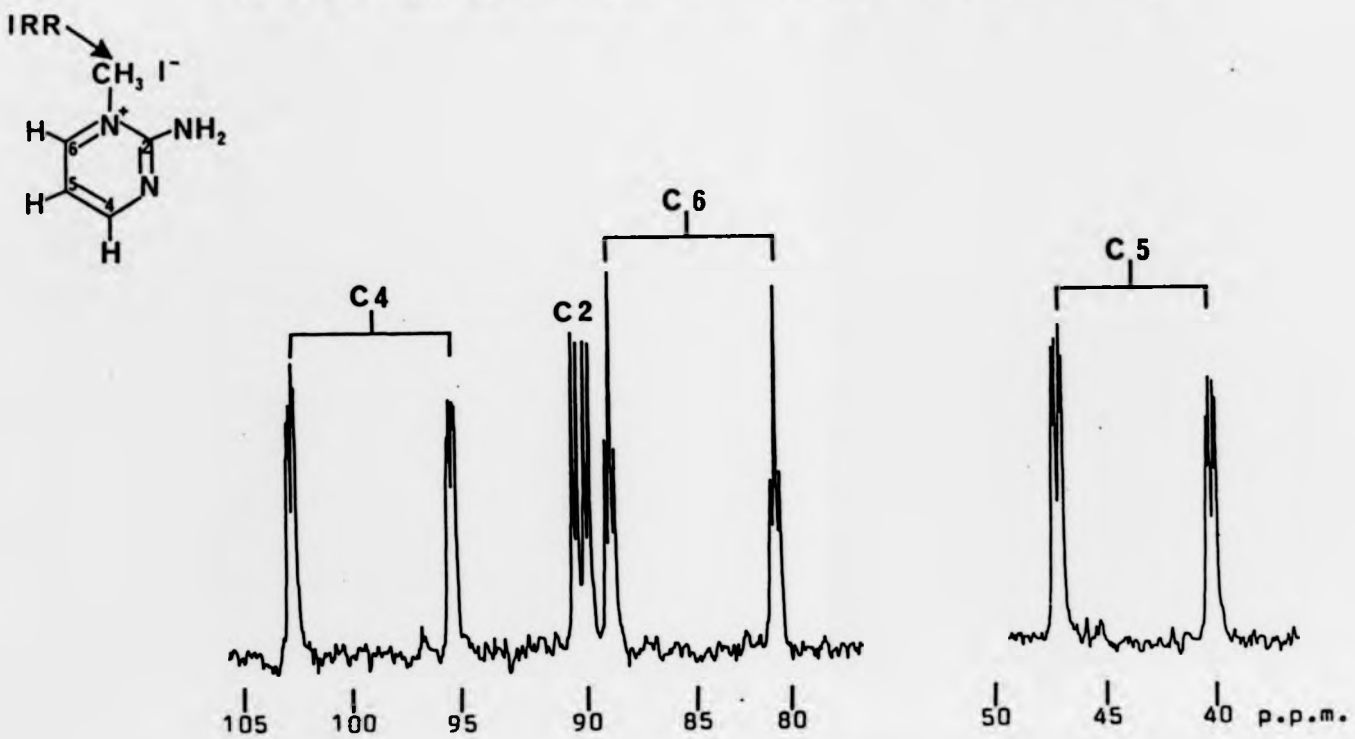
It is interesting to note that the vicinal through nitrogen coupling constants $^{3}JC2H4/6$ decreases from 11.7 to 9.9 Hz on protonation. This type of effect has also been seen in pyridine where a reduction in the through nitrogen vicinal coupling of 4.7 Hz was observed[31]. The smaller effect in the pyrimidine system is not unexpected since the observed coupling, $^{3}JC2H4/6$, reflects the average of two vicinal through nitrogen couplings since only one nitrogen at any instant of time is protonated. It is not possible, however, to evaluate the effect of protonation on the vicinal through nitrogen ($^{3}JC2H6$) coupling in the 2-aminopyrimidine simply by doubling the observed decrease in $^{3}JC2H4/6$ since the effect of protonation at N-1 may well have an effect on the through nitrogen coupling $^{3}JC2H4$ and vice versa.

In order to expose the $^{3}JC2H4$ and $^{3}JC2H6$ values in 2-amino-1-methylpyrimidinium iodide it was necessary to remove the long range coupling to the N-methyl protons. This was achieved by the selective decoupling of the N-methyl protons using low-power irradiation at the frequency of the centre of the methyl resonance in the ^{1}H n.m.r. spectrum, as previously discussed (see introduction). These low-power selective decoupling experiments need to be carried out with great care. While it is important to use enough power to remove the long-range couplings to the N-methyl protons it is also essential that the irradiation should not significantly reduce those coupling constants which are to be determined. The data from the spectra of 2-amino-1-methylpyrimidinium iodide with and without selective low-power irradiation of the N-methyl protons are compared in Table 2. It can be seen that by careful adjustment of the decoupling power it was possible to remove the long-range couplings to the N-methyl protons while at the same time having minimal effect(<1%) on the other couplings in the pyrimidine ring.

The effect of the low-power selective irradiation is shown in Plate 4. The C-2 resonance is now clearly visible as a doublet of doublets due to couplings with C-4 and C-6. Since it is known that protonation reduces the through-nitrogen vicinal coupling constant the smaller coupling of 5.8 Hz is assigned to $^{3}JC_{2}H6$ while that of 13.9 Hz is assigned to $^{3}JC2H4$. It is also interesting to note that methylation at N-1 increases

PLATE 4

^{13}C N.M.R. SPECTRUM OF 2-AMINO-1-METHYLPYRIMIDINIUM IODIDE IN D6 D.M.S.O. WITH N-METHYL PROTONS SELECTIVELY IRRADIATED

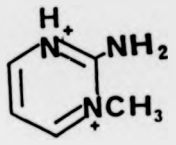


the value of $^{3}JC2H4$ by 2.2 Hz relative to the non-protonated system. Considered from another point of view this means that $^{3}JC2H6$ will be sensitive not only to methylation at N-1 but also at N-3.

In order to compare the effect of methylation with that of protonation it is necessary to compare the average of $^{3}JC2H6$ and $^{3}JC2H4$ (since this is the only parameter available from protonation studies). In T.F.A. and aqueous hydrochloric acid the mono-protonated form of 2-aminopyrimidine gave values for $^{3}JC2H4/6$ of 9.9 Hz and 10.0 Hz respectively. These are in excellent agreement with the value of 9.85 Hz calculated from the values for $^{3}JC2H4$ and $^{3}JC2H6$ in the N-methylated system in water. The differences in value are within the limits of experimental error since the couplings were determined to an accuracy of $\pm$ 0.2 Hz.

These observations would therefore support the view that 2-aminopyrimidinium iodide is a suitable model system to provide $^{3}JC2H6$ values for the interconverting protonated forms of 2-aminopyrimidine.

Further evidence for the similarity in the effects of protonation and methylation on the ^{3}JCH vicinal through-nitrogen coupling constants was provided by a study of the protonated form of 2-amino-1-methyl-pyrimidinium iodide (20).



(20)

Since diprotonation of pyrimidines is only achieved in extremely strong acids it was necessary to use concentrated sulphuric acid to protonate the 2-amino-1-methylpyrimidinium iodide. The ^{1}H n.m.r. spectrum of (20) in 100% sulphuric acid was poorly resolved due to the viscosity of the solvent but showed only two "aromatic" resonances, a triplet corresponding to H-5 and a doublet corresponding to H-4 and H-6 Addition of a little water to the n.m.r. sample caused the spectrum to revert to the three doublets of doublets characteristic of 2-amino-1-methylpyrimidinium iodide showing that the acid treatment had not caused an irreversible change. The ^{13}C n.m.r. spectrum of the 2-amino-1-methylpyrimidine iodide in 100% sulphuric acid with the N-methyl protons selectively irradiated (Plate 5) also shows C-2 as a triplet ($\sim$ 7.2 Hz) showing that $^{3}JC2H4 = {}^{3}JC2H6$. This again provides evidence that the effects of methylation and protonation are similar. However, we must be cautious in placing too much emphasis on this latter evidence since the situation in a dicationic species such as (20) may not be comparable to the situation found in the monocationic systems.

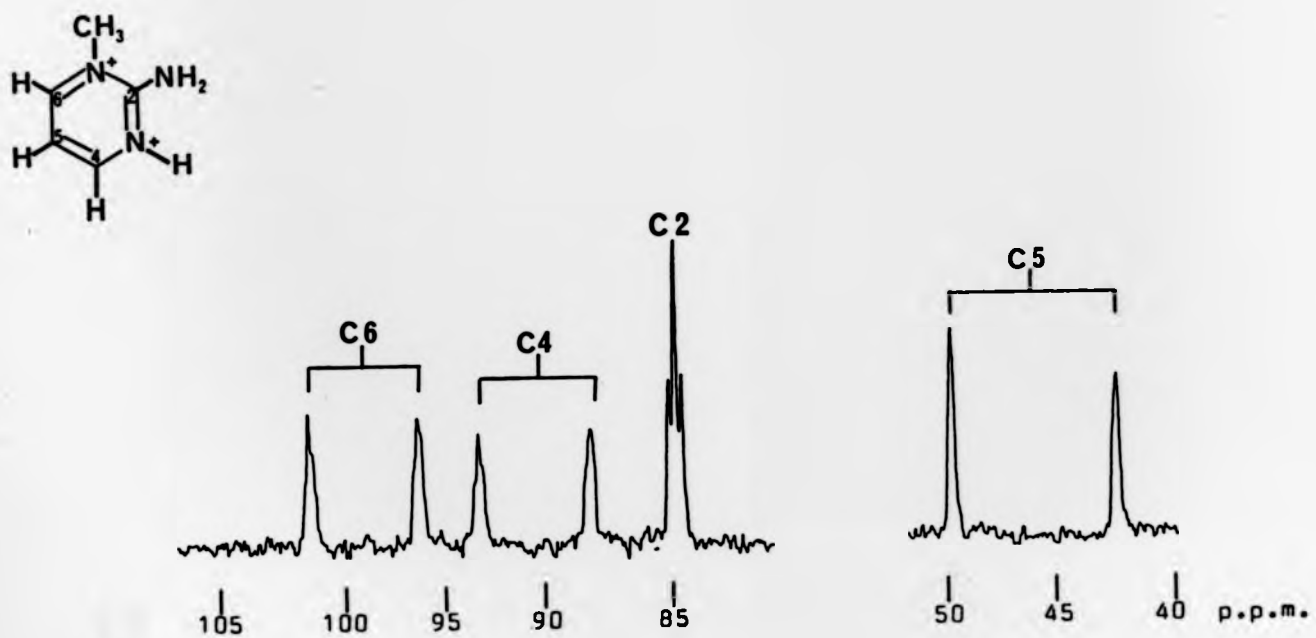
<u>The 2-amino-4-methylpyrimidine system</u>

Having obtained evidence that N-methylpyrimidinium iodides are suitable model systems for providing information about the effects of protonation on vicinal through nitrogen coupling constants it was of interest to determine the N-1:N-3 protonation ratio in an

PLATE 5

^{13}C N.M.R. SPECTRUM OF 2-AMINO-1-METHYLPYRIMIDINIUM IODIDE

IN SULPHURIC ACID WITH N-METHYL PROTONS SELECTIVELY IRRADIATED



asymmetrically substituted system. The system chosen was the 2-amino-4-methylpyrimidine system because we would not expect the methyl group to have a major effect on the relative basicities of N-1 and N-3 so that the protonation ratio would not be expected to be extreme. Furthermore, the monoprotonation of the 2-amino-4-methylpyrimidine system had already been studied by a ^{13}C n.m.r. method involving calculations based on the chemical shifts changes resulting from protonation [30].

The ^{13}C n.m.r. spectra of 2-amino-4-methylpyrimidine were determined in $CDCl_3$, T.F.A. and aqueous hydrochloric acid (pH 1) and this data is given in Table 3. In the proton coupled ^{13}C n.m.r. spectrum of 2-amino-4-methylpyrimidine $CDCl_3$, C-2 was observed as a doublet due to coupling to H-6. Interestingly, the value of this coupling (11.7 Hz) was identical to that observed in 2-aminopyrimidine indicating that the introduction of the methyl group had had minimal effect on $^{3}JC2H6$. However, in aqueous hydrochloric acid (Plate 6) the value of $^{3}JC2H6$ (9.5Hz) differed slightly from that in 2-aminopyrimidine (10.0Hz) under similar conditions. In T.F.A. a more significant difference was observed ($^{3}JC2H6$ = 8.9 Hz). These effects can be attributed to changes in the N-1:N-3 protonation ratio.

In order to obtain parameters for the effects on $^{3}JC2H6$ of protonation at N-1 and N-3 it was necessary to synthesise the 2-amino-1,4-dimethylpyridinium iodide and the previously unreported 2-amino-3,4-dimethyl-

TABLE 3

25MHz ^{13}C n.m.r. spectral data

2-Amino-4-methylpyrimidine

Chemical shifts/p.p.m.

Solvent:	$COCl_3$	T.F.A.	H_2O/HCl[a]
C2	95.76	88.56	88.54
C4	100.89	108.40	106.21
C5	43.79	44.85	44.51
C6	90.53	85.54	86.20
CH_3	-43.06	-44.69	-43.77

Coupling constants/Hz

Solvent:	$COCl_3$	T.F.A.	H_2O/HCl[a]
$^3J_{C2H6}$	11.7	8.9	9.5
$^2J_{C4H5}$ / $^3J_{C4H6}$	} b	} b	} b
$^1J_{C5H5}$	167.2	180.1	178.2
$^2J_{C5H6}$	7.2	4.6	5.1
$^1J_{C6H6}$	176.7	190.1	188.0
$^2J_{C6H5}$	3.5	4.1	4.2
$^1J_{CH(CH_3)}$	127.0	130.6	130.0

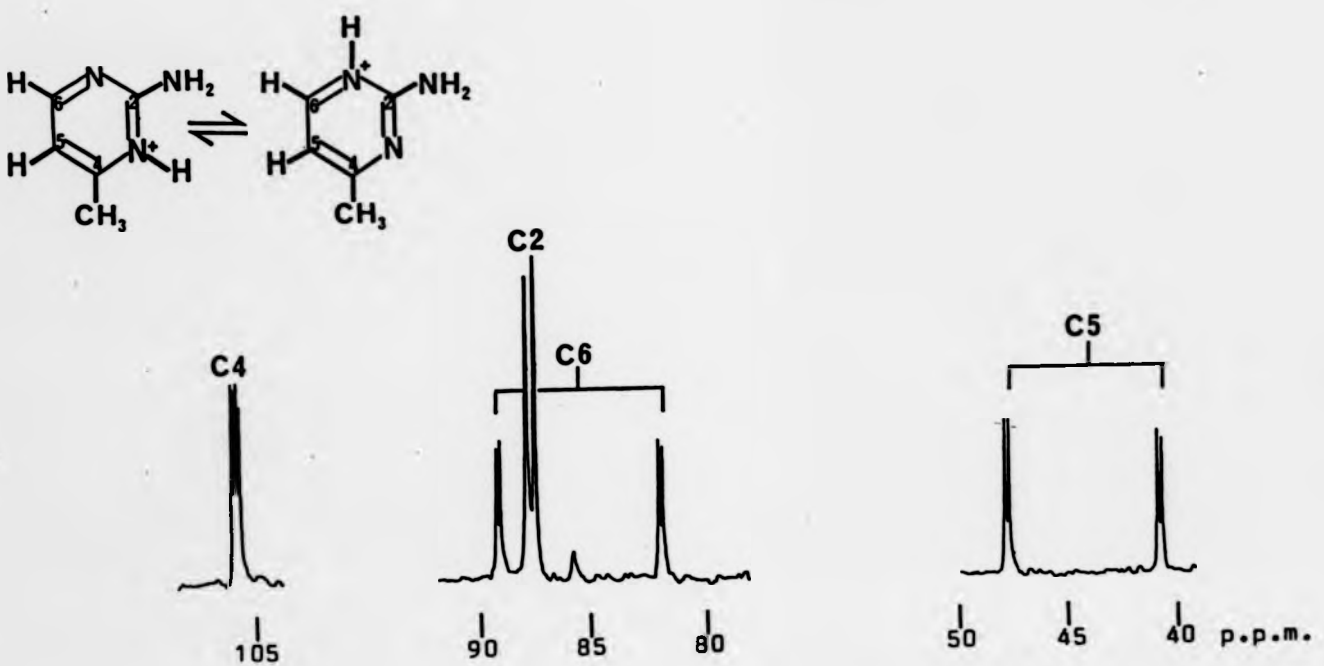
(a) pH of solution adjusted to 3 pH units below pka of 2-amino-4-methylpyrimidine.

(b) Complex multiplet, couplings not resolved.

PLATE 6

^{13}C N.M.R. SPECTRUM OF 2-AMINO-4-METHYLPYRIMIDINE

IN WATER OF PH 0·3 (PROTON COUPLED)



pyrimidinium iodide.

The synthesis of 2-amino-1,4-dimethylpyrimidinium iodide (21) and 2-amino-3,4-dimethylpyrimidinium iodide (22)

2-Amino-4-methylpyrimidine was readily quaternised with iodomethane to give 2-amino-1,4-dimethylpyrimidinium iodide (21). The site of methylation was readily confirmed by ^{13}C n.m.r. spectroscopy (see later discussion). No evidence for the formation of the corresponding N-3 methiodide was observed.

In order to prepare 2-amino-3,4-dimethylpyrimidinium iodide (22) it was therefore necessary to devise a suitable synthetic route (see Fig. 25). Interestingly, it had been reported that 2-amino-4-chloro-6-methylpyrimidine (23) reacts with methyl sulphate in nitrobenzene at 80 - 90° to give 2-amino-4-chloro-1,6-dimethylpyrimidinium methosulphate (24)[6,37].

(24)

If this were the case then removal of the chlorine atom would provide a route to the desired methiodide (22).

2-Amino-4-chloro-6-methylpyrimidine (23) was prepared by the action of phosphoryl chloride on 2-amino-6-methyl-4(3H)pyrimidinone (25) which in turn was prepared in almost quantitative yield by the

Fig. 25.

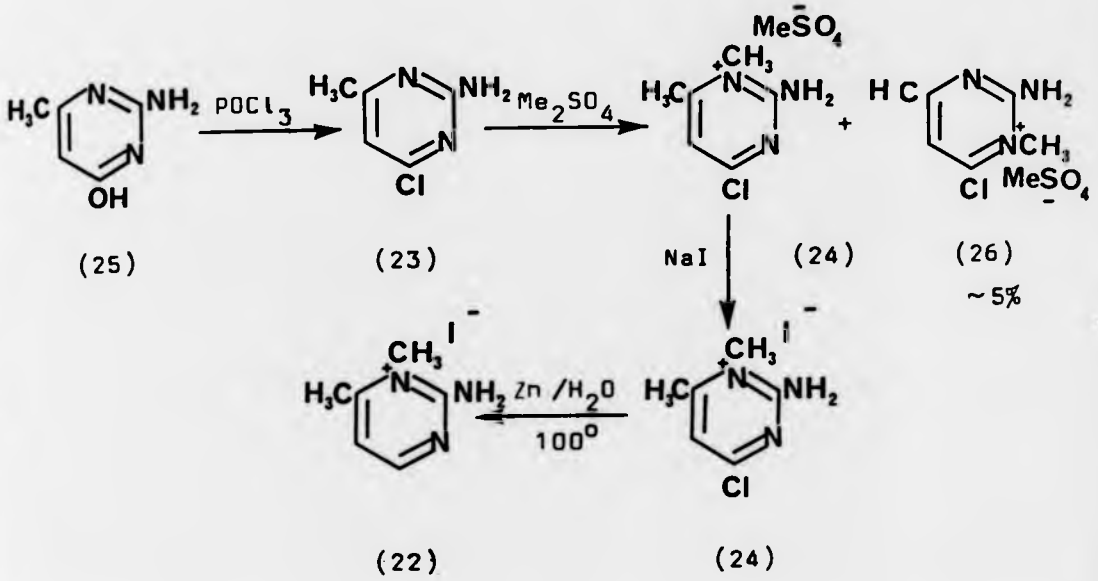
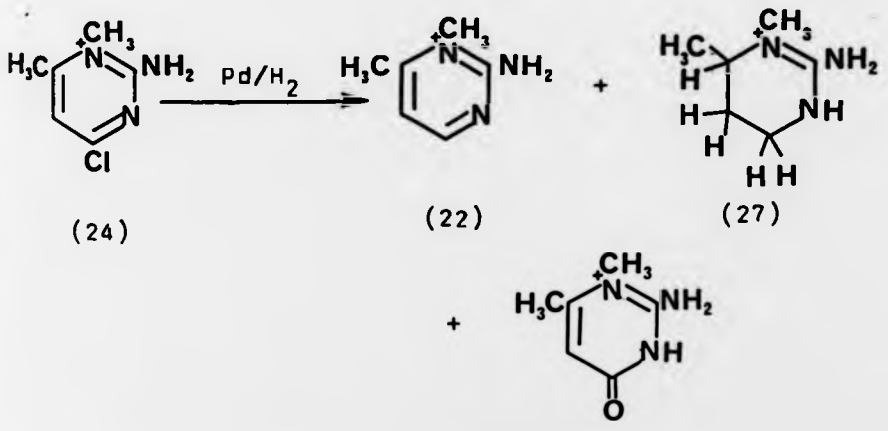


Fig. 26.

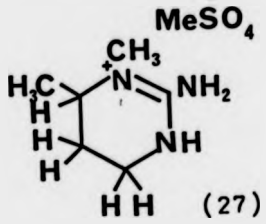


reaction of ethyl acetoacetate with guanidine carbonate in the presence of aqueous base. The reaction was mildly exothermic and demonstrates the ease of reaction of guanidine with β-keto esters due to the strong basicity of the N-C-N fragment in this reaction.

The reaction of the 2-amino-4-chloro-6-methylpyrimidine (23) with methyl sulphate, as previously described, yielded the desired 2-amino-4-chloro-1,6-dimethyl-pyrimidinium methosulphate (24) together with a little 2-amino-4-chloro-3,6-dimethylpyrimidinium methosulphate (26). Treatment of this mixture with aqueous sodium iodide resulted in the precipitation of the 2-amino-4-chloro-1,6-dimethylpyrimidinium iodide (24). It was later found that this material was also produced by the action of iodomethane on 2-amino-4-chloro-6-methyl-pyrimidine in acetone at room temperature. This is contrary to previous reports which suggested that both N-1 and N-3 isomers are formed with iodomethane . It should be noted, however, that the earlier work had been carried out at elevated temperatures which may account for the formation of the N-3 methiodide.

Since it had been reported that the removal of the chlorine atom from chloro-pyrimidines can be achieved by catalytic hydrogenation with a palladium on charcoal catalyst in the presence of magnesium oxide[21], this was the method initially investigated to convert (24) into the desired 2-amino-3,4-dimethyl pyrimidinium system (22). Unfortunately, although 2-amino-4-chloro-6-methylpyrimidine was found to be readily converted

to 2-amino-4-methylpyrimidine by this method, complications arose with the N-methyl compounds. With 2-amino-4-chloro-1,6-dimethylpyrimidinium methosulphate the uptake of hydrogen was three times greater than expected and this together with N.M.R. spectroscopic evidence suggested that the product was a tetrahydro pyrimidine (27).



(27)

This behaviour would suggest that the positively charged pyrimidine ring is more susceptible to reduction than the neutral ring system.

Attempts to stop the reduction after the uptake of only one equivalent of hydrogen to minimise reduction of the ring were unsuccessful. The product did contain a little of the desired 2-amino-3,4-dimethylpyrimidinium methosulphate (22) but the major component was unreacted starting material (24) (60%) together with some of the tetrahydropyrimidine (27) (Fig. 26). The catalytic hydrogenation of 2-amino-4-chloro-1,6-dimethyl-pyrimidinium iodide was also investigated. With the iodide the uptake of hydrogen was much slower and the reaction was stopped after one equivalent of hydrogen was taken up. A significant quantity (50%) of the desired product (22) was present. However, in addition

there were also significant quantities of the tetrahydropyrimidine (27) and 2-amino-3,4-dimethyl-6-oxo pyrimidine iodide (28) (Fig. 26).

A variety of other hydrogenation catalysts were therefore investigated in an attempt to find a suitable method for removing the chlorine atom from the ring without causing ring reduction. Unfortunately the more active catalysts, such as Lindlars catalyst, still led to some ring reduction whilst the less active catalysts, such as copper chromite, were unable to remove the chlorine before nucleophillic substitution led to the formation of the 6-oxo derivative (28).

Fortunately a reagent was discovered, namely zinc dust in boiling water, which enabled the chlorine to be removed without loss of the aromatic ring. However, even with this reagent the reaction conditions were critical if the desired product was to be produced without the formation of the 6-oxo derivative (28).

The optimum conditons required to convert 2-amino-4-chloro-1,6dimethylpyrimidinium iodide (24) to 2-amino-3,4-dimethylpyrimidinium iodide (22) involved adding the (24) to a boiling suspension of zinc dust in water and then heating under reflux for precisely 5 minutes. A shorter time caused the product to be contaminated with the starting material (24) while a longer time caused production of the 6-oxo derivative (28) presumably due to nucleophilic attack on the desired product (22).

The use of 2-amino-1,4-dimethylpyrimidinium iodide (21) and 2-amino-3,4-dimethylpyrimidinium iodide (22) as models for protonation

2-Amino-1,4-dimethylpyrimidinium iodide and 2-amino-3,4-dimethylpyrimidinium iodide spectral data is given in Tables (4) and (5) respectively. The proton coupled ^{13}C n.m.r. spectrum of 2-amino-1,4-dimethylpyrimidinium iodide (Plate 7) shows C-2 to be a complex multiplet due to the extra couplings to the N-methyl protons. However, the spectrum with the N-methyl protons selectively irradiated (Plate 8) shows the $^{3}JC2H6$ coupling exposed and also a simplification of the C-6 resonance confirming the site of methylation as N-1. In contrast in the ^{13}C n.m.r. proton coupled spectrum of 2-amino-3,4-dimethylpyrimidinium iodide (Plate 9). C-2 was observed as a complex multiplet but C-6 did not show any long range couplings to the N-methyl protons. As expected selective irradiation of the N-methyl protons (Plate 10) simplified C-2 to a doublet and also simplified C-4 confirming N-3 as the site of methylation. The values of $^{3}JC2H6$ in the N-1 and N-3 methiodides are 6.0 and 13.6 Hz respectively and these are very similar to those observed for $^{3}JC2H6$ and $^{3}JC2H4$ in 2-amino-1-methylpyrimidinium iodide (5.8 and 13.9). If these parameters are applied to the 2-amino-4-methylpyrimidine system then in aqueous acid the N-1: N-3 protonation ratio can be calculated to be 54:46 while in T.F.A. the ratio becomes 62:38.

TABLE 4

25MHz ^{13}C n.m.r. spectral data

2-Amino-1,4-dimethylpyrimidinium iodide

Chemical Shifts/p.p.m.

Solvent:	D_2O
C2	88.64
C4	111.30
C5	45.20
C6	82.44
CH_3	-42.01
$N\text{-}CH_3$	-25.24

Coupling constants/Hz

Solvent:	D_2O	
$^3J_{C2H6}$	a	(6.0)
$^2J_{C4H5}$	a	a
$^3J_{C4H6}$		
$^1J_{C5H5}$	177.6	(174.6)
$^2J_{C5H6}$	3.4	(3.4)
$^1J_{C6H6}$	189.2	(187.4)
$^2J_{C6H5}$	a	(5.1)
$^1J_{CH(CH_3)}$	129.0	(-)
$^1J_{CH(N\text{-}CH_3)}$	144.3	(-)

Values in parenthesise are those obtained when selectively decoupling N-methyl protons with low power irradiation.

(a) Complex multiplet, couplings not resolved.

TABLE 5

25MHz ^{13}C n.m.r. spectral data

2-Amino-3.4-dimethylpyrimidinium iodide

Chemical shifts/p.p.m.

Solvent:	D_2O
C2	89.99
C4	94.81
C5	46.20
C6	96.75
CH_3	-45.59
$N\text{-}CH_3$	-30.10

Coupling constants/Hz

Solvent:	D_2O	
^3JC2H6	a	(13.6)
^2JC4H5	} a	} a
^2JC4HC4Hme		
^3JC4H6		
^1JC5H5	176.5	(172.4)
^2JC5H6	7.8	(7.8)
^1JC6H6	187.1	(185.3)
^2JC6H5	3.0	(3.0)
^1JCH(CH_3)	128.6	(-)
^1JCH($N\text{-}CH_3$)	143.9	(-)

Values in parenthesise are those obtained when selectively decoupling N-methyl protons with low power irradiation.

(a) Complex multiplet, couplings not resolved.

PLATE 7

^{13}C N.M.R. SPECTRUM OF 2-AMINO-1,4-DIMETHYLPYRIMIDINIUM IODIDE IN DEUTERIUM OXIDE (PROTON COUPLED)

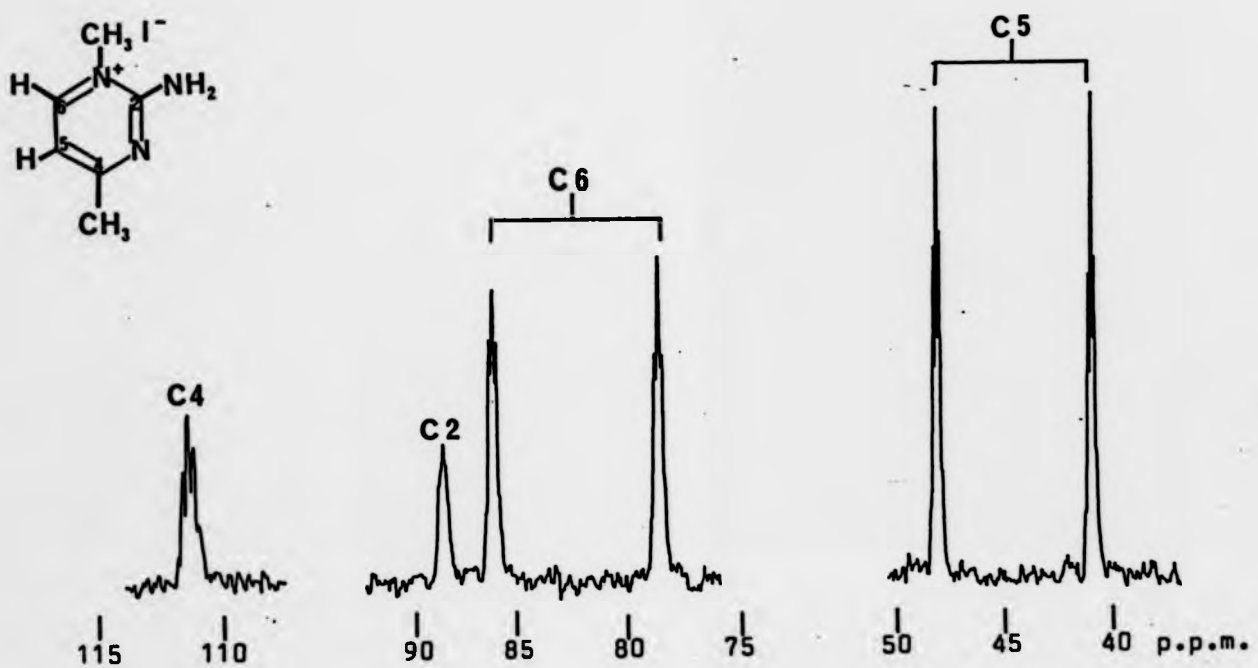


PLATE 8

^{13}C N.M.R. SPECTRUM OF 2-AMINO-1,4-DIMETHYLPYRIMIDINIUM IODIDE IN DEUTERIUM OXIDE WITH N-METHYL PROTONS SELECTIVELY IRRADIATED

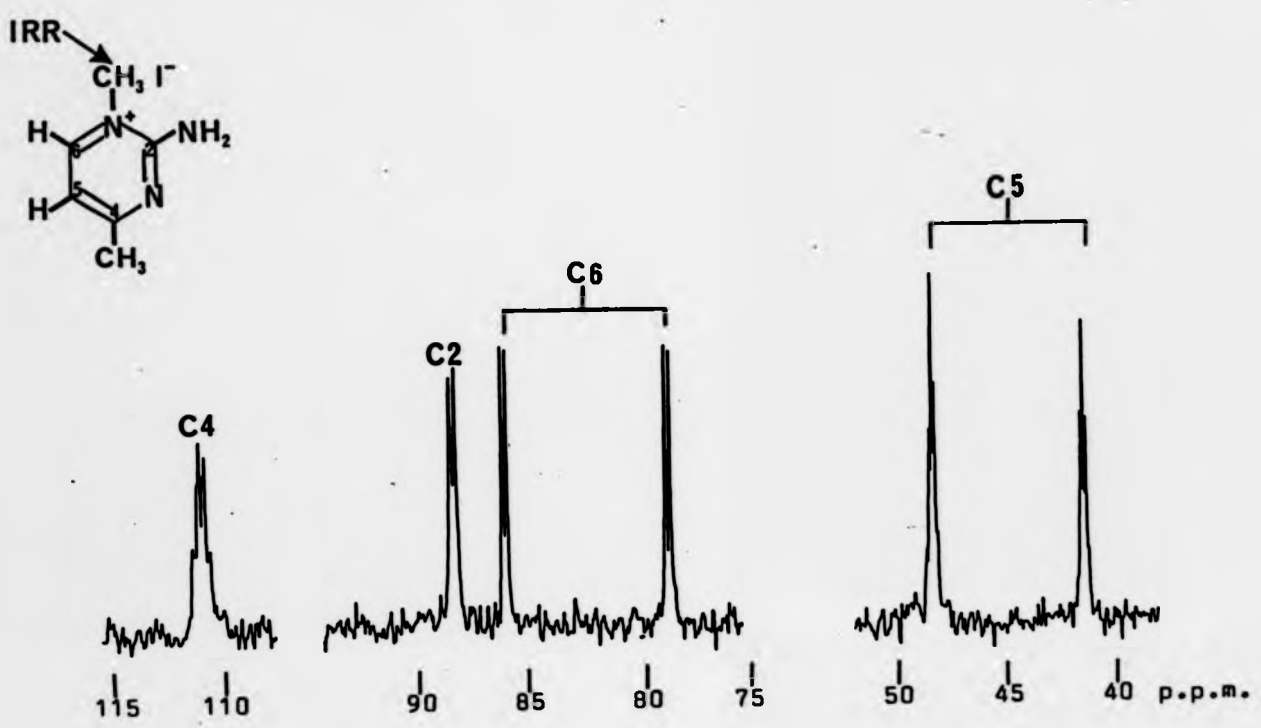


PLATE 9

^{13}C N.M.R. SPECTRUM OF 2-AMINO-3,4-DIMETHYLPYRIMIDINIUM IODIDE IN DEUTERIUM OXIDE (PROTON COUPLED)

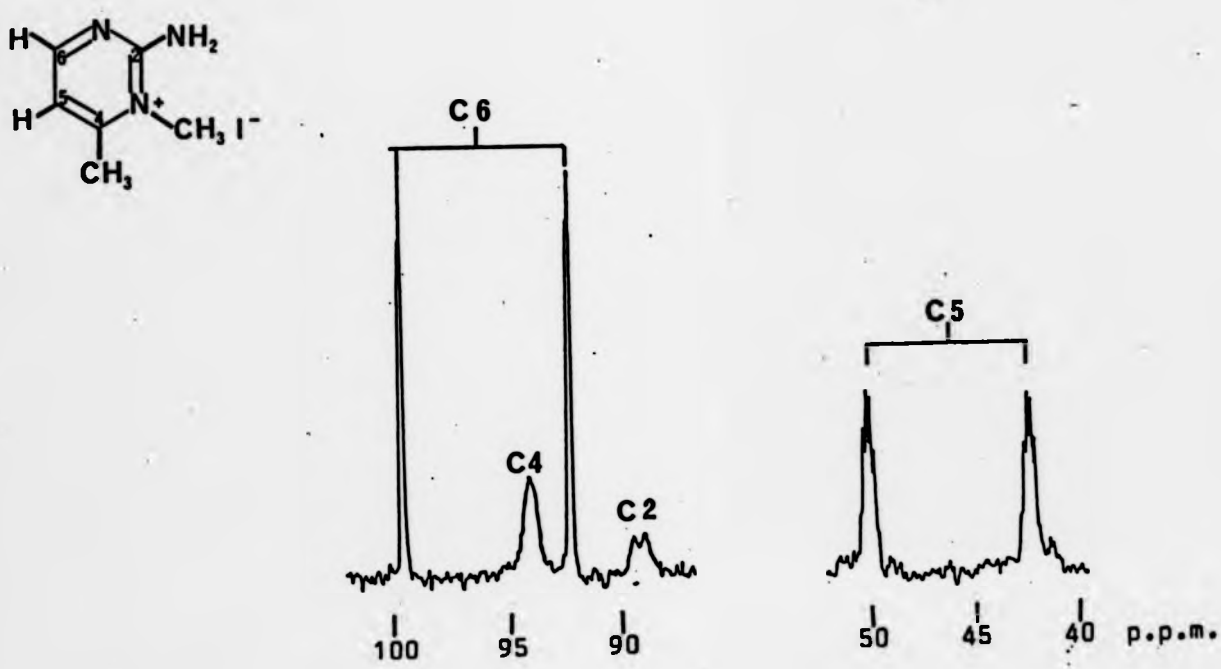
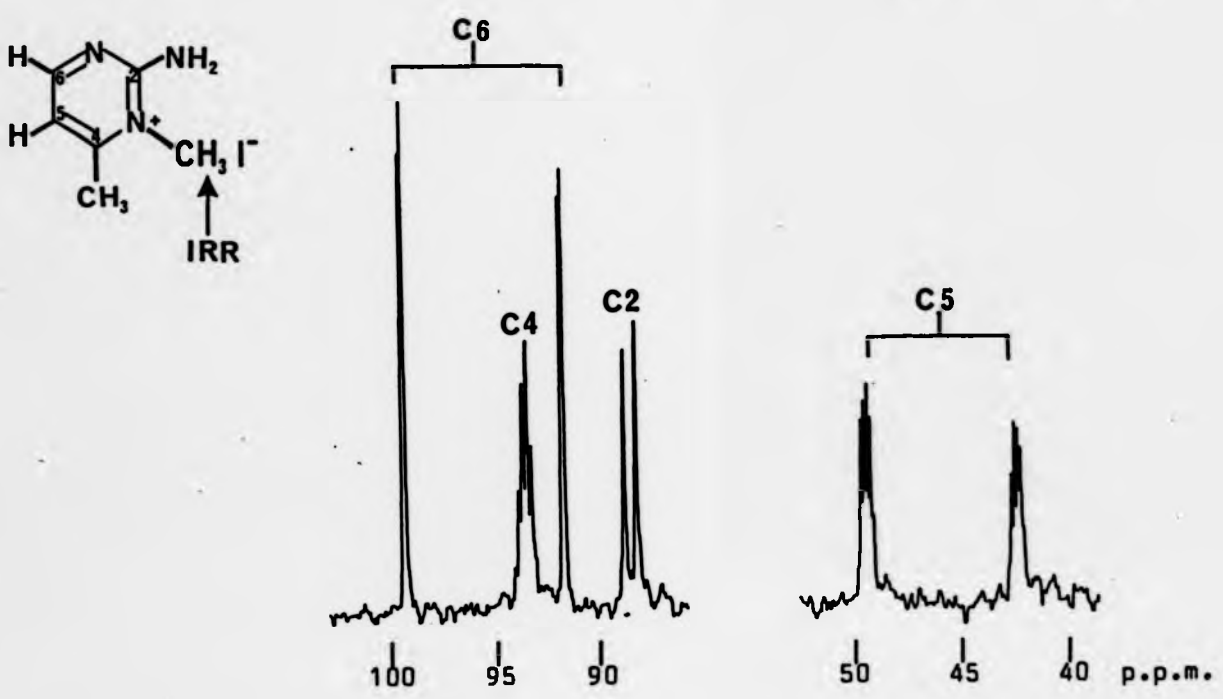


PLATE 10

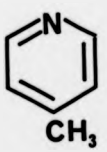
^{13}C N.M.R. SPECTRUM OF 2-AMINO-3,4-DIMETHYLPYRIMIDINIUM IODIDE IN DEUTERIUM OXIDE WITH N-METHYL PROTONS SELECTIVELY IRRADIATED



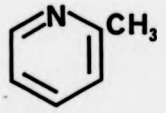
The increase in preference for N-1 protonation must be attributed to steric hindrance at N-3 due to the 4-methyl group since a consideration of the inductive effect of the methyl group would suggest that protonation at N-3 would be favoured.

The difference in the protonation ratios in the two acids is not totally unexpected since the stabilities of the protonated species are affected by the degree of solvation which may differ in the two solvents.

It is interesting to note that the N-1:N-3 protonation ratio of 2-amino-4-methylpyrimidine in water (54:46) implies that $pK_{N1} - pK_{N3} = 0.07$. This is in excellent agreement with the 0.07 predicted from a consideration of the model compounds (29) (pKa 7.48)[24] and (30) (pKa 7.41)[24].



(29)



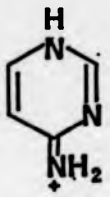
(30)

This provides further support for the use of methiodides as model compounds in these studies.

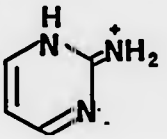
The 2,4-diaminopyrimidine system

Having successfully studied the protonation of 4-methylpyrimidine by the vicinal coupling approach we felt confident to extend this approach to the 2,4-diaminopyrimidine system. Previous workers had suggested that the N-1 site was exclusively

protonated in this molecule although no reasonable physical evidence was presented to support this view.[38] Their beliefs were based on the observation that the basicity of 2-aminopyrimidine (pKa 3.54) was significantly less than that of 4-aminopyrimidine (pKa 5.71). The greater basicity of 4-aminopyrimidine was considered to be due to the stability of the para-quinoid resonance form of the pyrimidine when protonated at N-1 (31), compared to the resonance forms of protonated 2-aminopyrimidine (32).

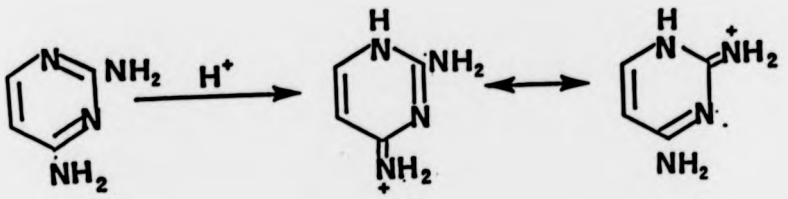


(31)

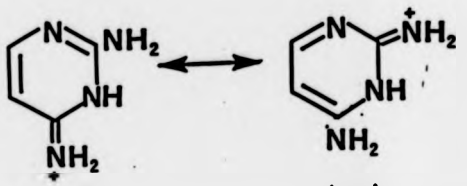


(32)

Similarly the high basicity of 2,4-diaminopyrimidine (7.40)[24] was considered to be due to the stability of the para-quinoid resonance structure (33) which in turn implied essentially exclusive protonation at N-1. However, it could also be argued that there is the possibility of delocalisation of the positive charge when N-3 is protonated and resonance forms such as (34) and (35) would also enhance the basicity of 2,4-diaminopyrimidine.



(33)



(34) (35)

Due to the insolubility of 2,4-diaminopyrimidine in $CDCl_3$ it was necessary to study the free base in water (pH ~ 11). T.F.A. and aqueous hydrochloric acid (pH ~ 4) were again chosen as solvents for studying the monoprotonated species. The ^{13}C n.m.r. spectral data for both the 2,4 diaminopyrimidine and its monoprotonated formsis given in Table 6. It is interesting to note that the ^{3}JC2H6 coupling constant observed for the free base in water was 11.7 Hz, the same value as that observed for 2-aminopyrimidine and 2-amino-4-methyl-pyrimidine in $CDCl_3$ indicating that the introduction of the NH_2 group in the 4 position had had no significant effect on this through nitrogen vicinal coupling. However when 2,4-diaminopyrimidine was studied in aqueous acid (Plate 11) this coupling had decreased substantially to 7.2 Hz while in T.F.A. the value of

TABLE 6

25MHz ^{13}C n.m.r. spectral data

2,4-Diaminopyrimidine

Chemical shifts/p.p.m.

Solvent:	$H_2O/NaOH$[a]	T.F.A.	H_2O/HCl[b]
C2	95.39	34.77	88.10
C4	97.63	92.47	98.19
C5	30.15	32.51	31.38
C6	88.87	77.49	74.92

Coupling constants/Hz

Solvent:	$H_2O/NaOH$[a]	T.F.A.	H_2O/HCl[b]
$^3J_{C2H6}$	11.6	7.6	7.2
$^2J_{C4H5}$	1.8	} c	1.8
$^3J_{C4H6}$	7.3		7.9
$^1J_{C5H5}$	169.7	183.7	177.0
$^2J_{C5H6}$	7.0	c	3.7
$^1J_{C6H6}$	177.0	192.8	187.4
$^2J_{C6H5}$	2.7	c	3.8

(a) pH of solution adjusted to 3 pH units above pka of 2,4 diaminopyrimidine.

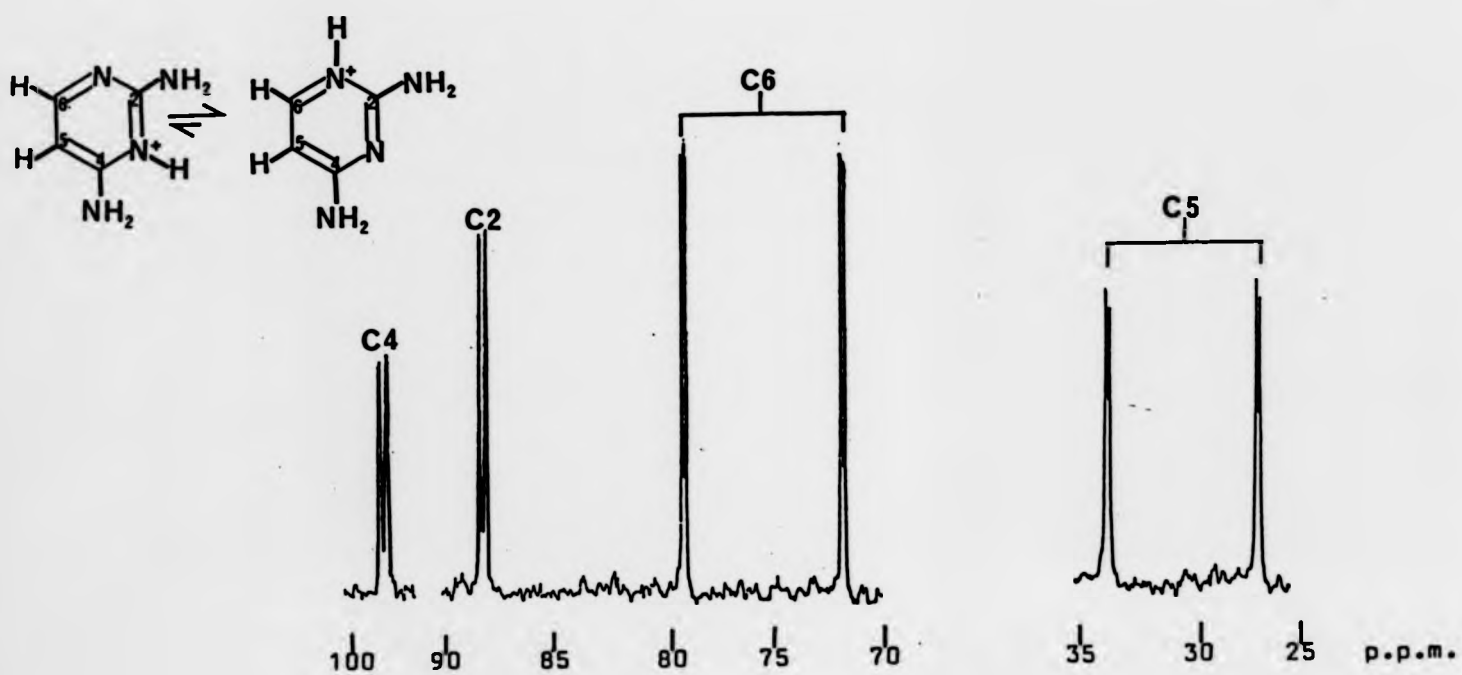
(b) pH of solution adjusted to 3 pH units below pka of 2,4 diaminopyrimidine.

(c) Couplings not resolved.

PLATE 11

^{13}C N.M.R. SPECTRUM OF 2.4-DIAMINOPYRIMIDINE

IN WATER OF PH 4·0 (PROTON COUPLED)



the coupling was 7.6 Hz. This reflects a more extreme protonation ratio in favour of N-1 in both media than is observed in the 2-amino-4-methyl system where couplings of 9.5 and 8.9 Hz respectively were observed. To determine the N-1:N-3 protonation ratio in 2,4-diaminopyrimidine accurately it was necessary to obtain values of $^{3}JC2H6$ for both the N-1 methiodide and the previously unreported N-3 methiodide.

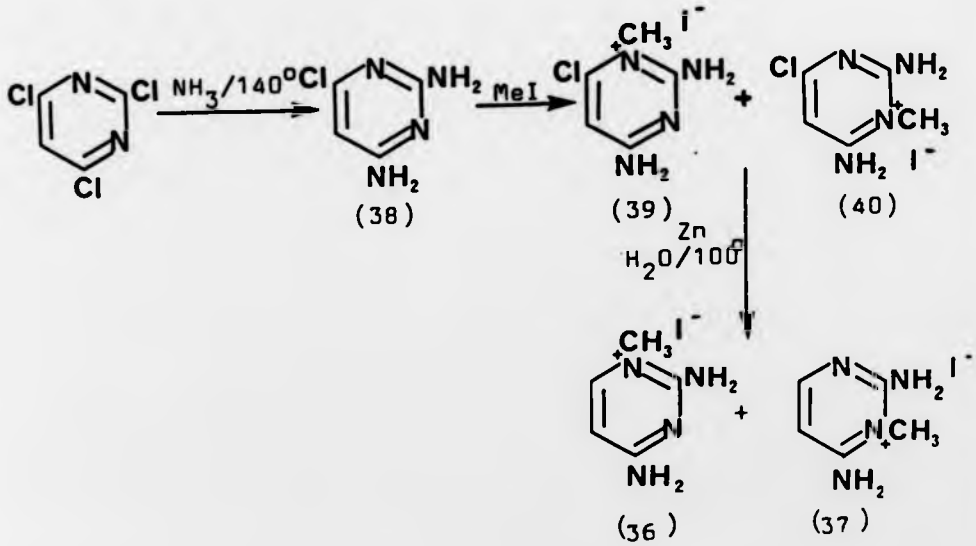
The Synthesis of 2.4-Diamino-1-methylpyrimidinium (36) and 2.4-diamino-3-methylpyrimidinium iodide (37)

Since 2,4-diamino-1-methylpyrimidinium iodide (36) was the only product formed when 2,4-diaminopyrimidine was treated with iodomethane it was necessary to investigate an alternative route for the production of the corresponding N-3 methiodide, 2,4-diamino-3-methyl pyrimidinium iodide.

Although we felt that a possible route to the N-3 isomer was via the 2,4-diamino-6-chloropyrimidine (38) in an analogous manner to that used for the preparation of 2-amino-3,4-dimethylpyrimidinium iodide previous workers had reported that methylation of 2,4-diamino-4-chloropyrimidine and its homologues leads to the formation of the N-1 methiodides.[39] Their conclusions were based on pKa value U.V. spectral data and the observation that dechlorination of the N-methylated 2,4-diamino-6-chloropyrimidine (38) by hydrogenation over a paladium-charcoal catalyst gave 2,4-diamino-1-methylpyrimidinium iodide (36). Nevertheless we felt

it wise to reinvestigate the reaction of 2,4-diamino-6-chloropyrimidine (38) with methyl iodide under the same conditions as those previously described. (Fig. 27) Interestingly the reaction led to the formation of both methiodides but it was found necessary to heat the mixture under reflux for 24 hours to achieve complete methylation. Fortunately the mixture of 2,4-diamino-6-chloro-1-methylpyrimidinium iodide (39) (70%) and 2,4-diamino-6-chloro-3-methylpyrimidinium iodide (40) (30%) could be successfully reduced with zinc powder in boiling water to give the desired methiodides (36) and (37). It is interesting to note that in this case the reaction took longer than with 2-amino-6-chloro-3,4-dimethylpyrimidinium iodide and no nucleophilic substitution of the chlorine to give the corresponding oxo- compound was observed.

Fig. 27.



The two isomers (36) and (37) could be separated either by fractional crystallisation or by chromatography on cellulose.

The use of 2,4-diamino-1-methylpyrimidinium iodide (36) and 2,4-diamino-3-methylpyrimidinium iodide (37) as models for protonation

The ^{13}C n.m.r. spectral data for 2,4-diamino-1-methylpyrimidinium iodide and 2,4-diamino-3-methylpyrimidinium is given in Tables 7 & 8 respectively. The proton-coupled ^{13}C n.m.r. spectrum of 2,4-diamino-1-methylpyrimidinium iodide (Plate 12) shows C-2 and C-6 as complex resonances due to long-range coupling to the N-methyl protons. However, the selectively decoupled spectrum resulting from low power irradiation of these methyl protons (Plate 13) clearly shows $^{3}JC2H6$ and simplification of the C6 resonance confirming the site of methylation as N-1. The proton-coupled ^{13}C n.m.r. spectrum of 2,4-diamino-3-methylpyrimidinium iodide (Plate 14) on the other hand shows extra couplings on the C-2 and C-4 resonances. Selective irradiation of the N-methyl protons (Plate 15), however, exposes $^{3}JC2H6$ and simplifies C-4 confirming the structure of the N-3 methiodide. The $^{3}JC2H6$ couplings observed for the N-1 and N-3 methiodides were 5.8 and 13.4 Hz respectively which again are in very close agreement with those derived from 2-aminopyrimidine (5.8 and 13.9 Hz). Using these parameters it is possible to calculate a protonation ratio of 77:23 in T.F.A. and one of 82:18 in aqueous acid, values which are much less extreme than

TABLE 7

25MHz ^{13}C n.m.r. spectral data
2,4-Diamino-1-Methylpyrimidinium iodide

Chemical shifts/p.p.m.

Solvent:	D_2O
C2	88.80
C4	97.51
C5	32.27
C6	79.97
N-CH_3	-27.11

Coupling constants/Hz

Solvent:	D_2O	
^3JC2H6	a	(5.8)
^2JC4H5	} a	} a
^3JC4H6		
^1JC5H5	176.7	(172·1)
^2JC5H6	3.4	(3.4)
^1JC6H6	187.1	(185·3)
^2JC6H5	a	(3.9)
^1JCH(N-CH_3)	142.8	(-)
^3JCH(N-CH_3H6)	3.8	(-)

Values in parenthesise are those obtained when selectively decoupling N-methyl protons with low power irradiation.

(a) Complex multiplet, couplings not resolved.

TABLE 8

25MHz ^{13}C n.m.r. spectral data

2,4 Diamino-3-methylpyrimidinium iodide

Chemical shifts/p.p.m.

Solvent:	D_2O
C2	88.33
C4	90.50
C5	29.29
C6	90.50
N-CH_3	-32.41

Coupling constants/Hz

Solvent:	D_2O	
^3JC2H6	a	(13.4)
^2JC4H5	} a	} a
^3JC4H6		
^1JC5H5	175.4	(172.3)
^2JC5H6	a	a
^1JC6H6	182.1	(180.3)
^2JC6H5	a	a
^1JCH(N-CH_3)	143.7	(—)

Values in parenthesise are those obtained when selectively decoupling N-methyl protons with low power irradiation.

(a) Complex multiplet, couplings not resolved.

PLATE 12

^{13}C N.M.R. SPECTRUM OF 2.4-DIAMINO-1-METHYLPYRIMIDINIUM IODIDE

IN DEUTERIUM OXIDE (PROTON COUPLED)

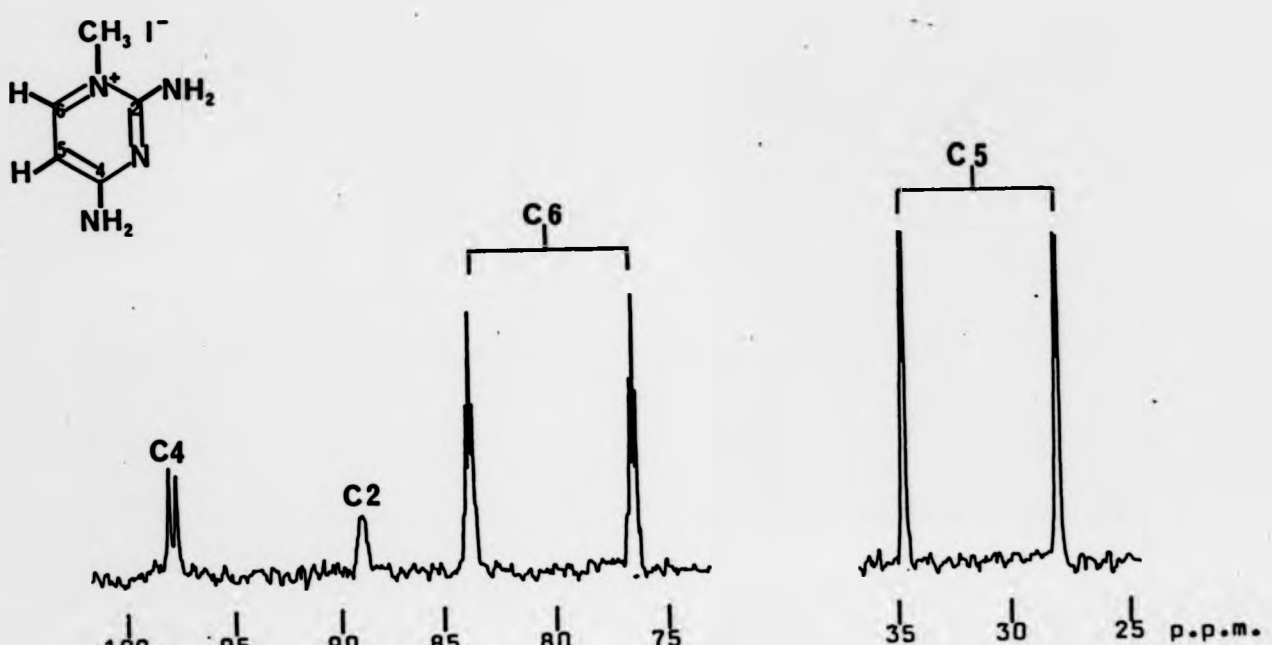


PLATE 13

^{13}C N.M.R. SPECTRUM OF 2,4-DIAMINO-1-METHYLPYRIMIDINIUM IODIDE IN DEUTERIUM OXIDE WITH N-METHYL PROTONS SELECTIVELY IRRADIATED

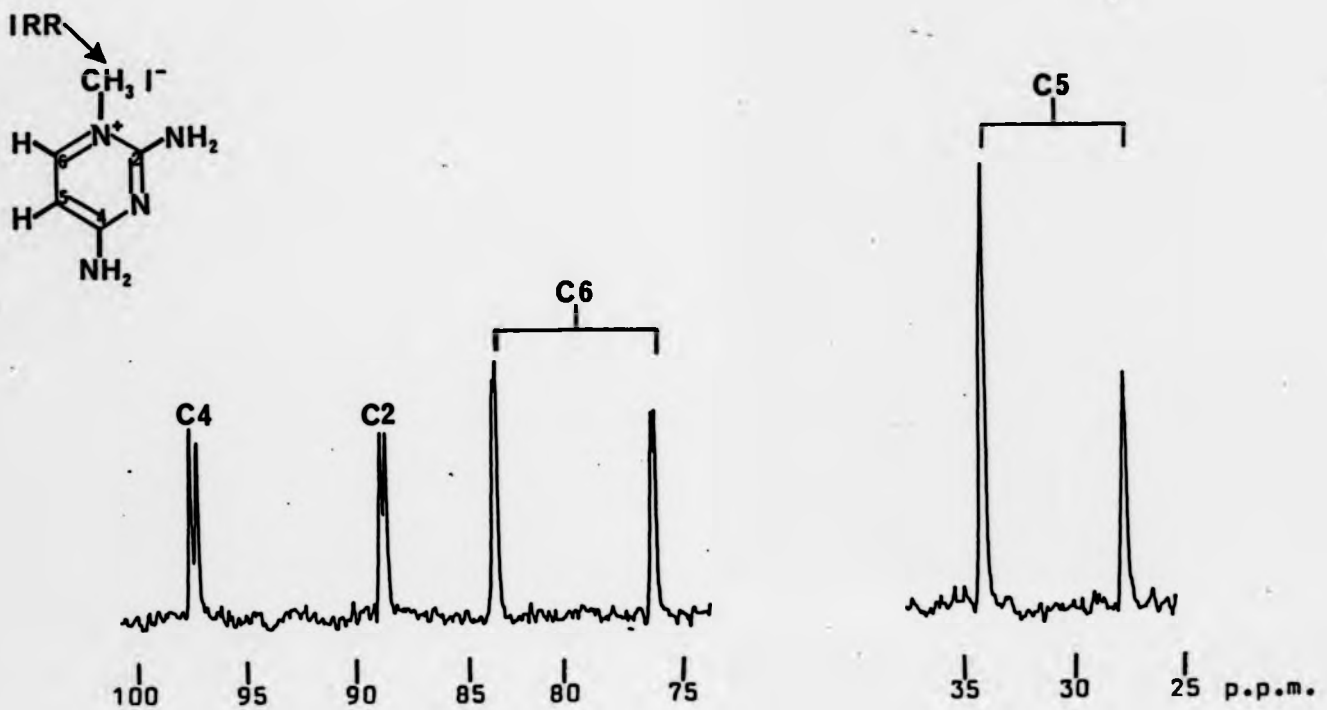
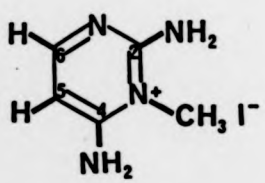


PLATE 14

^{13}C N.M.R. SPECTRUM OF 2,4-DIAMINO-3-METHYLPYRIMIDINIUM IODIDE IN D6 D.M.S.O. (PROTON COUPLED)



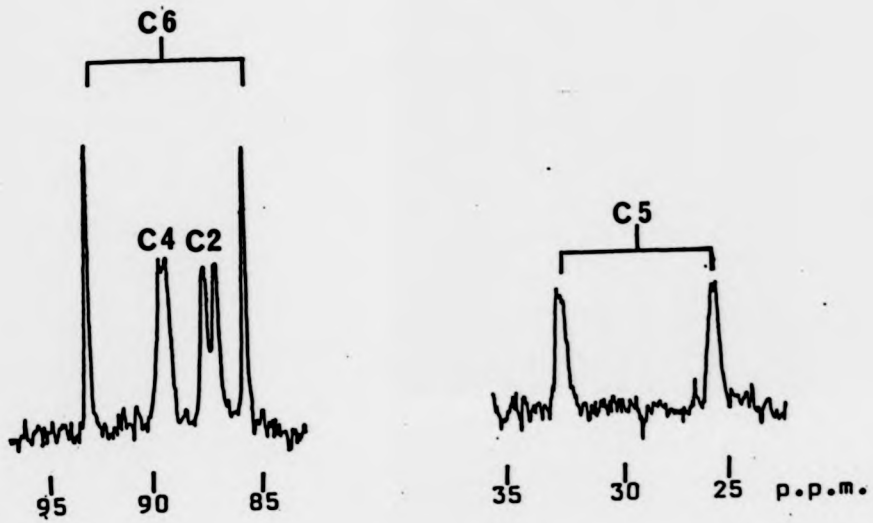
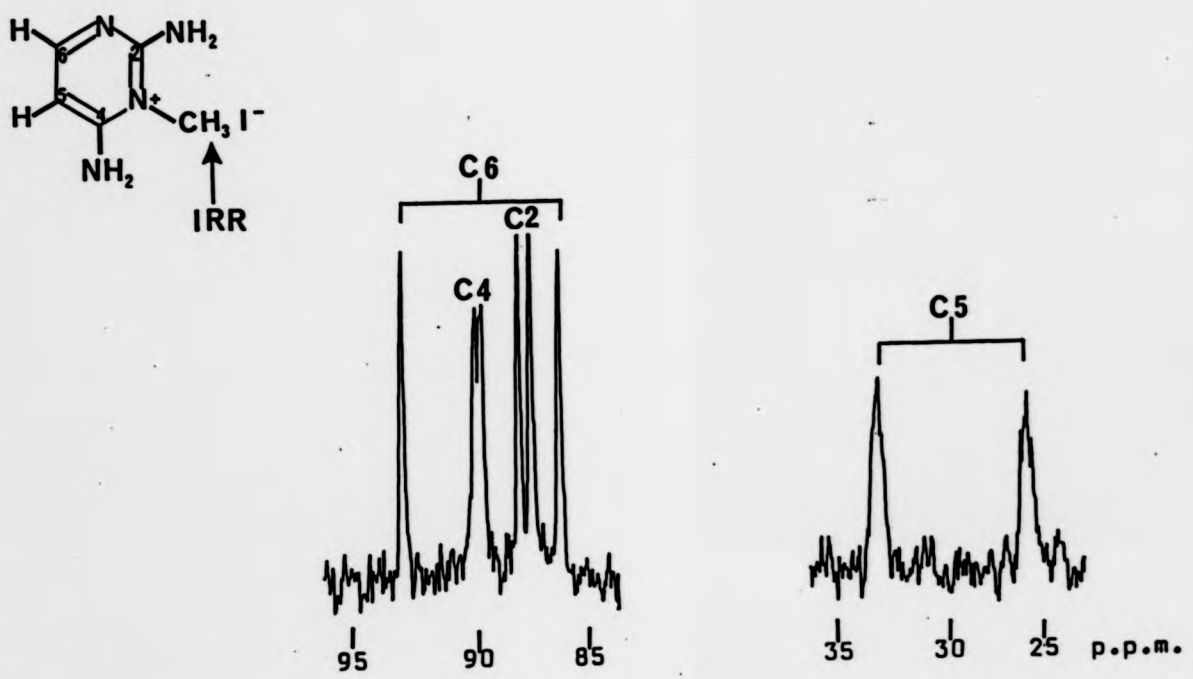


PLATE 15

^{13}C N.M.R. SPECTRUM OF 2,4-DIAMINO-3-METHYLPYRIMIDINIUM IODIDE IN D6 D.M.S.O. WITH N-METHYL PROTONS SELECTIVELY IRRADIATED



previously postulated. On the basis of these protonation ratios and the previously reported pKa value for the molecule (pK 7.40 ± 0.03).the"individual" basicities of the nitrogen atoms in 2,4-diaminopyrimidine were calculated. In aqueous acid these were found to be 7.31 and 6.66, for N-1 and N-3 respectively.

Subsequent criticism of the use of methiodides as models for protonation

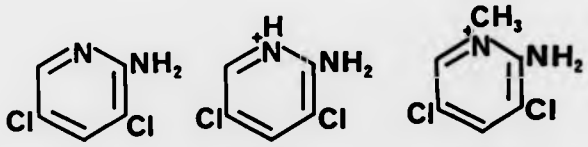
The publication of our preliminary results[40] on the use of the $^{3}JC2H6$ vicinal coupling constant for determining protonation ratios prompted a response by Riand[41]. He and his coworkers had previously reported protonation ratios for a number of methyl and amino substituted pyrimidines based on a consideration of the ^{13}C n.m.r. chemical shifts resulting from protonation.

For the 2-amino-4-methylpyrimidine system in T.F.A. they had calculated an N-1:N-3 protonation ratio of 67:33 slightly larger than the 62:38 ratio determined by us but significantly larger than the 54:46 ratio reported by us for 2-amino-4-methylpyridine in water. However, a more marked difference between the two approaches was observed with the 4-amino substituted pyrimidines. Although the 2,4-diaminopyrimidine system had not been studied by Riandet al, they had studied the 4 amino-6-methylpyrimidine system. In this system in T.F.A. they had found that the N-1:N-3 protonation ratio was quite extreme 95:5.[30] This is significantly higher than the N-1:N-3 protonation ratios we determined for 2,4 diamino-

pyrimidine in T.F.A., and aqueous hydrochloric acid, namely, 77:23 and 82:18. respectively.

Riand suggested that this difference could be explained if the decrease in the vicinal through-nitrogen coupling constants were greater as a result of methylation than for protonation.

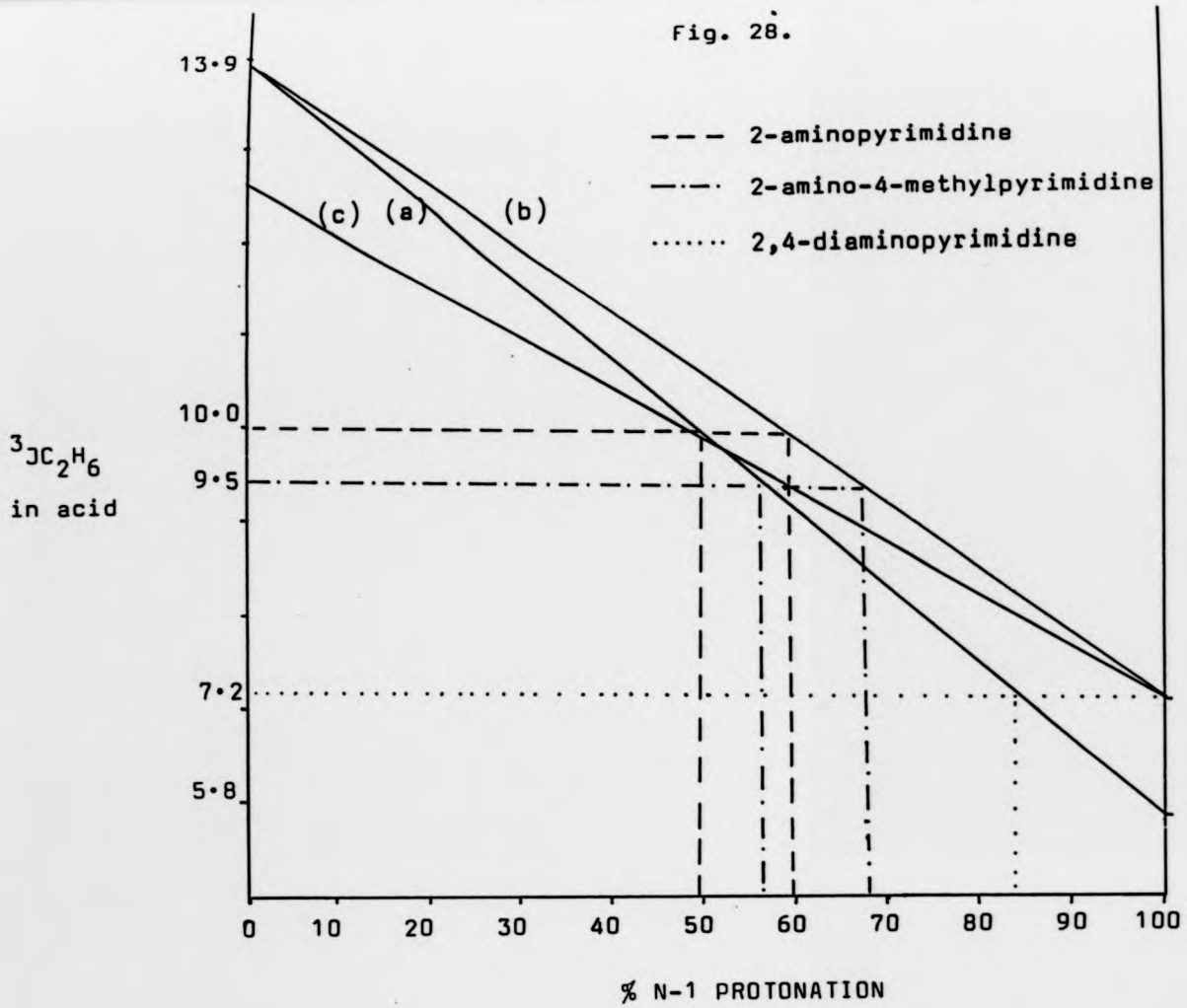
He suggested that a correction factor of +1.4 Hz to the 5.8 Hz parameter would take this difference into account. On this basis the corrected protonation ratios for 2,4-diaminopyrimidine and 2-amino-4-methylpyrimidine in aqueous acid were recalculated to be 100% and 66% respectively (Fig.28) bringing this ratio in line with those calculated by his chemical shift method despite the difference in solvent. The justification for the 1.4 Hz correction was based on a study of 2-amino-3,5-dichloropyridine (41), its protonated form (42) and the N-methylated salt (43). In this system the decrease in $^{3}J_{C2H6}$ observed on methylation of N-1 was found to be 1.4 Hz greater than that observed on protonation.



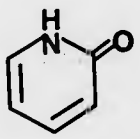
(41) (42) (43)

Further examples cited were the N-methyl derivatives of 2-hydroxypyridine (44), 2-mercaptopyridine (45) and 4-oxopyrimidine (46). In these latter cases the decrease

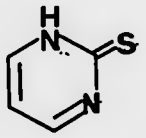
Fig. 28.



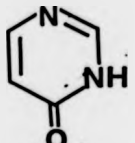
in the through nitrogen vicinal coupling ($^3JC2H6$) was greater for methylation than protonation by 1.8, 2.3 and 1.3 Hz respectively. A recent study of imidazole (7) by Guenther[34] was also cited where N-methylation was found to reduce the through-nitrogen vicinal coupling by 0.6 Hz more than protonation.



(44)



(45)



(46)

(7)

It was therefore argued that the substituent effect from an N-H to an N-Me group is always negative (-0.6 to -2.3 Hz) and that consequently a correction factor should be applied to our model systems before they can be used to evaluate protonation ratios.

There is, however, one serious defect in this line of arguement. If the $^3JC2H6$ coupling for 100% protonation at N-1 is taken as 7.2 Hz (5.8 + 1.4) and that for 100% protonation at N-3 is taken as 13.9 Hz (Fig. 28) then the protonation ratio for the symmetrical 2-aminopyrimidine would be 60:40 which is clearly unacceptable. Hence if a correction of 1.4 Hz in the value of $^3JC2H6$ for 100%

protonation at N-1 (5.8 Hz) is necessary then clearly this would also require that an equal correction, but of opposite sign, would have to be applied to the $^{3}JC2H6$ coupling for 100% protonation at N-3 (13.9 Hz) (Fig. 28) in order that the $^{3}JC2H6$ coupling in 2-aminopyrimidine (9.9 Hz) would agree a 50:50 protonation ratio. Since the correction of 1.4 Hz to the change in $^{3}JC2H6$ value (+2.2 Hz) when N-3 is 100% protonated is a much larger correction than a 1.4 Hz correction to the change in $^{3}JC2H6$ (-5.9 Hz) when N-1 is 100% protonated we can see no justification for such a change.

Furthermore, one justification for the original 1.4 Hz correction had been that the recalculated protonation ratios (in aqueous acid) were now more in line with results from the chemical shift approach in (in T.F.A.). This is invalid since our through nitrogen coupling constant method for 2-amino-4-methylpyrimidine in aqueous hydrochloric acid and T.F.A. shows significant differences in the protonation ratio in the two solvents. It is therefore inappropriate to compare the results from one method in a given solvent with the results from a different approach in a different solvent. A comparison of the two methods for 2-amino-4-methylpyrimidine in T.F.A. shows that both approaches give similar results without the need for a correction. Thus the chemical shift approach gives 67:33 while our vicinal coupling approach gives 62:38.

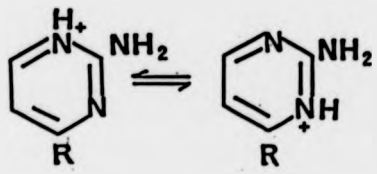
Other examples are given later in this dissertation where the application of a correction factor of 1.4 Hz to $^{3}JC2H6$ for the methiodide would result in the calculated percentage protonation at N-1 being in excess of 100%, which is clearly incorrect. We therefore conclude that while in some other systems there may be a need to correct for the relative effects of methylation and protonation we have no evidence to suggest that this applies to the 2-aminopyrimidines we have so far studied.

In conclusion, therefore, we feel that the vicinal coupling method we have developed which uses N-methyl-pyrimidinium iodides as models for protonation should provide a convenient means of studying the N-1:N-3 protonation ratios in a number of substituted pyrimidines.

CHAPTER 2

Further protonation studies of 2-amino-4-substituted pyrimidines.

In the previous chapter we described the successful development of a method for determining the N-1:N-3 protonation ratio in 2,4-diaminopyrimidine and 2-amino-4-methylpyrimidine based on a consideration of the values of $^{3}JC2H6$ for the monoprotonated systems. Having done this it was of interest to extend our study further to investigate the effects of a range of other substituents at C-4 on the N-1:N-3 protonation ratio. In theory this would have required the synthesis of both the N-1 and N-3 methiodides for each of the substituted systems studied. Fortunately, however, in our earlier studies we had observed that the $^{3}JC2H6$ coupling parameters for protonation at N-1 and N-3 were relatively insensitive to changes in the substituent at C-4. Thus, for example, similar values were observed for $^{3}JC2H6$ in the N-1 methiodides of 2-aminopyrimidine (5.8 Hz), 2-amino-4-methylpyrimidine (6.0 Hz) and 2,4-diaminopyrimidine (5.8 Hz). Likewise, similar values were observed for $^{3}JC2H6$ in the N-3 methiodides of 2-amino-4-methylpyrimidine (13.6 Hz) and 2,4-diaminopyrimidine (13.4 Hz). These latter values are also in good agreement with the corresponding coupling in 2-amino-1-methylpyridinium iodide ($^{3}JC2H4$ 13.8 Hz). We therefore concluded that providing caution was exercised it would be appropriate to use the $^{3}JC2H6$ and $^{3}JC2H4$ values obtained from 1-methylpyrimidinium iodide to represent the two possible protonation extremes (47) and (48) for a range of 4-substituted 2-aminopyrimidnes.



(47) (48)

This avoided the need to develop synthetic routes to the previously unreported N-3 methiodides of the systems studied. Where possible, however, the N-1 methiodide of the system being studied was prepared and the value of $^{3}JC2H6$ determined to alert us of any situations in which the substituent might have an unexpected impact on the value of $^{3}JC2H6$. In addition to this work on the value of $^{3}JC2H6$ we also wished to investigate the possible use of other $^{13}C - ^{1}H$ couplings for assessing N-1:N-3 protonation ratios.

The proton-coupled ^{13}C n.m.r. spectra of the protonated, non-protonated, and N-methylated pyrimidines could usually be analysed on a first order basis since even the introduction of a large ^{1}JCH coupling on either of the proton resonances of H-5 and H-6 did not lead to overlap of these proton signals. Where overlap did occur and a second order spectrum was expected the true couplings were obtained by spectrum simulation. In these latter cases it is worth noting that the presence of second order effects was clearly apparent in the ^{13}C n.m.r. spectrum.

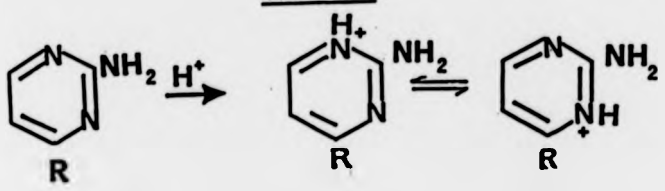
PROTONATION STUDIES BASED ON A CONSIDERATION OF $^3JC2H6$

A list of the 4-substituted 2-aminopyrimidines studied together with the values of $^3JC2H6$ for these pyrimidines in $CDCl_3$, aqueous HCl, and T.F.A. are given in Table 9. Also shown in the Table are the N-1:N-3 protonation ratios calculated for these systems from the $^3JC2H6$ values for the monoprotonated systems and a consideration of the $^3JC2H6$ parameters previously established for protonation at N-1 and N-3 in 2-amino-pyrimidine (5.8 and 13.9 Hz respectively). Evidence is presented later to support the view that these parameters are appropriate for the systems in Table 9. Interestingly, the value of $^3JC2H6$ for the free bases shows little variation (11.6 - 11.8 Hz) despite the wide range of substituents on C-4. It is not unreasonable therefore to propose that the $^3JC2H6$ parameters used to assess the N-1:N-3 protonation ratios in these systems will also be largely unaffected by the substituent at C-4. In the case of 2-amino-4-methoxypyrimidine, however, an unusually high value of $^3JC2H6$ for the free base (12.8 Hz) was observed. This case is considered separately later and in these circumstances alternative protonation parameters were used to assess the N-1:N-3 protonation ratio.

Factors influencing N-1:N-3 protonation ratios

From the initial studies of 2-amino-4-methylpyrimidine and 2,4-diaminopyrimidine it was established that steric,

TABLE 9



(X) (Z)

R	$CDCl_3$	H_2O/HCl	X:Z	T.F.A.	X:Z
	$^3JC2H6/Hz$[a]				
H	11.7	10.0	47:53	9.9	49:51
CH_3	11.7	9.5	54:46	8.9	61:39
Ph	11.6	7.3	81:19	7.6	77:23
CH:CHPh[b]	11.8	7.8	75:25	7.9	74:26
Cl[b]	11.6	7.8	75:25	7.7	76:24
NH_2[c]	11.6	7.2	82:18	7.6	77:23
$N(CH_3)_2$	11.8	7.2	82:18	7.5	79:21
$NHC_6H_4OCH_3$	11.7	7.0	85:15	-	-
OCH_3	12.8	7.5	90:10	7.5	90:10

(a) Accurate to ± 0.2 Hz.

(b) D.M.S.O. used as solvent for non-protonated species.

(c) H_2O, pH 11 used as solvent for non-protonated species.

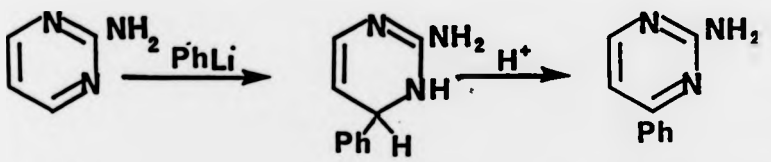
mesomeric and inductive effects were important factors which can influence the N-1:N-3 protonation ratio.

This study was extended to assess the effect of a variety of substituents at C-4 which are capable of exerting one or more of the above factors.

(i) 2-Amino-4-phenylpyrimidine

(a) Synthetic Aspects

2-amino-4-phenylpyrimidine (49) was prepared by the reaction of phenyl lithium with 2-aminopyrimidine (50), an example of direct nucleophilic attack on the pyrimidine ring.



(50) (51) (52)

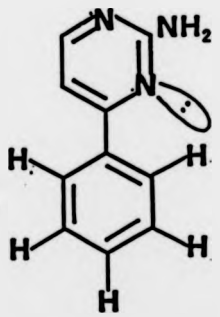
While the dihydropyrimidine intermediates in such reactions are usually quite stable, requiring oxidation with permanganate to rearomatise the ring system, in this case this was not found to be necessary. Treatment of the dihydro-derivative (51) with acid was all that was necessary to convert it into the desired pyrimidine (49). Reaction of the free base with iodomethane yielded only 2-amino-1-methyl-4-phenylpyrimidinium iodide.

(b) N.m.r. aspects

The ^{13}C n.m.r. spectral data for 2-amino-4-phenylpyrimidine is given in Table 10 and that for the

corresponding N-1 methiodide in Table 11. The value of $^{3}J_{C2H6}$ in the methiodide (5.8 Hz) was in good agreement with the value determined from 2-amino-1-methylpyrimidinium iodide (5.8 Hz). We therefore felt confident to use the $^{3}J_{C2H6}$ and $^{3}J_{C2H4}$ through-nitrogen vicinal coupling constants obtained from the methiodide of 2-aminopyrimidine to assess the N-1:N-3 protonation ratio of the mono-protonated form of 2-amino-4-phenylpyrimidine. The calculated N-1:N-3 protonation ratio for 2-amino-4-phenylpyrimidine in aqueous acid (81:19) was more extreme than in 2-amino-4-methylpyrimidine. This is readily understood if one considers the phenyl and pyrimidine rings to be coplanar. In this conformation the ortho-protons on the phenyl ring would be very close to the lone pair of electrons on N-3 (see Fig. 29).

Fig. 29.



While the ortho-proton would be unlikely to block the attack by a species as small as a proton it might prevent effective solvation of the resulting charged species, thus reducing the stability of this protonated form of the molecule.

A comparison of the U.V. spectra of the cations of 2-amino-4-phenylpyrimidine (pH 1: λ 282 nm, ε = 14,260 mol^{-1} dm^3 cm^{-1}; λ 318 nm, ε = 8,000 mol^{-1} dm^3 cm^{-1}; and 2-amino-pyrimidine [42] (pH 1: λ 224nm, ε =14,791 mol^{-1} dm^3 cm^{-1};

TABLE 10

25MHz ^{13}C n.m.r. spectral data
2-Amino-4-phenylpyrimidine

Chemical shifts/p.p.m.

Solvent:	$CDCl_3$	T.F.A.	H_2O/HCl[a]
C2	95.66	89.93	87.98
C4	98.89	105.91	104.20
C5	40.45	41.01	39.71
C6	90.51	81.40	79.76
C1'	69.83	66.05	67.26
C2'	60.04	61.95	61.47
C3'	61.69	62.92	62.49
C4'	63.74	65.42	66.16

Coupling constants/Hz

Solvent:	$CDCl_3$	T.F.A.	H_2O/HCl[a]
$^3J_{C2H6}$	11.6	7.6	7.3
$^2J_{C4H5}$	b	b	b
$^3J_{C4H6}$			
$^1J_{C5H5}$	167.8		
$^2J_{C5H6}$	7.3		second order effects
$^1J_{C6H6}$	178.2		
$^2J_{C6H5}$	2.7		

(a) pH of solution adjusted to 3 pH units below pka of 2-amino-4-phenylpyrimidine.

(b) Complex multiplet, couplings not resolved.

TABLE 11

25MHz ^{13}C n.m.r. spectral data

2-Amino-1-methyl-4-phenylpyrimidinium iodide

Chemical shifts/p.p.m.

Solvent:	D.M.S.O.
C2	89.03
C4	102.57
C5	40.02
C6	84.61
C1′	67.23
C2′	61.69
C3′	62.78
C4′	66.89
NCH_3	-25.07

Coupling constants/Hz

Solvent:	D.M.S.O.	
^3JC2H6	a	(5.8)
^2JC4H5	a	a
^3JC4H6		
^1JC5H5	}	second order effects
^2JC5H6		
^1JC6H6		
^2JC6H5		
^1JCH(NCH_3)	144.0	(–)

Values in parenthesises are those obtained when selectively decoupling N-methyl protons with low power irradiation.

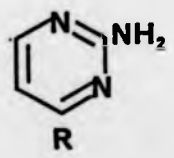
(a) Complex multiplet, couplings not resolved.

λ 302 nm, ε = 3,981 mol^{-1} dm^3 cm^{-1}) also supports the view that the two rings are conjugated. Characteristic red shifts of the absorption maxima are observed (i.e. to a longer wavelength) for 2-amino-4-phenylpyrimidine and this is also accompanied by an increase in the extinction coefficients.

By determining the pKa value of 2-amino-4-phenyl-pyrimidine we were also able to calculate the N-1 and N-3 values for the two ring nitrogens so that the effect of the 4-phenyl substituent could be directly compared with that of a 4-methyl substituent (see Table 12).

In the 2-amino-4-methylpyrimidine system the observed N-1:N-3 protonation ratio was 54:46 and the pKa value 4.15, yielding values for pKa N-1 and pKa N-3 of 3.88 and 3.81 respectively. However, in the case of the 2-amino-4-phenyl pyrimidine system the more extreme N-1:N-3 protonation ratio (81:19) appears to be offset by a reduced pKa value, which we were able to determine as 3.88. Combining this data enabled pKa N-1 and pKa N-3 values of 3.79 and 3.16 to be determined for the 2-amino-4-phenylpyrimidine. The methyl and phenyl groups thus appear to have remarkably similar effects at N-1. On the other hand the +M effect of the phenyl group is clearly offset by steric hindrance at N-3 since the pKa N-3 value in 2-amino-4-phenylpyrimidine is essentially unchanged from that in the system with no substituent at C-4, namely 2-aminopyrimidine, where, pKa N-1 = pKa N-3 = 3.24.

TABLE 12

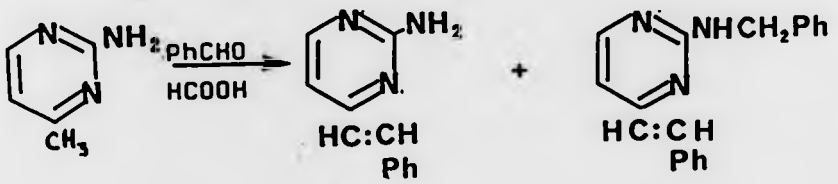


R	pka obs	pka N-1	pka N-3
H	3.54	3.24	3.24
CH_3	4.15	3.88	3.81
Ph	3.88	3.79	3.16
CH:CHPh	4.00	3.88	3.40
Cl	2.5	2.4	1.9
NH_2	7.40	7.31	6.52
	7.26	7.17	6.52
$N(CH_3)_2$	7.96	7.87	7.22
$NHC_6H_4OCH_3$	7.32	7.25	6.50
OMe	5.53	5.48	4.53

(ii) 2-Amino-4-styrylpyrimidine

(a) Synthetic aspects

2-Amino-4-styrylpyrimidine (52) was prepared by the reaction of benzaldehyde with 2-amino-4-methyl-pyrimidine in formic acid.



(52) (53)

This reaction also led to the formation of a quantity of the benzyl substituted system (53). It was clearly established by ^{1}H n.m.r. spectroscopy that only the trans isomer or (E) was produced since the observed coupling between the two protons on the carbon-carbon double bond was 16 Hz which is typical of a trans configuration.

(b) N.m.r. aspects

The ^{13}C n.m.r. spectral data for E-2-amino-4-styrylpyrimidine is given in Table 13 and that for its N-1 methiodide in Table 14. The value of $^{3}JC2H6$ in 2-amino-1-methyl-4-styrylpyrimidinium iodide (5.8 Hz) is once again identical to that observed in 2-amino-1-methyl-pyrimidinium iodide supporting the view that once again parameters from this latter system are suitable for

TABLE 13

25MHz ^{13}C n.m.r. spectral data
of 2-Amino-4-styrylpyrimidine

Chemical shifts/p.p.m.

Solvent:	$CDCl_3$	T.F.A.	H_2O/HCl[a]
C2	96.36	87.49	87.80
C4	96.39	102.18	102.44
C5	42.35	40.49	40.90
C6	91.49	82.14	80.19
C7	62.03	84.30	77.17
C8	59.11	52.30	56.19
C1′	68.71	66.53	67.16
C2′	61.69	62.83	62.34
C3′	60.42	62.63	61.71
C4′	69.24	66.94	64.61

Coupling constants/Hz

Solvent:	$CDCl_3$	T.F.A.	H_2O/HCl[a]
^3JC2H6	11.8	7.9	7.8
^2JC4H5	} b	} b	} b
^3JC4H6			
^1JC5H5	167.2	178.8	176.7
^2JC5H6	7.0	b	4.3
^2JC5H7	3.3	b	4.3
^1JC6H6	177.3	190.4	187.1
^2JC6H5	3.4	4.6	c

(a) pH of solution adjusted to 3 pH units below pka of 2-amino-4-styrylpyrimidine.
(b) Complex multiplet.
(c) Coupling not resolved.

TABLE 14

25MHz ^{13}C n.m.r. spectral data

E-2-Amino-4-styrylpyrimidinium iodide

Chemical shifts/p.p.m.

Solvent:	D.M.S.O.
C2	88.87
C4	102.18
C5	41.53
C6	83.68
N-CH_3	-25.12
C7	76.13
C8	57.88
C1'	67.99
C2'	62.65
C3'	62.02
C4'	64.32

Coupling constants/Hz

Solvent:	D.M.S.O.	
^3JC2H6	a	(5.8)
^2JC4H5	} a	} a
^3JC4H6		
^1JC5H5	176.4	(172.1)
^2JC5H6	3	(3)
^3JC5H7	3	(3)
^1JC6H6	189.2	(187.3)
^2JC6H5	a	(4.7)
^1JCH(N-CH_3)	142.2	(-)

Values in parenthesise are those obtained when selectively decoupling N-methyl protons with low power irradiation.

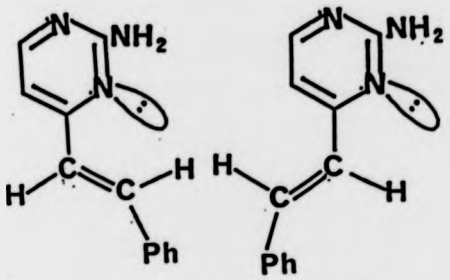
(a) Complex multiplet, couplings not resolved.

determining the N-1:N-3 protonation ratio in the styryl-substituted system.

The value of $^{3}JC2H6$ observed for 2-amino-4-styryl-pyrimidine in aqueous hydrochloric acid (7.8 Hz) lies between the values observed in 2-amino-4-methylpyrimidine (9.5 Hz) and 2-amino-4-phenylpyrimidine under similar conditions. This indicates a less extreme N-1:N-3 protonation ratio in the styryl-substituted system than in the phenyl substituted system.

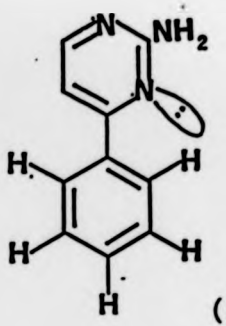
Using the pKa value of 4.00 which we determined for E-2-amino-4-styrylpyrimidine together with the calculated N-1:N-3 protonation ratio (75:25) enabled the individual pKa values for N-1 and N-3 to be determined as 3.88 and 3.40 respectively. It is again interesting to note that the value of pKa N-1 is essentially the same as that in both 2-amino-4-methylpyrimidine and 2-amino-4-phenylpyrimidine. The value of pKa N-3, however, is larger in the styryl substituted system than in 2-amino-4-phenylpyrimidine presumably reflecting a reduced degree of steric hindrance at N-3 in the former compound relative to the latter. Since the UV spectrum recorded for 2-amino-4-styrylpyrimidine indicates that the styrene system is in conjugation with the pyrimidine ring (pH 1, λ 354 nm, ε = 26,906 mol^{-1} dm^{3} cm^{-1}) we must presume that the protonated forms of two planar structures (54) and (55) both contribute to the overall structure. The reduced steric hindrance at N-3 in the styrene substituted system relative to the phenyl substituted system (56) suggests a significant

contribution of the less hindered planar structure (55).



(54)

(55)



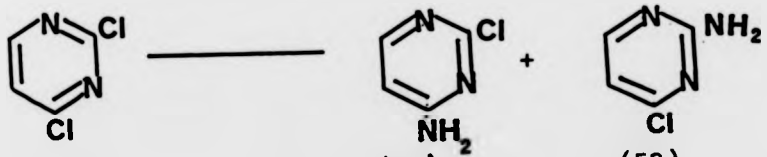
(56)

(iii) 2-Amino-4-chloropyrimidine

(a) Synthetic Aspects

Since 2-amino-4-chloropyrimidine is a useful precursor for a range of 4-substituted 2-aminopyrimidines two synthetic routes for its production were investigated.

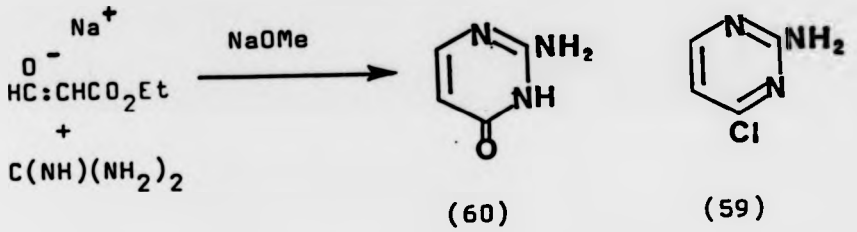
The reaction of 2,4-dichloropyrimidine (57) with ammonia was found to give both 4-amino-2-chloropyrimidine (58) and 2-amino-4-chloropyrimidine (59) with the 4-amino system as the major product (60%).



(57) (58) (59)

This is in agreement with the general observation that the C-4 position of pyrimidines is more susceptible to nucleophilic attack that the C-2 position. Previous reports[14] that 2-amino-4-chloropyrimidine is the major product in this reaction are therefore incorrect, and the reaction is not anomalous as previously claimed. However, since the two products (58) and (59) proved to

be difficult to separate on a large scale an alternative route based on iso-cytosine was used for the preparation of 2-amino-4-chloropyrimidine. Iso-cytosine (60) was first prepared by the reaction of guanidine carbonate with ethyl formylacetate(Na salt), the latter being conveniently prepared in an autoclave from ethyl acetate, methyl formate and sodium methoxide. The iso-cytosine was then converted to the desired chloro-compound (59) by reaction with phosphorus oxychloride.



(60) (59)

(b) N.m.r. aspects

The ^{13}C n.m.r. data for 2-amino-4-chloropyrimidine is given in Table 15. Using the parameters derived from 2-aminopyrimidine enabled the $^{3}JC2H6$ value of 7.8 Hz to be interpreted as an N-1:N-3 protonation ration of 75:25. However, in order to assess the effects of the chlorine atom on the ring nitrogens it was necessary to determine the pKa value for this system. Unfortunately, the UV spectra of the protonated and non-protonated molecules were so similar that the UV method we had used to determine the pKa values of the other 2-aminopyrimidines in our study proved to be unsatisfactory. It was therefore

TABLE 15

25MHz ^{13}C n.m.r. spectral data
2-Amino-4-chloropyrimidine

Chemical shifts/p.p.m.

Solvent:	D.M.S.O.	T.F.A.	H_2O/HCl^a
C2	97.08	88.58	88.22
C4	93.60	104.80	102.66
C5	42.51	45.37	44.80
C6	93.53	80.02	80.70

Coupling constants/Hz

Solvent:	D.M.S.O.	T.F.A.	H_2O/HCl^a
$^3J_{C2H6}$	11.6	7.7	7.8
$^2J_{C4H5}$	1	1	1
$^3J_{C4H6}$	9.8	9.7	9.7
$^1J_{C5H5}$	177.6	186.8	186.4
$^2J_{C5H6}$	7.6	4.0	4.3
$^1J_{C6H6}$	180.7	193.5	192.3
$^2J_{C6H5}$	2.7	4.5	4.6

(a) HCl (aq.) 6 mol dm^{-3}.

necessary to estimate the pKa value of 2-amino-4-chloropyrimidine as being approximately 2.5 from a consideration of the value of 2.53 previously reported for 2-methylamino-4-chloropyrimidine [24].

Using this pKa value gives pKa N-1 and pKa N-3 values of approximately 2.4 and 1.9 respectively showing, as anticipated, that the -I effect of the chlorine atom greatly reduces the pKa values at both ring nitrogens with the greater effect being at N-3. It is not possible in these circumstances to assess whether steric factors are also affecting the pKa of the N-3 nitrogen.

(iv) 2-Aminopyrimidines possessing an amino-substituent at C-4

(a) Synthetic Aspects

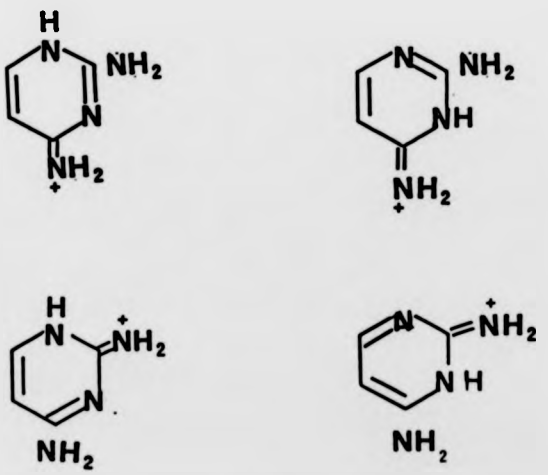
2,4-diaminopyrimidine was conveniently prepared from 2,4-dichloropyrimidine by the reaction with ethanolic ammonia at 180°C (autoclave). As previously discussed the first nucleophillic substitution occurs readily at room temperature to give a mixture of 2-amino-4-chloro and 4-amino-2-chloropyrimidines but much more vigorous conditions were required to effect the second nucleophillic substitution due to the deactivating effect of the first amino group.

Similarly, other amines such as dimethylamine and p-methoxyaniline reacted with 2-amino-4-chloropyrimidine to give substituted amino groups in the 4 position of the pyrimidine ring.

(b) N.m.r. aspects

We have previously discussed the determination of the N-1:N-3 protonation ratio in 2,4-diaminopyrimidine. From a consideration of the value of $^{3}JC2H6$ for the protonated system a N-1:N-3 protonation ratio of 82:18 was determined which was less extreme than previously proposed. As previously mentioned the belief that 2,4-diaminopyrimidine would be essentially exclusively protonated at N-1 stemmed from the belief that the para-quinoid resonance structure of the monoprotonated system was very much more favoured than those resonance structures involving the 2-amino group. (fig. 30). However, the observation that the pKa of 2,4-diaminopyrimidine (7.40) is considerably larger than that of 4-aminopyrimidine (5.71) clearly indicates that the 2-amino groups is providing a significant +M effect on the pyrimidine ring. Resonance structures involving the 2-amino group therefore clearly make a valuable contribution to the bonding in the monoprotonated 2,4-diaminopyrimidine molecule.

Fig. 30.



In order to try to enhance the stability of the para-quinoid resonance structures involving the amino-substituent at C-4 the amino group was replaced by a (para-methoxyphenyl)amino group. It was hoped that such a system might permit further delocalisation of the positive charge on the 4-amino group in the para-quinoid resonance structures thus increasing the stability of these resonance structures.

The ^{13}C n.m.r. spectra of 2-amino-4-(para-methoxyphenyl)aminopyrimidine and its N-1 methiodide are given in Tables 16 and 17 respectively. Although the value of $^{3}JC2H6$ is slightly smaller (7.0 Hz) for 2-amino-4-(para-methoxyphenyl)aminopyrimidine in aqueous hydrochloric acid than the 7.2 Hz observed for 2,4-diaminopyrimidine under similar conditions, it would be unwise to claim that this is conclusive evidence for an increase in the N-1:N-3 protonation ratio in favour of N-1 since $^{3}JC2H6$ values could only be determined with an accuracy of $\pm$ 0.2 Hz.

Interestingly the replacement of the 4-amino group in 2,4-diaminopyrimidine by the dimethylamino group does appear to increase the overall pKa of the molecule (see Table 12) although there is no significant change in the N-1:N-3 protonation ratio. The ^{13}C n.m.r. spectral data for 2-amino-4-dimethylaminopyrimidine and its N-1 methiodide are given in Tables 18 and 19 respectively.

(v) <u>2-Amino-4-methoxypyrimidine</u>

In an attempt to find a system with an N-1:N-3

TABLE 16

25MHz ^{13}C n.m.r. spectral data

2-Amino-4-(para-methoxyphenyl)aminopyrimidine

Chemical shifts/p.p.m.

Solvent:	$CDCl_3$	H_2O/HCl[a]
C2	95.80	89.37
C4	95.61	94.07
C5	27.90	32.88
C6	90.15	88.07
C1'	63.96	73.51
C2'	58.35	56.53
C3'	47.46	47.38
C4'	63.81	63.67
OCH_3	-11.57	-11.01

Coupling constants/Hz

Solvent:	$CDCl_3$	H_2O/HCl[a]
$^3J_{C2H6}$	12[b]	7.0
$^2J_{C4H5}$	} [c]	<1
$^3J_{C4H6}$	} [c]	8.2
$^1J_{C5H5}$	168.1	176.0
$^2J_{C5H6}$	7.2	4.3
$^1J_{C6H6}$	175.8	187.4
$^2J_{C6H5}$	<1	3.2

(a) pH of solution adjusted to 3 pH units below pka

(b) Resonance partially obscured by C4 resonance.

(c) Couplings not resolved.

TABLE 17

25MHz ^{13}C n.m.r. spectral data

2-Amino-1-methyl-4-(para-methoxyphenyl)aminopyrimidinium iodide

Chemical shifts/p.p.m.

Solvent:	D.M.S.O.
C2	88.61
C4	93.01
C5	33.27
C6	79.61
NCH_3	27.10
C1'	89.61
C2'	56.22
C3'	47.56
C4'	64.40
OCH_3	-10.94

Coupling constants/Hz

Solvent:	D.M.S.O.	
$^3J_{C2H6}$	a	(5.7)
$^2J_{C4H5}$	< 1	(< 1)
$^3J_{C4H6}$	3.9	(7.3)
$^1J_{C5H5}$	175.2	(165.4)
$^2J_{C5H6}$	a	a
$^1J_{C6H6}$	187.0	(183.7)
$^2J_{C6H5}$	a	(3.9)
$^1J_{CH(N-CH_3)}$	142.2	(-)

Values in parenthesise were those obtained when selectively decoupling N-methyl protons with low power irradiation.

(a) Complex multiplet, couplings not resolved.

TABLE 18

25MHz ^{13}C n.m.r. spectral data
2-Amino-4-dimethylaminopyrimidine

Chemical shifts/p.p.m.

Solvent:	$CDCl_3$	T.F.A.	H_2O/HCl[a]
C2	95.35	86.86	87.22
C4	97.76	93.98	95.00
C5	26.83	29.47	29.04
C6	88.80	74.19	73.89
$N\text{-}CH_3$	-30.10	-28.37[b]	-28.52[b]
$N'\text{-}CH_3$	"	-29.06[b]	-29.40[b]

Coupling constants/Hz

Solvent:	$CDCl_3$	T.F.A.	H_2O/HCl[a]
$^3J_{C2H6}$	11.8	7.5	7.2
$^2J_{C4H5}$ / $^3J_{C4H6}$	} c	} c	} c
$^1J_{C5H5}$	166.6	179.7	175.8
$^2J_{C5H6}$	7.6	3.7	4.0
$^1J_{C6H6}$	174.6	191.0	186.8
$^2J_{C6H5}$	c	c	c
$^1J_{CH(N\text{-}CH_3)_2}$	137.3	141.6	139.8
$^3J_{CH(N\text{-}CH_3)_2}$	3.3	c	3.3

(a) pH of solution adjusted to 3 units below pka of 2-amino-4-dimethylaminopyrimidine.
(b) Resonances coalesce at 50^o.
(c) Couplings not resolved.

TABLE 19

25MHz ^{13}C n.m.r. spectral data

2-Amino-4-dimethylamino-1-methylpyrimidinium iodide

Chemical shifts/p.p.m.

Solvent:	D.M.S.O.
C2	87.64
C4	94.27
C5	29.64
C6	79.90
N-CH_3	-27.11
N'-CH_3	-29.24[a]
N''-CH_3	-28.24[a]

Coupling constants/Hz

Solvent:	D.M.S.O.	
^3JC2H6	b	(6.0)
^2JC4H5	b	(<1)
^3JC4H6	b	(7.9)
^1JC5H5	175.2	(170.0)
^2JC5H6	3.9	(3.9)
^1JC6H6	186.8	(184.0)
^2JC6H5	b	(3.0)
^1JCH(N-CH_3)	142.2	(-)
^3JCH(N-CH_3H6)	3.7	(-)
^1JCH(N-CH_3)$_2$	139.7	(-)
^3JCH(N-CH_3)$_2$	3.4	(-)

Values in parenthesise were those obtained when selectively decoupling N-CH_3 protons with low power irradiation.

(a) Resonances coalesce at 50^o.

(b) Complex multiplet, couplings not resolved.

protonatidn ratio greater than that observed in 2,4-diaminopyrimidine we have examined the 2-amino-4-methoxy-pyrimidine system. The methoxy group has a strong +M effect which should have a base strengthening effect and also favour N-1 protonation due to the possibility of delocalisation of the positive charge at N-1 which would involve a para-quinoid type resonance structure. The stronger -I effect of a methoxy group as compared to an amino group however would have a base weakening effect and this is reflected in the reported pKa value of 2-amino-4-methoxypyrimidine (5.53)[24]. The strong -I effect would exert a greater influence at N-3 and hence also favour N-1 protonation and so in this case a more extreme N-1:N-3 protonation than for 2,4-diaminopyrimidine might be observed.

An additional complication was found to arise with the 2-amino-4-methoxypyrimidine system, however, in that the values of ^{3}JC2H6 for the free base (12.8 Hz) and N-1-methiodide (6.8 Hz) differ significantly from those values observed for the other 4-substituted 2-amino-pyrimidines examined in our study (11.6-11.8 and 5.8-6.0 Hz). In both cases the values of ^{3}JC2H6 appear to be approximately 1 Hz larger than expected. In the absence of a sample of the N-3 methiodide of 2-amino-4-methoxy-pyrimidine it has been necessary to assume that this same correction applies to the ^{3}JC2H6 parameter for N-3 protonation determined from the value of ^{3}JC2H4 in 2-amino-1-methylpyrimidinium iodide. Fortunately, since the observed value of ^{3}JC2H6 for the protonated form of 2-amino-4-methoxypyrimidine (7.5 Hz) is relatively close

TABLE 20

25MHz ^{13}C n.m.r. spectral data
2-Amino-4-methoxypyrimidine

Chemical shifts/p.p.m.

Solvent:	$CDCl_3$	T.F.A.	H_2O/HCl[a]
C2	95.98	89.92	89.47
C4	103.20	106.70	105.85
C5	30.89	34.24	33.32
C6	90.70	77.25	78.18
OCH_3	-13.79	-11.16	-10.84

Coupling constants/Hz

Solvent:	$CDCl_3$	T.F.A.	H_2O/HCl[a]
$^3J_{C2H6}$	12.8	7.5	7.5
$^2J_{C4H5}$ / $^3J_{C4H6}$	}[b]	}[b]	}[b]
$^1J_{C5H5}$	170.9	181.3	180.7
$^2J_{C5H6}$	7.3	3.4	3.7
$^1J_{C6H6}$	176.7	189.8	189.5
$^2J_{C6H5}$	2.7	4.3	4.0
$^1J_{CH\ (OCH_3)}$	146.5	149.5	149.5

(a) pH of solution adjusted to 3 units below pka of 2-amino-4-methoxypyrimidine.

(b) Complex multiplet, couplings not resolved.

TABLE 21

25MHz ^{13}C n.m.r. spectral data
2-Amino-1-methyl-4-methoxypyrimidinium iodide

Chemical shifts/p.p.m.

Solvent:	D_2O
C2	89.59
C4	104.04
C5	33.24
C6	84.15
OCH_3	-10.84
NCH_3	-26.00

Coupling constants/Hz

Solvent:	D_2O	
$^3J_{C2H6}$	a	(6.8)
$^2J_{C4H5}$	} a	} a
$^2J_{C4H6}$		
$^1J_{C5H5}$	180.4	(177.0)
$^2J_{C5H6}$	3.7	(3.7)
$^1J_{C6H6}$	189.8	(187.9)
$^2J_{C6H5}$	a	a
$^1J_{CH(OCH_3)}$	148.5	(-)
$^1J_{CH(NCH_3)}$	143.1	(-)

Values in parenthesise are those obtained when selectively decoupling N-methyl protons with low power irradiation.
(a) Complex multiplet, couplings not resolved.

to that of the established value of $^{3}JC2H6$ for N-1 protonation (6.8 Hz) the calculation of the N-1:N-3 protonation ratio is less critically dependent on the value of the parameter for N-3 protonation. It is therefore possible to assess the N-1:N-3 protonation ratio as approaching 90:10 which does, in fact, appear more extreme that the 2,4-diaminopyrimidine case. It is also interesting to note that the difference in the $^{3}JC2H6$ value for the protonated and methylated forms is only 0.7 Hz. This is much less than the 1.4 Hz correction value proposed by Riand et al as being necessary to allow for the differences in $^{3}JC2H6$ for the protonated and N-methylated forms and is further evidence to support our view that such a correction is unnecessary.

THE USE OF $^{13}C - {}^{1}H$ COUPLING CONSTANTS OTHER THAN $^{3}JC2H6$ FOR THE DETERMINATION OF N-1:N-3 PROTONATION RATIOS

There has been relatively little $^{13}C - {}^{1}H$ coupling constant data reported on substituted pyrimidines. Where it has been reported the data has mainly been used as an aid to the assignment of resonances andthe accuracy of the values quoted is thus usually limited. Even fewer studies have been concerned with the changes in these couplings which occur on protonation of the pyrimidine ring, and even here the conclusions have been of a rather general nature. Thus, for example, it has been observed that all ^{1}JCH couplings increase on protonation.

Since commencing this work Riand et al published coupling constant data for amino and methyl substituted pyrimidines and the effects of protonation on these parameters [33]. It was concluded that all one bond ^{13}C - ^{1}H coupling constants increased on protonation with the largest effect being observed on the carbon adjacent to the site of protonation. Also it was observed that the vicinal through nitrogen couplings decreased on protonation.

They also attempted to correlate both the increase in ^{1}JCH values and the decrease in $^{3}JCNH$ with the N-1:N-3 protonation ratio but in the absence of any model compounds they could only assume that the ratios determined in their previous chemical shift study were correct, (i.e. a methyl group in the 4 position results in a N-1:N-3 protonation ratio of 70:30, whilst a 4-amino group strongly favours N-1 protonation (95%) . Hence the conclusions from this study were of a qualitative rather than a quantitative nature and did not provide a method for accurately assessing N-1:N-3 protonation ratios. During the course of our investigations on substituted pyrimidines we were able to acquire a considerable quantity of coupling constant data. This data has been analysed to see if it can be used to determine the N-1:N-3 protonation ratios in those systems studied. The conclusions of this investigation will now be considered.

(i) <u>One bond ^{13}C - ^{1}H coupling constants (^{1}JCH)</u>

A summary of the observed ^{1}JCH values of 4-substituted pyrimidines in both the non-protonated and

the protonated state (T.F.A. and aqueous hydrochloric acid) is given in Table 22.

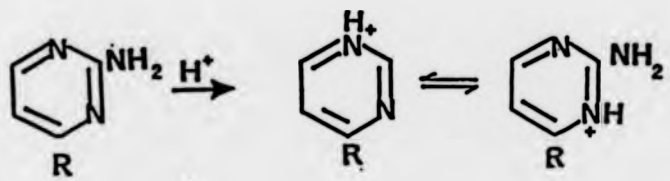
The non-protonated systems

For solubility reasons it was not possible to determine the ^{13}C n.m.r. spectra of all the non-protonated species in the same solvent. In most cases $CDCl_3$ was satisfactory but for 2-amino-4-chloropyrimidine and 2-amino-4-styryl-pyrimidine deuterated dimethyl sulphoxide (d_6-D.M.S.O.) was used as the solvent and for 2,4-diaminopyrimidine aqueous base was used. Although these changes in solvent may affect the ^{1}JCH couplings it is clear that the values of both ^{1}JC5H5 and ^{1}JC6H6 are also affected by the nature of the substituent at C-4. It is interesting to contrast this with the ^{3}JC2H6 coupling, which, under the same conditions, showed little variation in the non-protonated pyrimidines, (11.6-11.8 Hz), with the exception of 2-amino-4-methoxy pyrimidine (12.8 Hz).

The mono-protonated systems

Before the data from the pyrimidines in their monoprotonated state could be assessed it was necessary to establish valid protonation parameters for the effects of protonation at N-1 and N-3 on the ^{1}JCH couplings. Since ^{1}JC5H5 is "meta" to both ring nitrogen atoms it would not be expected to be particularly sensitive to changes in the N-1:N-3 protonation ratio, initial investigations were therefore concentrated on a study of ^{1}JC6H6. Since it has been reported that the effects of protonation are greatest on the carbon adjacent to

TABLE 22.



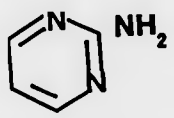
R		$COCl_3$	T.F.A.	H_2O/HCl
H	$^1JC5H5$	169.7	181.9	180.1
	$^1JC6H6$	177.9	190.1	188.9
Me	$^1JC5H5$	167.3	180.1	178.2
	$^1JC6H6$	176.7	190.1	188.0
Ph	$^1JC5H5$	167.8	-	-
	$^1JC6H6$	178.2	-	-
CH:CH Ph[a]	$^1JC5H5$	167.2	178.8	176.7
	$^1JC6H6$	177.3	190.4	187.1
Cl[a]	$^1JC5H5$	177.6	186.8	186.4
	$^1JC6H6$	180.7	193.5	192.3
NH_2[b]	$^1JC5H5$	169.7	183.7	177.0
	$^1JC6H6$	177.0	192.8	187.4
NMe_2	$^1JC5H5$	166.6	179.7	175.8
	$^1JC6H6$	174.6	191.0	186.8
$NHC_6H_4OCH_3$	$^1JC5H5$	168.1	-	176.0
	$^1JC6H6$	175.8	-	187.4
OMe	$^1JC5H5$	170.9	181.3	180.7
	$^1JC6H6$	176.7	189.8	189.5

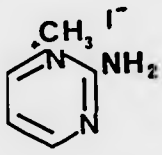
(a) D.M.S.O. used as solvent for non-protonated species.

(b) Aqueous sodium hydroxide used as solvent for non-protonated species.

the site of protonation[33] it is reasonable to expect that the value of $^{1}JC6H6$ would be sensitive to changes in the N-1:N-3 protonation ratio.

Since 2-amino-1-methylpyrimidinium iodide had proved to be a suitable model for establishing the effects of protonation on $^{3}JC2H6$ in the 2-aminopyrimidines it was also investigated as a model for the effects on $^{1}JC6H6$. The effects of N-methylation on the ^{1}JCH couplings in 2-aminopyrimidine are shown below.

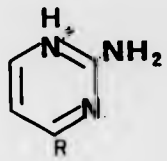




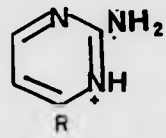
Solvent:	$CDCl_3$	D_2O	D.M.S.O
$^{1}JC4H4$	177.9	187.4	186.8
$^{1}JC5H5$	169.7	180.0	178.8
$^{1}JC6H6$	177.9	191.0	190.4

As can be seen, all the one bond ^{1}JCH coupling constants increase on methylation, the largest effect being observed on the carbon adjacent to the site of methylation.

Nevertheless, at first sight, it does appear that the values obtained for $^{1}JC6H6$ and $^{1}JC4H4$ from 2-amino-1-methylpyrimidinium iodide may be suitable as protonation parameters for the two extreme positions of protonation (61) and (62).



(61)



(62)

The average value of $^{1}JC4H4$ and $^{1}JC6H6$ in 2-amino-1-methylpyrimidinium iodide in D_2O (189.2 Hz) is in reasonably good agreement with the value in the protonated form of 2-aminopyrimidine in aqueous hydrochloric acid (188.9 Hz). However, it should be noted that the parameters determined from 2-amino-1-methylpyrimidinium iodide do appear to be affected by changes in the solvent (D.M.S.O. or H_2O). If the parameters for protonation are equally affected it can be seen that significant errors may arise if data obtained from one solvent is used to calculate protonation ratios in another. It would therefore be unwise, for example, to assume the validity of the parameters derived from 2-amino-1-methylpyrimidinium iodide in D_2O for calculating protonation ratios in T.F.A.

In addition to the effects of solvent, the value of the $^{1}JC6H6$ coupling constant also seems to be sensitive to the nature of the substituent on the ring at C-4. Thus, while $^{1}JC6H6$ has a value of 191.0 Hz (D_2O) in 2-amino-1-methylpyrimidinium iodide the corresponding value in 2-amino-1,4-dimethylpyrimidinium iodide is 189.2 Hz (D_2O) and in 2,4-diaminopyrimidinium iodide it has a value of only 187.1 Hz (D_2O) which is comparable with the value of $^{1}JC4H4$ in 2-amino-1-methylpyrimidinium iodide (187.4 Hz). The variation in the value of $^{1}JC6H6$ in the N-1 methiodides of 2-aminopyrimidine, 2,amino-4-methylpyrimidine and 2,4-diaminopyrimidine is thus larger than the difference in the values of $^{1}JC4H4$ and $^{1}JC6H6$ in 2-amino-1-methylpyrimidinium iodide.

Since $^{1}JC6H6$ appears to vary with the nature of the substituent at C-4 it is clear that the protonation parameters derived from 2-amino-1-methylpyrimidinium iodide would be unsuitable for assessing the N-1:N-3 protonation ratio in those 2-aminopyrimidines possessing a substituent at C-4. It would therefore appear to be necessary to obtain protonation parameters for each substituted system from the N-1 and N-3 methiodides of that system.

Fortunately, since for 2-amino-4-methylpyrimidine both the N-1 and N-3 methiodides had been prepared, it was possible to obtain $^{1}JC6H6$ parameters for the effects of protonation at both N-1 and N-3 in this system (189.2 and 185.6 Hz respectively). These values, as expected are significantly different than those determined for the 2-aminopyrimidine system (191.0 and 187.4 Hz). The observed $^{1}JC6H6$ coupling for 2-amino-4-methylpyrimidine in aqueous hydrochloric acid (188.0 Hz) therefore suggests a protonation ratio in favour of N-1 ($\sim$2:1). Considering the accuracy to which these couplings were determined ($\pm$ 0.3 Hz) and the difference in protonation parameters for the two extreme forms, 61 and 62, R = Me, (3.6 Hz) it would be unwise to quote an exact ratio for this pyrimidine based on these parameters.

For the 2,4-diaminopyrimidine system the $^{1}JC6H6$ protonation parameters for N-1 and N-3 protonation (derived from the appropriate methiodides in D_2O) were found to be 187.1 Hz and 182.1 Hz respectively. Once

again these parameters are different to those found for 2-aminopyrimidine and 2-amino-4-methylpyrimidine. The greater difference between the two values thus means that potentially a more accurate N-1:N-3 protonation ratio could be determined. The value observed for 2,4-diaminopyrimidine in aqueous acid (187.4 Hz) thus suggests that the protonation ratio is quite extreme in favour of N-1. However, in view of the limited evidence for the suitability of the ^{1}JC6H6 protonation parameters derived from the N-1 and N-3 methiodides and the accuracy of these couplings, it would be unwise to place too much emphasis on this result. We feel that the ^{3}JC2H6 parameters derived from 2-amino-1-methylpyrimidinium iodide (5.8 and 13.9 Hz) provide more reliable N-1:N-3 protonation ratios. The 8.1 Hz difference in these values means that, potentially, more accurate ratios can be determined. Evidence that these parameters are valid is provided from the ^{3}JC2H6 coupling in 2-aminopyrimidine in both T.F.A. and aqueous hydrochloric acid. T.F.A. also provides a useful check for the calculated N-1:N-3 protonation ratios of the 2-aminopyrimidines substituted at C-4. For the method based on the ^{1}JC6H6 coupling constant the T.F.A. data is not very meaningful due to the variation of ^{1}JCH couplings with solvent.

(ii) Two bond ^{13}C - ^{1}H coupling constants (^{2}JCH)

(a) ^{2}JC5H6

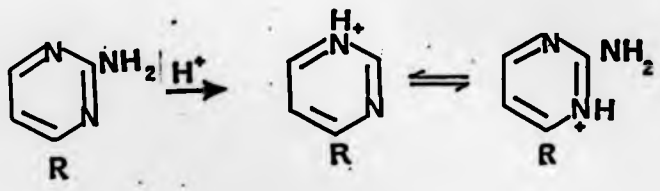
A summary of the ^{2}JC5H6 values observed for the neutral and protonated pyrimidines studied is given

in Table 23. As can be seen this coupling decreases in the protonated systems and there appears to be some correlation between the reduction in $^{2}JC5H6$ on protonation and the percentage protonation at N-1.

Fortunately, as with the $^{3}JC2H6$ coupling previously considered the N-methylpyrimidinium iodides do appear to be satisfactory models for determining $^{2}JC5H6$ protonation parameters for protonation at N-1 and N-3. Thus, the proton coupled ^{13}C n.m.r. spectrum of 2-amino-1-methylpyrimidinium iodide in D_2O and D.M.S.O. shows couplings of 7.9 and 3.3 Hz for $^{2}JC5H4$ and $^{2}JC5H6$ respectively. The average of these two couplings is thus 5.6 Hz, which is in very good agreement with the value of $^{3}JC5H6$ and $^{2}JC5H4$ observed for 2-aminopyrimidine in aqueous acid (5.5 Hz). Furthermore, the $^{2}JC5H6$ values for the N-1 and N-3 methiodides of the 4-substituted 2-aminopyrimidines studied agree closely with the appropriate couplings in 2-amino-1-methylpyrimidinium iodide. Thus, for example, the N-1 methiodides of 2-amino-4-methylpyrimidine and 2,4-diaminopyrimidine both have values of 3.4 Hz while the corresponding N-3 methiodides gave values of 7.8 and 7.7 Hz respectively.

The values of $^{2}JC5H6$ for the protonated systems may therefore also provide a means of assessing the N-1:N-3 protonation ratios. Assuming that the $^{2}JC5H6$ parameter values for N-1 and N-3 protonation are 3.3 Hz and 7.9 Hz respectively it is therefore possible to interpret the value of 5.1 Hz observed for $^{2}JC5H6$ in protonated 2-amino-4-methylpyrimidine as indicating 59% protonation

TABLE 23



R	$^2J_{CSH6}$/Hz[a]	
	$COCl_3$	H_2O/HCl
H	7.0	5.5
CH_3	7.2	5.1
Ph	7·3	-
CH-CHPh	7.0	4.3
Cl[b]	7.6	4.3
NH_2	7.0	3.7
$N(CH_3)_2$	7.6	4.0
$NHC_6H_4OCH_3$	7.2	4.3

(a) Accurate to ± 0.3Hz.

(b) D.M.S.O. used as solvent for non-protonated species.

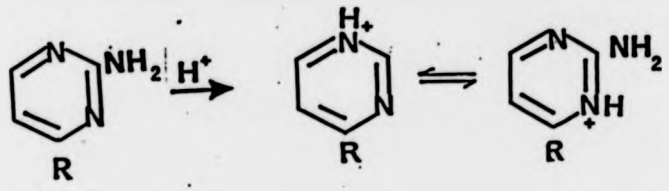
(c) H_2O, pH 11, used as solvent for non-protonated species.

at N-1. Similar calculations on 2,4-diaminopyrimidine give 85% protonation at N-1. These are in good agreement with the values determined from a consideration of $^{3}JC2H6$ values particularly when it is realised that the values for $^{2}JC5H6$ are only accurate to ± 0.3 Hz. and hence that the derived protonation ratios are only accurate to ± 7%. The major disadvantage when using $^{2}JC5H6$ values to determine protonation ratios stems from the rather small difference in the parameter values for N-1 and N-3 protonation. The difference of 4.6 Hz is only just over half the value found for $^{3}JC2H6$ (8 Hz). As a consequence of this the accuracy of the associated protonation ratio calculations will be less than those based on $^{3}JC2H6$.

(b) $^{2}JC6H5$

A summary of the $^{2}JC6H5$ coupling observed for both the neutral and protonated forms of the pyrimidines studied is given in Table 24. It can be seen that this coupling is smaller that that observed for $^{2}JC5H6$ particularly in the case of the neutral species. On protonation the values of $^{2}JC6H5$ increase slightly, this contrasting with the values of $^{2}JC5H6$ which decreased on protonation. However, while it is possible to suggest that the increase in $^{2}JC6H5$ correlates with an increase in the protonation at N-1 it is not possible to determine these coupling parameters with an accuracy which would enable a more quantitative measure to be made.

TABLE 24



R	$^2JC6H5/Hz$[a] $CDCl_3$	H_2O/HCl
H	3.8	4.0
CH_3	3.5	4.2
Ph	2.7	-
CH-CHPh[b]	3.4	4.6
Cl[b]	2.7	4.6
NH_2	2.7	3.8
$N(CH_3)_2$	<1	2.8
$NHC_6H_4OCH_3$	<1	3.2

(a) Accurate to ± 0.3 Hz.

(b) D.M.S.O. used as solvent for non-protonated species.

(c) H_2O, pH11, used as solvent for non-protonated species.

2-amino-1-methylpyrimidinium iodide can be used as a model for establishing the range of values for $^{2}JC6H5$. In this system the values of $^{2}JC4H5$ and $^{2}JC6H5$ were found to be 2.7 and 5.3 Hz respectively, thus confirming that $^{2}JC6H5$ increases as the percentage protonation at N-1 increases. However, the small range of values available make it unlikely that accurate protonation ratios could be determined from a consideration of this parameter.

(c) $^{2}JC4H5$

Due to the complexity of the spin system associated with the C-4 resonance in a number of the systems studied very few values for $^{2}JC4H5$ were obtained. It is therefore not possible to draw conclusions about this parameter except to say that it is clearly not generally suitable for determining protonation ratios.

(iii) Three bond ^{13}C - ^{1}H coupling constants (^{3}JCH)

(a) $^{3}JC4H6$

Once again due to the complexities of the C-4 resonances in a number of the systems studied few values for this parameter were obtained. In general it appears that the value of $^{3}JC4H6$ is greater than $^{2}JC4H5$ in the neutral molecules. Furthermore, it appears that there is not a large change in the values of this coupling as a result of protonation. Also there does not appear to be a large difference for the parameters for N-1 and N-3 protonations. Support for this view comes from a considerationoof the values of $^{3}JC4H6$ and $^{3}JC6H4$ in 2-amino-1-methylpyrimidinium iodide which

have values of 6.4 and 5.3 Hz respectively.

Conclusions

While it appears that, in theory, it may be possible to use couplings other than $^{3}JC2H6$ to determine protonation ratios, in practical terms they offer no advantages. As anticipated the through-nitrogen vicinal couplings were found to be most sensitive to the site of protonation and a study of this parameter therefore appears to offer the best opportunity for establishing accurate protonation ratios.

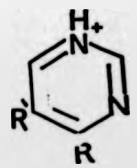
CHAPTER 3

Protonation studies of 4-alkyl and 4-aryl pyrimidines to assess the effect of steric factors on the N-1:N-3 protonation ratio.

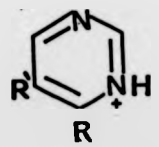
In the previous chapter we were able to clearly establish that steric factors play an important part in determining the N-1:N-3 protonation ratio in 4-substituted 2-aminopyrimidines. It was therefore decided to conduct a more systematic study to evaluate the effect of increasing the size of the substituent at C-4 on the N-1:N-3 protonation ratio. However, to avoid possible complications arising from the presence of a substituent at C-2, it was decided to prepare a range of 4-substituted pyrimidines rather than extend the range of 4-substituted 2-aminopyrimidines already discussed.

Initially a number of 4-alkyl and 4-aryl substituted pyrimidines were prepared and their N-1:N-3 protonation ratios determined. Subsequently, a number of these systems containing an additional methyl substituent at C-5 were synthesised to see if the additional substituent at C-5 had any effect on the N-1:N-3 protonation ratio.

The approach used to evaluate the N-1:N-3 protonation ratio in both the 4- and 4,5-substituted systems was the same as that used for the 2-aminopyrimidine systems previously discussed. However, it was found necessary to use different coupling parameters to represent the two extreme positions of protonation, (63) and (64), to those previously used for our studies on the 2-amino-pyrimidines.



(63)

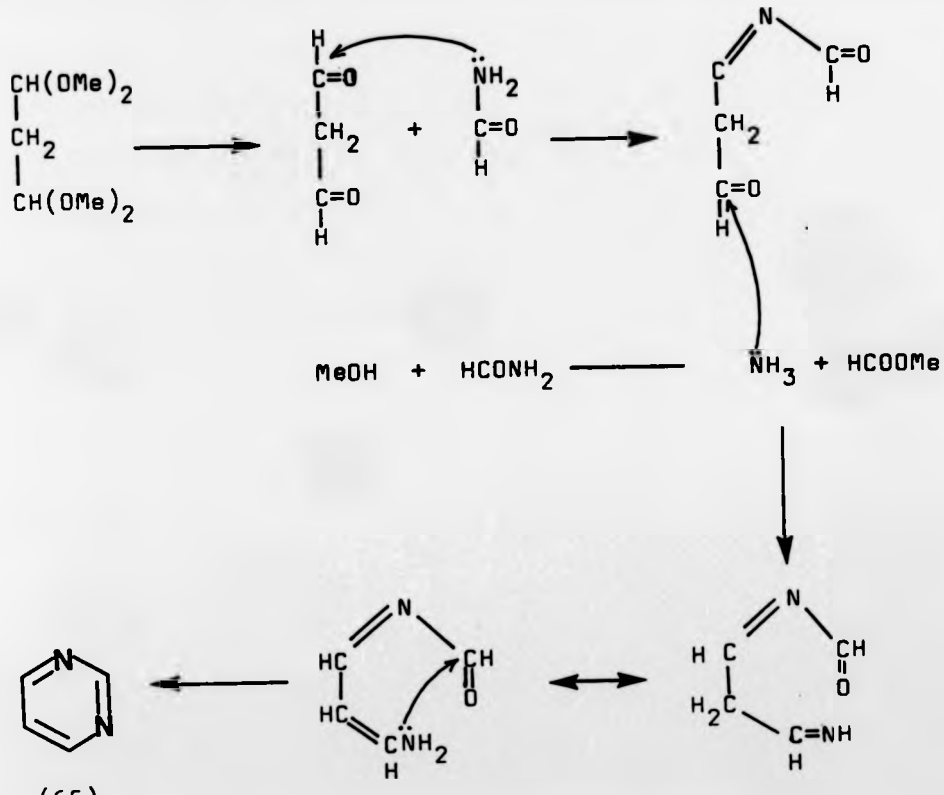


(64)

Synthetic Aspects

(a) Pyrimidine

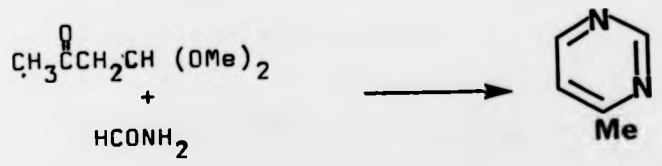
The parent ring system, pyrimidine, (65) has been synthesised by a variety of indirect means but the most convenient method was found to be one-step synthesis from the acyclic precursors. The reaction of 1,1,3,3-tetramethoxypropane with formamide at 180 - 190° gave a 50% yield of pyrimidine. The presence of ammonium salts and a trace of water was necessary to hydrolyse the dimethyl acetals to the aldehyde functions. A possible mechanistic route of this reaction has been proposed.[43]



(65)

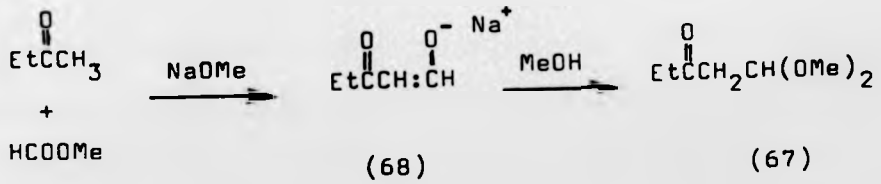
(b) 4-substituted pyrimidines

4-Methylpyrimidine (66) was synthesised in the same manner as that used for pyrimidine by using 4,4-dimethoxybutan-2-one in place of 1,1,3,3-tetramethoxypropane.



(66)

Unfortunately, the higher homologs of the β-keto-dimethylacetals were found to be difficult to prepare. Thus, for example, while an attempt to prepare 5,5-dimethoxypentan-3-one (67) from butan-2-one and methyl-formate in the presence of sodium methoxide led to the isolation of the intermediate sodium salt of the hydroxymethylene ketone (68) subsequent treatment with methanolic HCl caused the formation of polymeric material rather than the desired acetal. (67)



(68) (67)

However, since previous reports have suggested that[44] any compound with the general formula (69) will react with formamide to give pyrimidines the reaction of the sodium salt of the hydroxymethylene ketone previously prepared (68) with formamide was investigated. Unfortunately, when the reaction was carried out in the usual manner no 4-ethylpyrimidine was isolated.

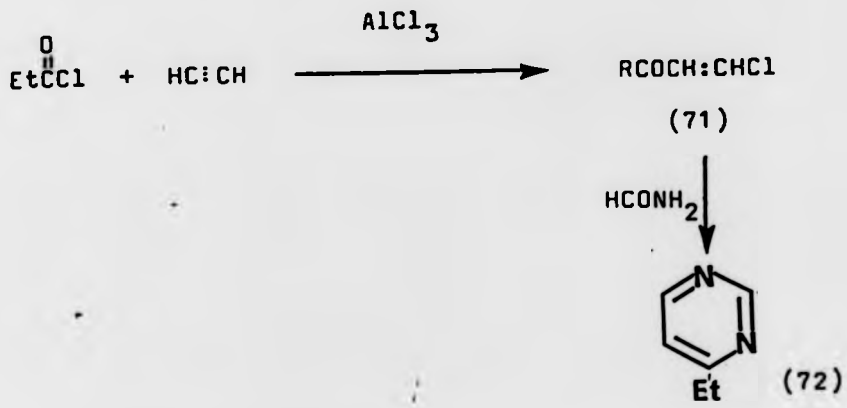
(69)

X = OH, ONa, OR, OCOR, NH_2, Cl

β-chlorovinylketones (70) also fall into the category of compounds of general formula (69) and are readily synthesised from the appropriate acid chloride and acetylene in the presence of a Friedel-Crafts catalyst.[45]

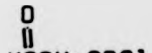
(70)

Thus, for example, propionyl chloride was reacted with acetylene in the presence of aluminium chloride to give trans-1-chloropenten-3-one (71). This was then reacted with formamide to give 4-ethylpyrimidine (72).



4-isopropylpyrimidine was similarly prepared in a 51% yield from trans-1-chloro-3-methylpenten-3-one.

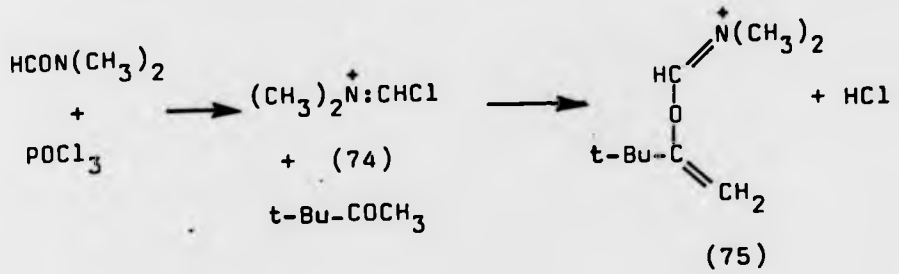
3-chloroacroleins (73) also fall into the category of compounds having the general formula (69) and were successfully used by us in the synthesis of a number of 4-alkyl and 4,5-dialkylpyrimidines.

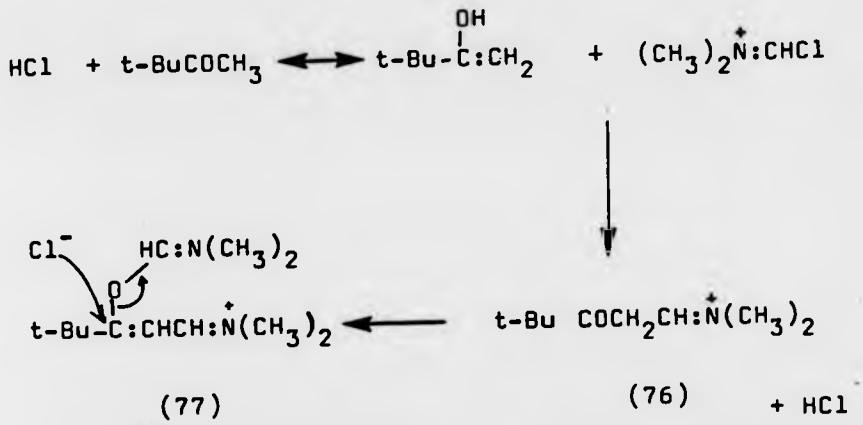


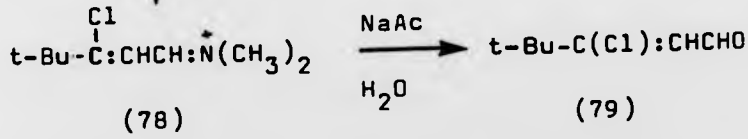
(73)

The 3-chloroacroleins were synthesised in a good yield from the chloromethyleniminiumsalt (74), prepared from dimethylformamide and phosphorous oxychloride, and the appropriate ketone. Thus, for example, the reaction of (74) with 3,3-dimethylbutan-2-one was used to prepare 3-chloro-4,4-dimethylpent-2-enal (2) (79). A possible mechanism for the chloroformylation of methyl and methylene ketones has been proposed. (Fig. 31)[46].

Fig. 31.

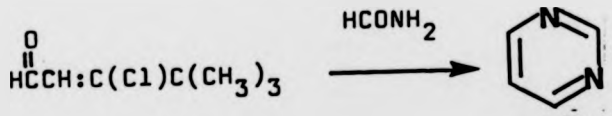

HCON(CH3)2
+
POCl3
(CH3)2N:CHCl
+ (74)
t-Bu-COCH3
HC
O
t-Bu-C
CH2
(75)
+ HCl


HCl + t-BuCOCH3
OH
t-Bu-C:CH2
+ (CH3)2N:CHCl
t-Bu COCH2CH:N(CH3)2
(76)
+ HCl
Cl⁻
HC:N(CH3)2
O
t-Bu-C:CHCH:N(CH3)2
(77)


Cl
t-Bu-C:CHCH:N(CH3)2
(78)
NaAc
H2O
t-Bu-C(Cl):CHCHO
(79)

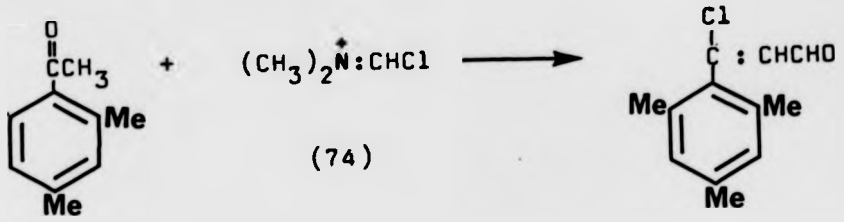
The initial electrophillic attack by the chloro-iminium salt (74) on the ketone yields (75) and, more importantly, produces HCl. The enolisation of the ketone is favoured by acid and the enol can itself react with the chloro-iminium salt (74) to give (76) and more HCl hence catalysing the reaction with the enol. In practice an induction period is often observed followed by an increasingly exothermic reaction which agrees with this autocatalytic mechanism. Further electrophilic attack of the iminium salt (74) on (76) results in the labile bis-iminium salt (77) which is readily attacked by chloride ion to yield the chloroacrolein iminium salt (78) which hydrolysestothe 3-chloroacrolein (79)

The reaction of the 3-chloroacrolein (79) with formamide gave a good yield of 4-tert-butylpyrimidine (80).



(79) (80)

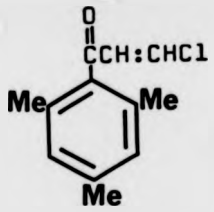
4-Phenylpyrimidine, 4,5-dimethylpyrimidine, 4-ethyl-5-methylpyrimidine and 5-methyl-4-phenylpyrimidine were all successfully synthesised by this route. However, attempts to prepare 4-(2,4,6-trimethylphenyl) pyrimidine (83) by the 3-chloroacrolein route were unsuccessful. The reaction of 2,4,6-trimethylphenyl-ethanone with the chloromethyliminium salt (74) did not yield the desired 3-chloroacrolein (81), only 2,4,6-trimethylbenzoic acid was isolated from the reaction.



(74)

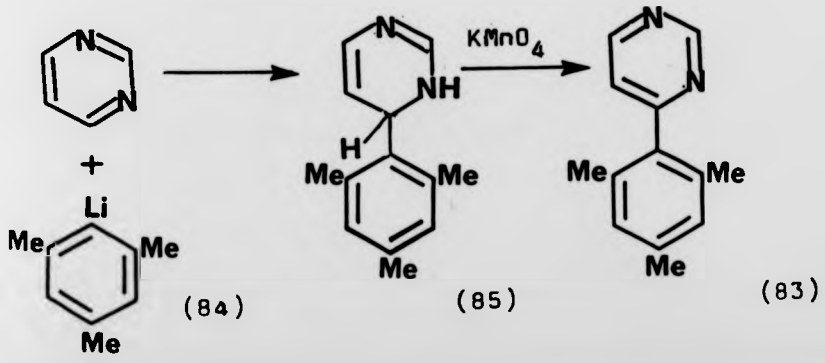
(81)

An attempt was also made to synthesise the β-chlorovinyl ketone (82) by the reaction of 2,4,6-trimethylbenzoyl chloride with acetylene in the presence of aluminium chloride. Once again, however, none of the desired product (82) was isolated from this reaction.



(82)

The failure of both these reactions was probably due to the bulk of the 2,4,6-trimethylphenyl group blocking attack at the carbon atom. We were, however, able to synthesise 4-(2,4,6-trimethylphenyl)pyrimidine (83) successfully from the aryllithium compound (84) and pyrimidine.



(84)

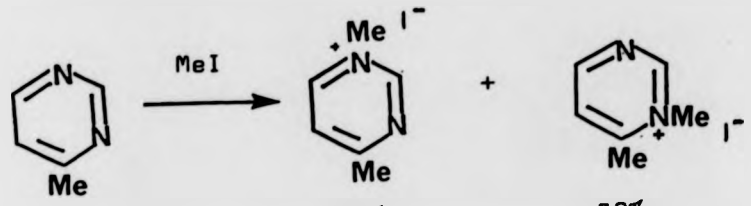
(85)

(83)

Interestingly, it had previously been reported that 2,4,6-trimethylphenyl lithium could not be satisfactorily prepared by direct reaction with lithium metal[47,48] and it was necessary to prepare it by an exchange reaction of 2,4,6-trimethylphenylbromide and tert-butyllithium. However, although the reaction of 2,4,6-trimethylphenyl bromide with lithium metal was slow it was found that the metal did slowly react on refluxing in ether to give the desired aryl lithium compound (84). The intermediate dihydropyrimidine (85) formed on reaction of (84) with pyrimidine was quite stable and required oxidation with permanganate before re-aromatisation of the pyrimidine ring occurred. The reaction of aryllithium reagents with pyrimidine was also used successfully to prepare 4(2-methylphenyl)pyrimidine and 4-tert-butyl-5-methylpyrimidine since experience with the 4-(2,4,6-trimethylphenyl) pyrimidine suggested that difficulty with the method involving chloroacroleins or β-chlorovinyl ketones may have occurreddue to steric factors.

Methiodide formation

The reaction of 4-alkyl and 4-aryl pyrimidines with iodomethane resulted in the formation of both N-methyl isomers in some cases and only the N-1 isomer in others. Steric effects seem to be the most important factor and generally speaking methylation at N-1 becomes increasingly favoured as the size of the 4-substituent increases. Thus, for example, 4-methylpyrimidine gave both 1,4-dimethylpyrimidinium iodide (86) (70%) and 3,4-dimethyl-pyrimidine (87) (30%).

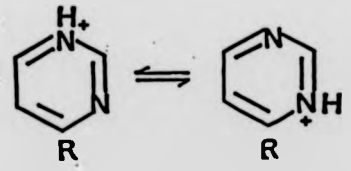


(86) (87)

The reaction of iodomethane with 4-tert-butylpyrimidine and 4-phenylpyrimidine, on the other hand, gave only the N-1 methiodides. Fortunately, in those cases where both methiodides were produced the desired spectral parameters could usually be obtained from the mixture thus avoiding the need to separate the isomeric mixture.

AN ASSESSMENT OF THE USE OF 1-METHYLPYRIMIDINIUM IODIDE AS A MODEL FOR PROTONATION IN 4-ALKYL AND 4-ARYL SUBSTITUTED PYRIMIDINES

As with the 2-aminopyrimidines previously discussed, in order to evaluate the N-1:N-3 protonation ratios in the 4-substituted pyrimidines lacking a substituent at C-2 it was necessary to establish protonation parameters for the two extreme protonation states (88) and (89).



(88) (89)

In view of our success in using the values of $^{3}JC2H4$ and $^{3}JC2H6$ from 2-amino-1-methylpyrimidinium iodide as protonation parameters for $^{3}JC2H6$ in our studies on the 4-substituted 2-aminopyrimidines, it seemed sensible to begin our study of the 4-substituted pyrimidines by assessing whether the $^{3}JC2H6$ and $^{3}JC2H4$ couplings in 1-methylpyrimidinium iodide might provide suitable values for $^{3}JC2H6$ in (88) and (89). The vicinal through-nitrogen coupling $^{3}JC2H6$ was chosen for our preliminary studies since we had already shown this coupling to be the most sensitive to the protonation state of the associated ring nitrogen.

Unfortunately, an additional complication was observed in the proton decoupled ^{13}C n.m.r. spectrum of 1-methylpyrimidinium iodide in water in that the ^{13}C resonances, particularly those for C-2 and C-6, were observed as broad lines (see Fig. 32 (a)). Thus the ^{13}C n.m.r. proton coupled spectrum of 1-methylpyrimidinium iodide was ill-resolved preventing us from obtaining data about the smaller couplings such as $^{3}JC2H6$ and $^{3}JC2H4$. Since the cause of this line broadening was believed to be coupling between the ring carbons and the quadrupole relaxed ^{14}N nucleus (N-1), efforts were made to remove the coupling to the ^{14}N nucleus by reducing the relaxation time of this quadrupolar nucleus to the point where the spin states of the ^{14}N nucleus could be regarded as being averaged. The pyrimidine ring carbon atoms would then be effectively "decoupled" from the ^{14}N nucleus.

As previously discussed in the introduction, a quadrupolar nucleus, such as ^{14}N, often has a short relaxation time due to the efficient relaxation mechanism which arises as a result of the oscillating electric field created by the unsymmetrical electronic distribution around the nucleus. However, if the rate of molecular tumbling increases the electronic field around the quadrupolar nucleus becomes more symmetrical and the relaxation time of the ^{14}N nucleus increases. Under these circumstances coupling between the ^{14}N nucleus and the adjacent carbons may be observed.

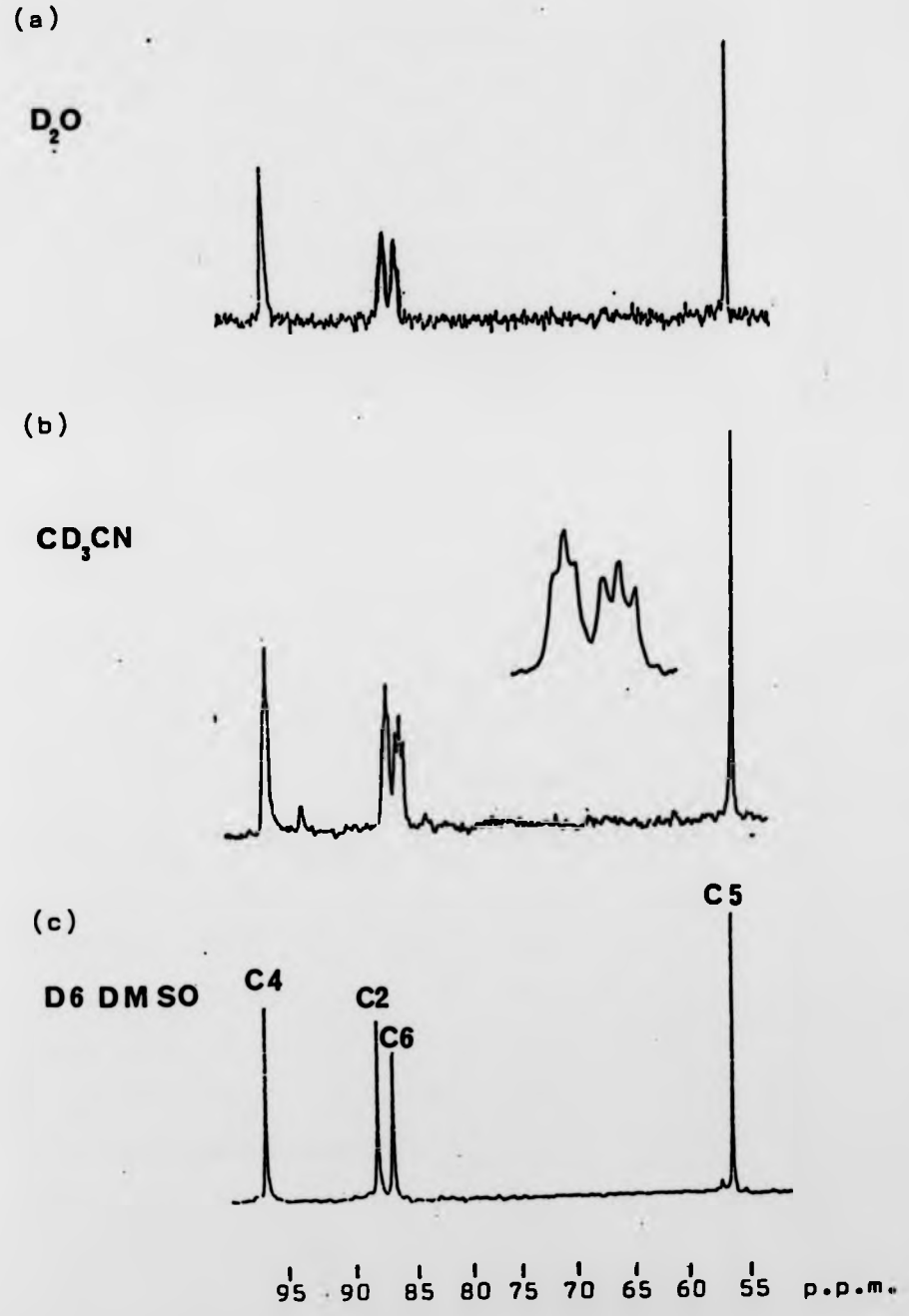
In the case of the 1-methylpyrimidinium iodide in water the relaxation times of the ^{14}N nucleus is somewhere between these two extremes in that while broadening is observed in the ^{13}C n.m.r. spectrum the 1:1:1 triplet pattern expected for coupling with ^{14}N is not. As expected, however, the use of a less viscous solvent such as acetonitrile (see Fig. 32 (b)) increased the rate of molecular tumbling and hence the relaxation time of the ^{14}N nucleus to the point where the triplet character of the ^{13}C resonances became apparent.

In an attempt to remove the ^{14}N coupling from the ^{13}C n.m.r. spectrum of 1-methylpyrimidinium iodide attempts were therefore made to increase the viscosity of the n.m.r. sample. The use of d_6 dimethyl sulphoxide as the solvent caused a marked improvement in the sharpness of the ^{13}C n.m.r. resonances which was further enhanced by cooling the sample to 10^{o} C. The dramatic effects of decreasing the rate of molecular tumbling by

Fig. 32

^{13}C N.M.R. PROTON DECOUPLED SPECTRA

OF 1-METHYLPYRIMIDINIUM IODIDE



increasing the viscosity of the sample are clearly seen in Fig. 32 (c).

By using this approach to remove the coupling to the nitrogen, and selective irradiation of the protons of the N-methyl group to remove couplings to C-2 from these protons, it was therefore possible to observe both $^{3}JC2H4$ and $^{3}JC2H6$. These couplings were found to have values of 11.3 and 4.9 Hz respectively, significantly smaller that the values (5.8 and 13.9 Hz) observed for these couplings in 2-amino-1-methylpyrimidinium iodide.

In order to assess the suitability of these values as models for the effects of protonation at N-1 and N-3 respectively it was necessary to compare the average of these values with the averaged coupling observed in the protonated form of pyrimidine. (Table 26).

Since it is generally accepted that pyrimidines are mono-protonated in T.F.A. this solvent was used initially to obtain information about protonated pyrimidine. Fortunately, the observed through-nitrogen vicinal couplings ($^{3}JC2H4$ and $^{3}JC2H6$) were found to be 8.2 Hz, in excellent agreement with the 8.1 Hz value predicted from a consideration of the coupling parameters obtained from 1-methylpyrimidinium iodide in D.M.S.O. at 10^{o} C.

The determination of the ^{13}C n.m.r. spectrum of the protonated form of pyrimidine in aqueous medium, however, caused more problems. Due to the reduced basicity of the pyrimidine lacking substituent at C-2 relative to the 2-amino substituted systems studied earlier it was necessary to run the spectra in strongly acidic solutions. Pyrimidine, for example, has been reported to have a

PLATE 16

^{13}C N.M.R. SPECTRUM OF 1-METHYLPYRIMIDINIUM IODIDE

IN D6 D.M.S.O. AT 10°C WITH N-METHYL PROTONS SELECTIVELY IRRADIATED

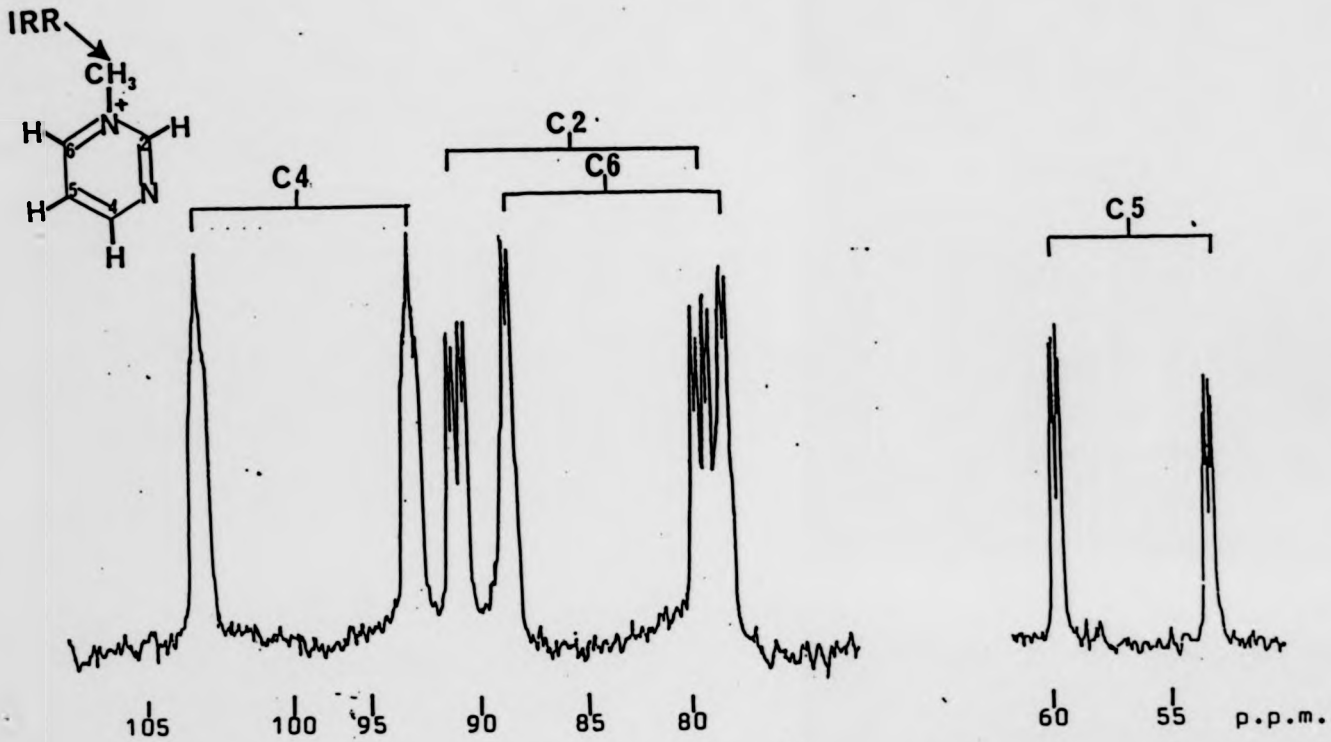


TABLE 25

25MHz ^{13}C n.m.r. spectral data
1-Methylpyrimidinium iodide

Chemical shifts/p.p.m.

Solvent:	D.M.S.O. (10°C)
C2	87.64
C4	97.43
C5	56.73
C6	86.30
N-CH_3	-20.86

Coupling constants/Hz

Solvent:	D.M.S.O.(10°C)	
$^1J_{C2H2}$	220.9	(219.1)
$^3J_{C2H4}$	} b	(11.3)
$^3J_{C2H6}$		(4.9)
$^1J_{C4H4}$	191.6	(189.8)
$^2J_{C4H5}$	} b	} b
$^3J_{C4H2}$		
$^3J_{C4H6}$		
$^1J_{C5H5}$	179.4	(176.4)
$^2J_{C5H4}$	8.0	(8.0)
$^2J_{C5H6}$	3.7	(3.7)
$^1J_{C6H6}$	195.9	(193.1)
$^2J_{C6H5}$	} b	} b
$^3J_{C6H2}$		
$^1J_{C6H4}$		
$^1J_{CH(N-CH_3)}$	145.8	(-)

Values in parenthesise are those obtained when selectively decoupling N-methyl protons with low power irradiation.

(a) Broad resonances observed.

(b) Complex multiplet, couplings not resolved.

TABLE 26

25MHz ^{13}C n.m.r. spectral data

Pyrimidine

Chemical shifts/p.p.m.

Solvent:	$CDCl_3$	T.F.A.	H_2O/HCl[a]
C2	91.72	84.94	84.72
C4,6	89.78	91.24	90.99
C5	54.61	57.63	57.34

Coupling constants/Hz

Solvent:	$CDCl_3$	T.F.A.	H_2O/HCl[a]
$^1J_{C2H2}$	203.2	220.9	219.1
$^3J_{C2H4/C2H6}$	10.4	8.2	8.5
$^1J_{C4H4/C6H6}$	181.0	195.3	193.7
$^2J_{C4H5/C6H5}$	2.7	} b	3.6
$^3J_{C4H2/C6H2}$	8.9		7.2
$^3J_{C4H6/C6H4}$	5.5		5.5
$^1J_{C5H5}$	168.1	181.9	180.3
$^3J_{C5H6/C5H4}$	7.3	5.5	5.5
$^4J_{C5H2}$	1.5	b	1.2

(a) 50% conc. HCl.

(b) Couplings not resolved.

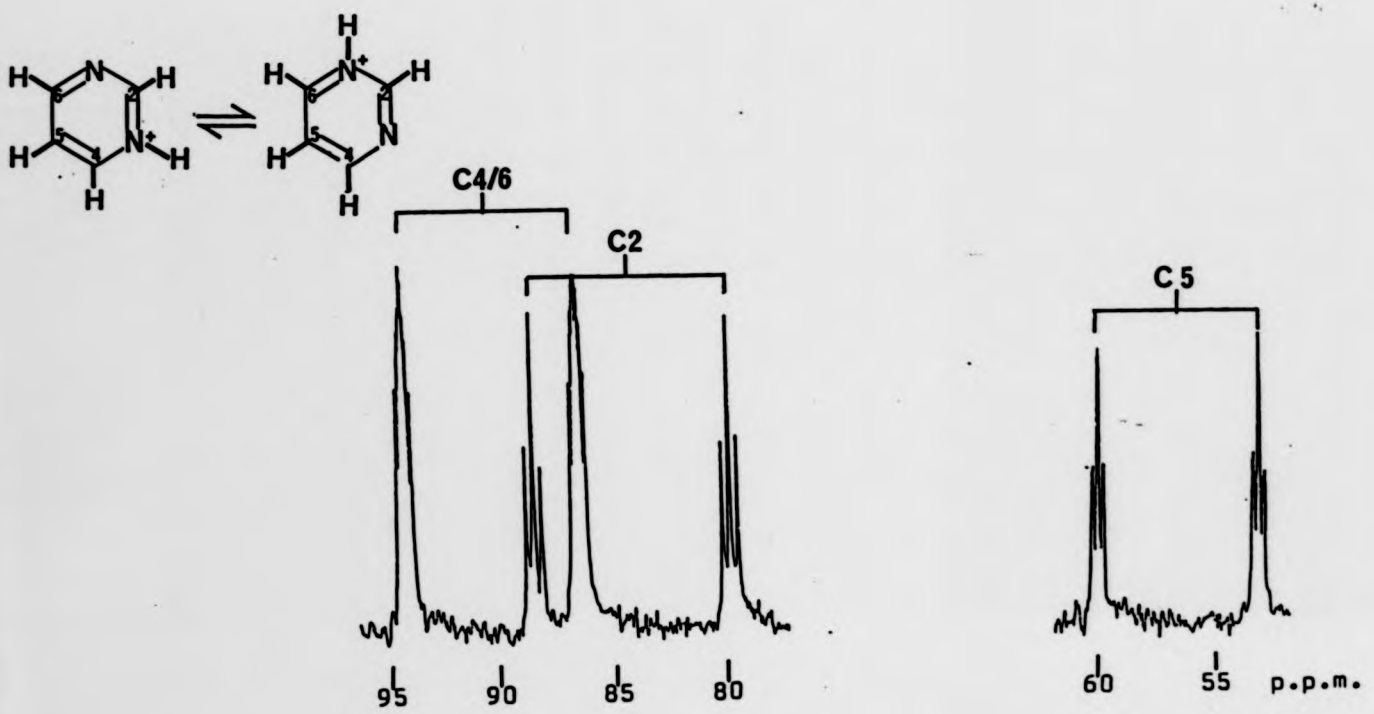
pKa value of approximately 1.3[24] so that to achieve complete protonation it was necessary to operate at very low pH values which could not be monitored by a standard pH meter. At the same time it was necessary to avoid very strongly acidic solutions since diprotonation of the pyrimidine ring might begin to occur under these conditions. Fortunately, however, the pKa value for the diprotonation of pyrimidine has been determined as -6.3 so that diprotonation is unlikely to become a problem unless the pyrimidine is dissolved in extremely strong acids (for example 100% sulphuric acid)[27].

Initial studies were carried out on solutions prepared by dissolving the pyrimidine in 50% concentrated hydrochloric acid (~6M). Since the concentration of the pyrimidine was 1.5M this resulted in a solution of the pyrimidinium chloride in 4.5M hydrochloric acid which would be expected to achieve monoprotonation of pyrimidine ensuring complete monoprotonation. Nevertheless, the value of $^{3}JC2H4$ and $^{3}JC2H6$ observed for pyrimidine in the 50% hydrochloric acid was 8.5 Hz, Plate 17, slightly larger than the value in T.F.A. and in less good agreement with the value predicted from a consideration of the values from 1-methylpyrimidinium iodide. If, as seems likely, the 1-methylpyrimidinium iodide is a good model for the effects of protonation on the vicinal through-nitrogen couplings then the value of 8.5 Hz in aqueous acid would suggest that the pyrimidine is not completely protonated in the hydrochloric acid solution. If this were the case this effect would also be apparent in the other couplings in the molecule . The ^{1}JCH couplings have been shown

PLATE 17

^{13}C N.M.R. SPECTRUM OF PYRIMIDINE

IN WATER OF PH<0 (PROTON COUPLED)



previously to increase significantly when the ring is protonated. The observed increase in ^{1}JCH values was smaller in aqueous hydrochloric acid that the observed increases in T.F.A. This is best interpreted as indicating that the pyrimidine is not fully protonated when dissolved in 50% hydrochloric acid. Furthermore, if the values of $^{3}JC2H4$ and $^{3}JC2H6$ are corrected on the basis of this interpretation it is interesting to note that these decrease to a value of 8.2 Hz in excellent agreement with the value determined in T.F.A.

To explain the apparent incomplete protonation of the pyrimidine in the 50% hydrochloric acid it is therefore necessary to re-assess the "effective pH" of the resulting solution. For pH values below zero the Hammett acidity function (H_o) has been developed as a measure of acid strength. However, it should be noted that the H_o acidity scale was established for essentially pure acids, only the presence of a small quantity of an indicator (usually a para-nitroaniline) being additionally present. The Hammett functions for solutions of hydrochloric and sulphuric acid are shown below.

Hydrochloric acid		Sulphuric acid	
Conc / mol dm^{-3}	H_o	Conc. / mol dm^{-3}	H_o
1	-0.20	1	-0.26
3	-1.05	3	-1.38
4	-1.40	4	-1.85

Hydrochloric acid		Sulphuric acid	
Conc./mol dm^{-3}	H_o	Conc./mol dm^{-3}	H_o
		mol dm^{-3}	
5	-1.76	5	-2.28
6	-2.12	6	-2.76
10	-3.68	10	-4.89
		70% w/w	-5.04
		100% w/w	-11.10

Although T.F.A. does not behave as a typical strong acid the H_o value for the pure acid has been determined as approximately -4 by extrapolating the values which can determined for aqueous solutions of this acid.

Although we would predict from a consideration of the information above that the H_o value of the pyrimidine in 50% hydrochloric acid would be approximately -1.15 it is important to remember that we are not dealing with pure hydrochloric acid. In the case of the pyrimidine solution used for our ^{13}C n.m.r. studies there is a high concentration of the pyrimidinium chloride in the acid. The presence of this salt may therefore cause the effective pH of the solution to be significantly different from that observed for the pure hydrochloric acid.

For this reason it was decided to determine the ^{13}C n.m.r. spectrum of pyrimidine in several more strongly acidic solutions, namely 50%, 70% and 100% sulphuric acid. The chemical shifts (relative to 1,4 dioxan), the ^{1}JCH coupling values and the values of

$^{3}J_{C2H4}$ and $^{3}J_{C2H6}$ for pyrimidine in these three solutions are given in Table 27 together with the data obtained in chloroform, T.F.A. and 50% hydrochloric acid.

In 50% sulphuric acid the $^{1}J_{CH}$ values for pyrimidine are significantly larger than those in 50% hydrochloric acid and are approaching those values observed in T.F.A. Similarly the values of $^{3}J_{C2H4}$ and $^{3}J_{C2H6}$ (8.3 Hz) are comparable to the value observed in T.F.A. This would suggest that in 50% sulphuric acid the pyrimidine is approaching complete mono-protonation.

In 70% sulphuric acid a further small increase in the $^{1}J_{CH}$ values is observed. This suggests that the acidity of the 70% sulphuric acid is now exceeding that of T.F.A. We can therefore be reasonably confident that monoprotonation of the pyrimidine ring is complete at this stage. However, in this strongly acidic medium it is difficult to rule out the possibility of some diprotonation beginning to occur.

In 100% sulphuric acid the pyrimidine would be expected to be in its diprotonation state and this is clearly reflected in the significantly increased values for the $^{1}J_{CH}$ couplings. The slightly larger values of $^{1}J_{CH}$ observed for pyrimidine in 70% sulphuric acid relative to those in T.F.A. are thus consistent with a modest degree of diprotonation occurring.

We have therefore been able to confirm that in sufficiently acidic solutions of aqueous acids the ^{13}C n.m.r. spectral parameters for protonated pyrimidine are comparable with those values observed in T.F.A. where the system would be expected to be completely

TABLE 27

Solvent:	$CDCl_3$	T.F.A.	50% HCl	50%	H_2SO_4 70%.	100%
C2	91.72	84.94	84.72	84.67	84.45	82.97
C4,6	89.78	91.24	90.99	91.15	91.23	91.16
C5	54.61	57.63	57.34	57.58	58.04	60.77
$^1J_{C2H2}$	203.2	220.9	219.1	220.4	221.8	231.3
$^1J_{C4H4}$	181.0	195.3	193.7	194.1	195.3	206.9
$^1J_{C5H5}$	168.1	181.9	180.3	181.3	182.5	193.3
$^3J_{C2H6}$	10.3	8.2	8.5	8.3	8.0	not resolved

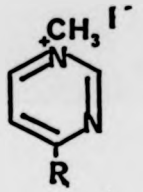
mono-protonated.

At first sight it might therefore appear sensible to avoid the complications of using aqueous acids for our studies of the N-1:N-3 protonation ratios in 4-substituted pyrimidines and to use T.F.A. for all further studies. However, our work on the 4-substituted 2-aminopyrimidines had indicated that the solvent could affect the N-1:N-3 protonation ratio. It was therefore desirable to compare the effects of both T.F.A. and aqueous acids to see if similar differences were observed in the N-1:N-3 protonation ratio in the 4-substituted pyrimidines. Fortunately, the value of $^{1}JC5H5$ in the 4-substituted pyrimidines would be expected to be sensitive to the extent of protonation of the ring system while remaining insensitive to the N-1:N-3 protonation ratio. It was therefore possible to obtain the ^{13}C n.m.r. data for a given pyrimidine in 50% hydrochloric acid and thus correct for the effects of incomplete protonation by comparing the value of $^{1}JC5H5$ in the aqueous hydrochloric acid with that in T.F.A.

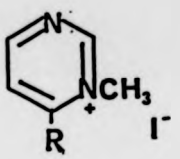
AN ASSESSMENT OF THE N-1:N-3 PROTONATION RATIO IN A NUMBER OF 4-SUBSTITUTED PYRIMIDINES BASED ON A CONSIDERATION OF THE VALUE OF $^{3}JC2H6$

Having established that the values for $^{3}JC2H6$ and $^{3}JC2H4$ from 1-methylpyrimidinium iodide appear to be suitable models for the effects of protonation at N-1 and N-3 in pyrimidine it was of interest to extend our study to a number of 4-alkyl and 4-aryl substituted

pyrimidines to assess the importance of steric factors in influencing the N-1:N-3 protonation ratio and the relative pKa values of the individual nitrogen atoms. Fortunately changes in the substituent at C-4 in the alkyl and aryl substituted systems studied appeared to have little effect on the value of $^{3}JC2H6$ in the free bases in $CDCl_3$ (Table 28), or the value $^{3}JC2H6$ in the N-1 methylated system (90 : R = H, 4.9 Hz; R = Me, 4.8 Hz; R = Ph, 4.8 Hz; R = 2,4,6-trimethylphenyl, 4.9 Hz).



(90)



(91)

Furthermore the values of $^{3}JC2H5$ in N-3 methylated system (91 : R = Me, 11.4 Hz; R = Ph, 11. Hz; R = 2,4,6-trimethylphenyl, 11.6 Hz) are in good agreement with the value of $^{3}JC2H4$ in 1-methylpyrimidinium iodide (11.3 Hz). It would therefore seem reasonable to assume that the $^{3}JC2H6$ parameters determined from 1-methyl pyrimidinium iodide for the effects of N-1 and N-3 protonation may be used to assess the N-1:N-3 protonation ratio in those 4-alkyl and 4-aryl substituted pyrimidines studied.

(i) <u>4-Alkylpyrimidines</u>

The ^{13}C n.m.r. spectral data for 4-methyl, 4-ethyl, 4-isopropyl and 4-tert-butyl pyrimidines are displayed in Tables 29 - 32. For each compound data

TABLE 28

R	R'	$^3J_{C2H6}$/Hz[a]				
		$CDCl_3$	T.F.A.	N-1:N-3	H_2O/HCl[b]	N-1:N-3
H	H	10.4	8.2	48:52	8.2	48:52
Me	H	10.6	7.7	56:44	8.0	52:48
Et	H	10.6	7.3	62:38	7.8	55:45
i-Pr	H	10.4	7.0	67:33	7.6	58:42
t-But	H	10.6	6.5	75:25	6.8	70:30
Ph	H	10.6	5.8	86:14	5.8	86:14
2-methyl-Phenyl	H	10.6	6.6	73:27	6.6	73:27
2,4,6-tri-methylphenyl	H	10.4	8.1	50:50	7.5	60:40
H	Me	10.0	8.2	48:52	8.2	48:52
Me	Me	10.4	8.2	48:52	8.2	48:52
Et	Me	10.3	7.6	58:42	7.8	55:45
t-But	Me	10.9	7.7	56:44	7.8	55:45
Ph	Me	10.8	7.6	58:42	7.1	66:34

(a) Accurate to $\pm$ 0.2 Hz.

(b) Values corrected for incomplete protonation.

TABLE 29

25MHz ^{13}C n.m.r. spectral data

4-Methylpyrimidine

Chemical shifts/p.p.m.

Solvent:	$CDCl_3$	$H_2O/NaOH$[a]	T.F.A.	H_2O/HCl[b]
C2	91.26	89.97	84.23	84.21
C4	99.73	101.25	108.28	105.44
C5	53.93	55.20	57.39	57.36
C6	89.24	89.58	86.64	88.10
CH_3	-42.82	-43.62	-43.94	-43.58

Coupling constants/Hz

Solvent:	$CDCl_3$	$H_2O/NaOH$[a]	T.F.A.	H_2O/HCl
$^1J_{C2H2}$	202.0	203.8	219.1	217.3
$^3J_{C2H6}$	10.6	10.4	7.7	8.3
$^2J_{C4H5}$	} c	} c	} c	} c
$^3J_{C4H2}$				
$^3J_{C4H6}$				
$^1J_{C5H5}$	165.4	167.8	177.6	177.9
$^2J_{C5H6}$	7.9	7.6	5.5	
$^1J_{C6H6}$	180.3	183.1	193.5	191.6
$^2J_{C6H5}$	2.8	3.0	4.3	3.9
$^3J_{C6H2}$	9.3	8.8	6.1	6.9
$^1J_{CH(CH_3)}$	127.2	128.5	131.2	130.6

(a) pH 9.

(b) 50% conc. HCl.

(c) Complex multiplet, couplings not resolved.

TABLE 30

25MHz ^{13}C n.m.r. spectral data

4-Ethylpyrimidine

Chemical shifts/p.p.m.

Solvent:	$CDCl_3$	$H_2O/NaOH$[a]	T.F.A.	H_2O/HCl[b]
C2	91.20	90.03	84.18	84.35
C4	103.76	105.97	113.68	110.50
C5	52.14	53.91	55.98	55.93
C6	89.57	89.87	86.05	87.49
CH_2	-36.16	-36.50	-35.99	-34.56
CH_3	-53.98	-54.37	-55.88	-54.93

Coupling constants/Hz

Solvent:	$CDCl_3$	$H_2O/NaOH$[a]	T.F.A.	H_2O/HCl[b]
$^1J_{C2H2}$	202.2	203.9	219.1	217.3
$^3J_{C2H6}$	10.6	10.4	7.3	8.1
$^2J_{C4H5}$ / $^3J_{C4H2}$ / $^3J_{C4H6}$	}c	}c	}c	}c
$^1J_{C5H5}$	167.3	169.1	177.6	177.0
$^2J_{C5H6}$	c	c	c	c
$^1J_{C6H6}$	180.7	183.1	193.5	191.7
$^2J_{C6H5}$	2.7	2.7	4.2	4.2
$^3J_{C6H2}$	9.0	8.5	6.1	7.0
$^1J'CH(CH_2)$	128.1	128.8	130.0	130.0
$^1J\,CH(CH_3)$	127.0	127.6	128.9	128.7

(a) pH 9.

(b) 50% conc. HCl.

(c) Complex resonance, couplings not resolved.

TABLE 31

25MHz ^{13}C n.m.r. spectral data
4-iso-Propylpyrimidine

Chemical shifts/p.p.m.

Solvent:	$CDCl_3$	$H_2O/NaOH$[a]	T.F.A.	H_2O/HCl[b]
C2	91.26	90.07	84.18	84.35
C4	108.18	109.68	118.49	114.97
C5	51.38	52.67	54.62	54.71
C6	89.61	90.14	85.03	86.64
CH	-31.05	-31.17	-29.64	-30.76
$(CH_3)_2$	-45.25	-45.62	-46.49	-45.84

Coupling constants/Hz

Solvent:	$CDCl_3$	$H_2O/NaOH$[a]	T.F.A.	H_2O/HCl[b]
$^1J_{C2H2}$	201.7	203.2	219.1	217.0
$^3J_{C2H6}$	10.4	10.4	7.0	7.7
$^2J_{C4H5}$ $^3J_{C4H2}$ $^3J_{C4H6}$	} c	} c	} c	} c
$^1J_{C5H5}$	164.8	168.5	177.0	176.7
$^2J_{C5H6}$	c	c	4.3	c
$^1J_{C6H6}$	180.1	182.8	192.9	191.3
$^2J_{C6H5}$	3.0	2.4	4.3	4.2
$^3J_{C6H2}$	9.1	9.2	6.0	7.0
$^1J_{CH}$	128.2	128.3	131.6	131.3
$^1J_{CH(CH_3)}$	127.0	127.6	128.7	128.4

(a) pH 9.
(b) 50% conc. HCl.
(c) Complex resonance, couplings not resolved.

TABLE 32

25MHz ^{13}C n.m.r. spectral data

4-tert-Butylpyrimidine

Chemical shifts/p.p.m.

Solvent:	$CDCl_3$	$H_2O/NaOH$[a]	T.F.A.	H_2O/HCl[b]
C2	90.92	89.87	83.54	84.08
C4	110.32	111.76	122.43	118.81
C5	49.70	51.41	53.96	53.35
C6	89.61	90.19	83.54	84.65
C	-29.49	-29.52	-26.65	-27.57
$(CH_3)_3$	-37.62	-38.02	-38.78	-38.08

Coupling constants/Hz

Solvent:	$CDCl_3$	$H_2O/NaOH$[a]	T.F.A.	H_2O/HCl[b]
$^1J_{C2H2}$	201.7	202.9	219.7	217.3
$^3J_{C2H6}$	10.6	10.4	6.5	7.1
$^2J_{C4H5}$ / $^3J_{C4H2}$ / $^3J_{C4H6}$	} c	} c	} c	} c
$^1J_{C5H5}$	164.2	168.5	second order effects	
$^2J_{C5H6}$	7.9	7.9		
$^1J_{C6H6}$	179.7	182.5		
$^3J_{C6H5}$	2.4	3.0		
$^3J_{C6H2}$	9.1	8.8		
$^1J_{CH(CH_3)}$	126.7	127.0	128.0	128.2

(a) pH 9.

(b) 50% conc. HCl.

(c) Complex multiplet, couplings not resolved.

is presented for the free base in $COCl_3$ (where solubility permitted data is also presented in aqueous base) and for the mono-protonated form in T.F.A. and in 50% hydrochloric acid. A summary of the values of $^3JC2H6$ in the 4-alkyl substituted pyrimidines is given in Table 28. The effects of incomplete protonation in the hydrochloric acid have been allowed for as previously described. (Comparison of $^1JC2H2$ values in T.F.A. and aqueous acid.)

Both the N-1 and N-3 methiodides of 4-methylpyrimidine and the N-1 methiodide of 4-tert-butylpyrimidine were prepared. The ^{13}C n.m.r. spectral data for these compounds is given in Table 33 - 35, once again the close similarity of the $^3JC2H6$ parameters obtained from these methiodides with those obtained for 1-methylpyrimidinium iodide and hence it seems valid to use the $^3JC2H6$ values from the latter to assess the N-1: N-3 protonation ratio.

In both T.F.A. and hydrochloric acid the value of $^3JC2H6$ decreases steadily as the bulk of the alkyl substituent at C-4 increases. It is interesting to note, however, that the effect seems more marked in T.F.A. than in hydrochloric acid indicating that the N-1:N-3 protonation ratio is slightly different in the two solvents. As expected the most extreme case in the 4-alkyl-substituted pyrimidines was that of 4-tert-butyl-pyrimidine where the N-1:N-3 protonation ratio was calculated to be 75:25 in T.F.A. and 70:30 in aqueous hydrochloric acid. Using Equation 4 (see Chapter 1) these

TABLE 33

25MHz ^{13}C n.m.r. spectral data
1,4-Dimethylpyrimidinium iodide

Chemical shifts/p.p.m.

Solvent:	D.M.S.O.
C2	86.68
C4	109.25
C5	55.95
C6	85.59
CH_3	-41.17
N-CH_3	-21.74

Coupling constants/Hz

Solvent:	D.M.S.O.	
$^1J_{C2H2}$	219·6	(217.3)
$^3J_{C2H6}$	a	(4.8)
$^2J_{C4H5}$	} a	} a
$^3J_{C4H2}$		
$^3J_{C4H6}$		
$^1J_{C5H5}$	177.3	(173.9)
$^2J_{C5H6}$	3·7	3·8
$^1J_{C6H6}$	194.7	(191.9)
$^2J_{C6H5}$	4.7	(4.7)
$^3J_{C6H2}$	4.7	(4.7)
$^1J_{CH(CH_3)}$	130.9	(-)
$^1J_{CH(N\text{-}CH_3)}$	145.2	(-)

Values in paranthesise are those obtained when selectively decoupling N-methyl protons with low power irradiation.

(a) Complex multiplet, couplings not resolved.

TABLE 34

25MHz ^{13}C n.m.r. spectral data
3,4-Dimethylpyrimidinium iodide

Chemical shifts/p.p.m.

Solvent:	D.M.S.O.
C2	87.76
C4	98.09
C5	58.07
C6	95.88
CH_3	-45.67
N-CH_3	-23.73

Coupling constants/Hz

Solvent:	D.M.S.O.	
$^1J_{C2H2}$	219.6	(217.9)
$^3J_{C2H6}$	a	(11.4)
$^2J_{C4H5}$	} a	} a
$^3J_{C4H2}$		
$^3J_{C4H6}$		
$^1J_{C5H5}$	177.3	(173.9)
$^2J_{C5H6}$	a	a
$^1J_{C6H6}$	191.3	(188.0)
$^2J_{C6H5}$	3.0	(3.0)
$^3J_{C6H2}$	9.4	(9.4)
$^1J_{CH(CH_3)}$	130.9	(-)
$^1J_{CH(N\text{-}CH_3)}$	145.2	(-)

Values in parenthesise are those obtained when selectively decoupling N-methyl protons with low power irradiation.

(a) Complex multiplet, couplings not resolved.

TABLE 35

25MHz n.m.r. spectral data

1-Methyl-4-tert-butylpyrimidinium iodide

Chemical shifts/p.p.m.

Solvent:	D.M.S.O.
C2	86.44
C4	118.13
C5	52.79
C6	85.48
N-CH_3	-21.88
C	-27.21
$(CH_3)_3$	-37.64

Coupling constants/Hz

Solvent:	D.M.S.O.	
$^1J_{C2H2}$	219.7	(218.2)
$^3J_{C2H6}$		(4.9)
$^2J_{C4H5}$	} a	} a
$^3J_{C4H2}$		
$^3J_{C4H6}$	a	a
$^1J_{C5H5}$		
$^2J_{C5H6}$		
$^1J_{C6H6}$	} second order effects	
$^2J_{C6H5}$		
$^3J_{C6H2}$		
$^1J_{CH(CH_3)}$	127.6	(124.5)
$^1J_{CH(N-CH_3)}$	145.5	(-)

Values in paranthesise are those obtained when selectively decoupling N-methyl protons with low power irradiation.

(a) Complex multiplet, couplings not resolved.

ratios can be interpreted as implying that pKa N-1 - pKa N-3 = 0.4 pH units. It is interesting to compare this with a 4-methyl group where pKa N-1 - pKa N-3 is only 0.07 pH units. Since inductive effects of the alkyl group at C-4 would be expected to enhance rather than weaken the basicity of N-3 relative to N-1 the reduced basicity of N-3 can be attributed solely to increased steric hindrance in the vicinity of N-3.

(ii) 4-Aryl pyrimidines

The ^{13}C n.m.r. spectral data for 4-phenyl 4-(2-methylphenyl) and 4-(2,4,6-trimethylphenyl) pyrimidines are shown in Tables 36 - 38. As with the 4- alkyl substituted system previously discussed these Tablescontain ^{13}C n.m.r. data about the free base in $CDCl_3$ and the protonated state in T.F.A. and 50% hydrochloric acid.

The ^{13}C spectral data for 1-methyl-4-phenylpyrimidinium iodide and both 1-methyl-2,4,6-(trimethylphenyl) pyrimidinium iodide and 3-methyl 2,4,6-(trimethylphenyl)-pyrimidinium iodide is given in Tables 39-41. The $^{3}JC2H6$ protonation parameters were once more in close agreement with those from the parent system.

For 4-phenylpyrimidine the value of $^{3}JC2H6$ is 5.8 Hz in both T.F.A. and aqueous hydrochloric acid (corrected for incomplete protonation). This indicates that the N-1:N-3 protonation ratio is 86:14 which corresponds to a difference in pKa N-1 and pKa N-3 of

TABLE 36

25MHz ^{13}C n.m.r. spectral data

4-Phenylpyrimidine

Chemical shifts/p.p.m.

Solvent:	$CDCl_3$	T.F.A.	H_2O/HCl[a]
C2	91.79	84.04	84.33
C4	96.49	106.34	103.16
C5	49.82	51.59	51.67
C6	90.17	81.38	82.19
C1'	69.21	65.62	65.80
C2'	59.94	62.65	62.10
C3'	61.84	63.20	62.93
C4'	63.92	69.23	67.96

Coupling constants/Hz

Solvent:	$CDCl_3$	T.F.A.	H_2O/HCl[a]
$^1J_{C2H2}$	202.6	219.1	217.3
$^3J_{C2H6}$	10.6	5.8	6.1
$^2J_{C4H5}$ $^3J_{C4H2}$ $^3J_{C4H6}$	}[b]	}[b]	}[b]
$^1J_{C5H5}$	165.4	177.0	176·7
$^2J_{C5H6}$			
$^1J_{C6H6}$	180.7	194.7	193·8
$^2J_{C6H5}$	2.4	4·1	4·2
$^3J_{C6H2}$	9.1	4.9	5·2

(a) 50% conc. HCl.

(b) Complex multiplet, couplings not resolved.

TABLE 37

25MHz ^{13}C n.m.r. spectral data

4-(2-methylphenyl)pyrimidine

Chemical shifts/p.p.m.

Solvent:	$CDCl_3$	T.F.A.	H_2O/HCl[a]
C2	91.28	84.00	84.04
C4	99.89	108.18	106.22
C5	54.19	56.37	53.37
C6	89.56	83.07	83.34
C1'	68.86	66.41	66.99
C2'	70.37	71.90	71.09
C3'	62.43	64.23	65.27
C4'	64.02	66.78	65.88
C5'	62.43	65.71	64.04
C6'	59.04	60.32	60.11
CH_3	-46.56	-46.98	-46.54

Coupling constants/Hz

Solvent:	$CDCl_3$	T.F.A.	H_2O/HCl[a]
$^1J_{C2H2}$	202.9	219.4	219.1
$^3J_{C2H6}$	10.6	6.6	6.8
$^2J_{C4H5}$	}	}	}
$^3J_{C4H2}$	}[b]	}[b]	}[b]
$^3J_{C4H6}$	}	}	}
$^1J_{C5H5}$	166.6	177.8	177.6
$^2J_{C5H6}$	7.9	b	5.5
$^1J_{C6H6}$	181.0	194·3	193·4
$^2J_{C6H5}$	2.7	b	b
$^3J_{C6H2}$	9.1	b	b

(a) 50% conc. HCl.

(b) Complex multiplet, couplings not resolved.

TABLE 38

25MHz ^{13}C n.m.r. spectral data
4-(2,4,6-Trimethylphenyl)pyrimidine

Chemical shifts/p.p.m.

Solvent:	$CDCl_3$	T.F.A.	H_2O/HCl[a]
C2	92.00	85.15	85.32
C4	100.84	106.38	105.89
C5	55.44	59.53	58.47
C6	89.72	88.56	86.45
C1'	68.12	62.40	64.69
C2'	67.95	69.30	69.15
C3'	61.50	62.96	62.35
C4'	71.31	76.65	74.53
$(CH_3)_2$	-47.02	-46.66	-46.05
CH_3	-45.95	-47.88	-47.21

Coupling constants/Hz

Solvent:	$CDCl_3$	T.F.A.	H_2O/HCl[a]
$^1J_{C2H2}$	202.6	220.0	217.6
$^3J_{C2H6}$	10.4	8.1	7.6
$^2J_{C4H5}$ $^3J_{C4H2}$ $^3J_{C4H6}$	} b	} b	} b
$^1J_{C5H5}$	166.6	179.7	178.5
$^2J_{C5H6}$	7.9	5.2	4.9
$^1J_{C6H6}$	180.9	194.7	193.5
$^2J_{C6H5}$	3.1	3.3	3.3
$^3J_{C6H2}$	4.1	7.0	6.7

(a) 50% conc. HCl.
(b) Complex multiplet, couplings not resolved.

TABLE 39

25MHz ^{13}C n.m.r. spectral data
1-Methyl-4-phenylpyrimidinium iodide

Chemical shifts/p.p.m.

Solvent:	D.M.S.O ($10^{o}C$)
C2	86.53
C4	102.36
C5	51.63
C6	84.77
C1'	65.75
C2'	62.27
C3'	62.95
C4'	68.05
$N-CH_3$	22·30

Coupling constants/Hz

Solvent:	D.M.S.O.	
$^{1}J_{C2H2}$	220.3	(219.1)
$^{3}J_{C2H6}$	a	(4.8)
$^{2}J_{C4H5}$	} a	} a
$^{3}J_{C4H2}$		
$^{3}J_{CCH6}$		
$^{1}J_{C5H5}$	177.0	(176.7)
$^{2}J_{C5H6}$	3.6	(3.6)
$^{1}J_{C6H6}$	195.3	(193.5)
$^{2}J_{C6H5}$	4.6	(4.6)
$^{3}J_{C6H2}$	4.6	(4.6)
$^{1}J_{CH(N-CH_3)}$	145.3	(-)

Values in parenthesise are those obtained when selectively decoupling N-methyl protons with low power irradiation.

(a) Complex multiplet, couplings not resolved.

TABLE 40

25MHz ^{13}C n.m.r. spectral data

1-Methyl-4-(2,4,6-trimethylphenyl)pyrimidinium iodide

Chemical shifts/p.p.m.

Solvent:	D.M.S.O.
C2	87.25
C4	106.58
C5	57.78
C6	85.69
N-CH_3	-21.56

Other carbon resonances not assigned.

Coupling constants/Hz

Solvent:	D.M.S.O.	
$^1J_{C2H2}$	220.6	(220.3)
$^3J_{C2H6}$	a	(4.9)
$^2J_{C4H5}$, $^3J_{C4H2}$, $^3J_{C4H6}$	a	a
$^1J_{C5H5}$	179.3	(178.3)
$^2J_{C5H6}$	3	(3)
$^1J_{C6H6}$	195.7	(193.5)
$^2J_{C6H5}$	4.2	(4.2)
$^3J_{C6H2}$	4.2	(4.2)
$^1J_{CH(N-CH_3)}$	145.3	(-)

Values in parenthesise are those obtained when selectively decoupling N-methyl protons with low power irradiation.

(a) Complex multiplet, couplings not resolved.

TABLE 41

25MHz ^{13}C n.m.r. spectral data

3-Methyl-4-(2,4,6-trimethylphenyl)pyrimidinium iodide

Chemical shifts/p.p.m.

Solvent:	D.M.S.O.
C2	90.09
C4	95.10
C5	59.62
C6	98.01
N-CH_3	-23.68

Other carbon resonances not assigned.

Coupling constants/Hz

Solvent:	D.M.S.O.	
$^1J_{C2H2}$	220·6	(220·0)
$^3J_{C2H6}$	a	(11.6)
$^2J_{C4H5}$	} a	} a
$^3J_{C4H6}$		
$^3J_{C4H6}$		
$^1J_{C5H5}$	resonances obscured	
$^2J_{C5H6}$		
$^1J_{C6H6}$		
$^2J_{C6H5}$		
$^3J_{C6H2}$		
$^1J_{CH(N-CH_3)}$	145.2	(-)

Values in parenthesise are those obtained when selectively decoupling n-methyl protons with low power irradiation.

(a) Complex multiplet, couplings not resolved.

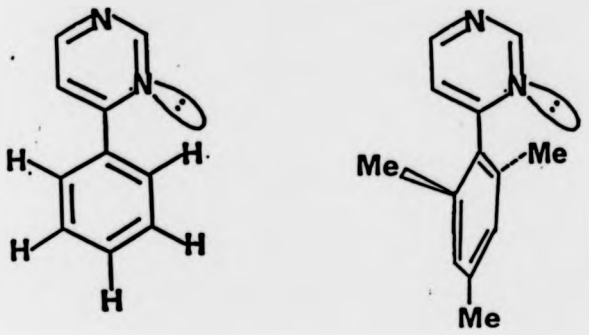
of 0.8 pH units. These values are more extreme than those observed for 4-tert-butylpyrimidine and can be explained by considering that the pyrimidine and phenyl rings are held in a coplanar arrangement (Fig. 33). In these circumstances the ortho protons of the phenyl ring would hinder the solvation of the protonated species formed when N-3 is the site of protonation. Further evidence for the coplanarity of the two rings is provided by the U.V. spectrum of 4-phenylpyrimidine which shows that the two rings are in conjugation in both the free base (λ max 275 nm, ε = 16,000 mol^{-1} dm^3 cm^{-1}) and the protonated form (λmax 304 nm, ε = 19,150 mol^{-1} dm^3 cm^{-1}).

In an attempt to increase the N-1:N-3 protonation ratio still further we have investigated the effects of introducing methyl groups into the ortho positions on the phenyl ring. It was hoped that the presence of these groups would further hinder the N-3 site thus making it less attractive as a site for protonation.

However, the value of $^3JC2H6$ observed in 4(2,4,6-trimethylphenyl) pyrimidine in T.F.A. was 8.1 Hz and 7.5 Hz in aqueous hydrochloric acid suggesting that the N-1:N-3 protonation ratio is only slightly in favour of the N-1 site. It would therefore appear that in the 4-(2,4,6-trimethylphenyl) system the presence of the methyl substituents forces the molecule into a non-coplanar arrangement of the rings. In these circumstances the interaction of the methyl substituents with N-3 would be minimal thus accounting for the apparent ease with which protonation at N-3 can occur. This view is supported by the U.V. spectral data for both the free

base (λ max 262 nm, ε = 6,500 mol^{-1} dm^3 cm^{-1}) the monoprotonated system (λ max 316 nm, ε = 4,370 mol^{-1} dm^3 cm^{-1}) which both indicate that the two rings are much less conjugated than 4-phenylpyrimidine and do not prefer the coplanar arrangement.

Fig. 33



The accessibilities of the N-3 site is even more clearly indicated by the observation that the reaction of 4-(2,4, 6-trimethylphenyl)pyrimidine with iodomethane yields both the N-1 and N-3 methiodides in contrast to the 4-phenylpyrimidines where only the N-1 methiodide is formed.

The effect of introducing one methyl group at the 2 position on the phenyl ring was also investigated in the hope that this might increase the steric interaction at N-3 while maintaining the two aromatic rings in a coplanar arrangement. However, the value of $^3JC2H6$ in 4-(2-methylphenyl)pyrimidine in both T.F.A. and aqueous acid was 6.6 Hz (Table 28), indicating a protonation ratio of 73:27 in favour of N-1. This situation is thus between those observed in 4-phenyl-pyrimidine and 4-(2,4,6-trimethylphenyl)pyrimidine and

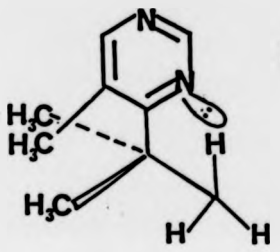
clearly indicates that even the introduction of one methyl group can affect the coplanarity of the phenyl and pyrimidine rings.

In conclusion, therefore, the 4-phenylpyrimidine was found to have the most extreme N-1:N-3 protonation ratio (86:14) in those 4-substituted pyrimidines studied and efforts to increase the ratio by increasing the steric interactions at N-3 proved unsuccessful.

(iii) 4-alkyl and 4-aryl pyrimidines with an additional methyl substituent at C-5

The degree of interaction between a given substituent at C-4 and the N-3 protonation site depends on the preferred conformation of that C-4 substituent with respect to the pyrimidine ring. It was therefore of interest to investigate whether the presence of a methyl substituent at C-5 would affect the preferred conformation and thus have an effect on the N-1:N-3 protonation ratio in the 4-substituted systems previously studied. Furthermore since the electronic effects of the 5-methyl group would be expected to be similar at both N-1 and N-3 any changes observed in the N-1:N-3 protonation ratio would be able to be interpreted as being due to a change in the preferred conformation of the 4-substituent. Thus, for example, since the ortho methyl group in 4-(2-methylphenyl)pyrimidine had already been observed to cause some loss of planarity between the pyrimidine and benzene rings one might expect the presence of the 5 methyl substituent on the pyrimidine ring would have a similar effect.

Similarly in the case of 4-tert-butyl pyrimidine it might be anticipated that the presence of the 5-methyl substituent might encourage the tert-butyl group to favour a conformation where two of its methyl groups straddled the 5 methyl group (92). This in turn might provide a way of increasing the interaction between the remaining methyl group and N-3.



(92)

A number of 4-substituted 5-methylpyrimidines were therefore prepared and their ^{13}C n.m.r. spectra determined. Tables 42 - 46 show the data obtained for these pyrimidines in $CDCl_3$, aqueous base (when solubility permitted), T.F.A. and 50% hydrochloric acid. The methiodides of several of these compounds were also prepared and the ^{13}C n.m.r. data for these compounds can be found in Table 47 - 49.

The introduction of the additional 5-methyl group was found to have little effect on the values of $^{3}JC2H6$ in $CDCl_3$ although this coupling was found to vary slightly within those 5-methylpyrimidines studied (Table 28). The $^{3}JC2H6$ and $^{3}JC2H4$ values for 1,5-dimethyl-pyrimidinium iodide (4.9 and 11.3 Hz) were almost identical to those found in 1-methylpyrimidinium iodide and the value of $^{3}JC2H6$ for the N-1 methiodides of 4-ethyl-5-

TABLE 42

25MHz ^{13}C n.m.r. spectral data
5-Methylpyrimidine

Chemical shifts/p.p.m.

Solvent:	$CDCl_3$	$H_2O/NaOH$[a]	T.F.A.	H_2O/HCl[b]
C2	89.03	87.64	82.31	81.94
C4	89.75	90.19	90.62	90.56
C5	63.78	65.75	69.47	68.81
C6	89.75	90.19	90.62	90.56
CH_3	-51.23	-51.65	-51.89	-50.94

Coupling constants/Hz

Solvent:	$CDCl_3$	$H_2O/NaOH$[a]	T.F.A.	H_2O/HCl[b]
$^1J_{C2H2}$	203.9	205.7	220.9	219.7
$^3J_{C2H6}$	10.2	10.0	8.2	8.5
$^3J_{C4H2/C6H6}$	178.8	181.9	191.6	191.0
$^3J_{C4H2/C6H2}$	9.8	9.2	} c	~6
$^3J_{C4H6/C6H4}$	5.2	5.2		~5
$^2J_{C5H4}$	6.7	6.1	5.5	5.5
$^1J_{CH_3}$	127.9	128.8	130.9	130.6

(a) pH 9.
(b) 50% conc. HCl.
(c) Couplings not resolved.

TABLE 43

25MHz ^{13}C n.m.r. spectral data

4,5-Dimethylpyrimidine

Chemical shifts/p.p.m.

Solvent:	$CDCl_3$	T.F.A.	H_2O/HCl[a]
C2	98.24	81.92	81.58
C4	89.07	105.10	1o3.54
C5	62.o5	67.63	67.65
C6	89.07	86.44	87.73
4-CH_3	-44.96	-46.08	-45.81
5-CH_3	-51.17	-51.87	-51.23

Coupling constants/Hz

Solvent:	$CDCl_3$	T.F.A.	H_2O/HCl[a]
^1JC2H2	201.1	217.6	217.3
^3JC2H6	10.4	8.2	8.5
^3JC4H2	} b	} b	} b
^3JC4H6			
^2JC5H6			
^1JC6H6	177.3	189.8	189.8
^2JC6H2	9.7	6.1	6.2
^1JCH(4-CH_3)	127.6	130.6	130.6
^1JCH(5-CH_3)	127.6	130.3	130.3

(a) 50% conc. HCl.

(b) Complex multiplet, couplings not resolved.

TABLE 44

25MHz ^{13}C n.m.r. spectral data

4-Ethyl-5-methylpyrimidine

Chemical shifts/p.p.m.

Solvent:	$CDCl_3$	$H_2O/NaOH$[a]	T.F.A.	H_2O/HCl[b]
C2	89.26	87.73	81.92	82.01
C4	102.32	103.79	111.27	108.72
C5	61.35	63.27	67.60	67.08
C6	89.41	89.36	84.98	86.84
CH_2	-39.03	-39.22	-38.54	-39.10
CH_3	-55.30	-55.27	-52.26	-51.66
$5\text{-}CH_3$	-51.60	-52.06	-56.78	-55.92

Coupling constants/Hz

Solvent:	$CDCl_3$	$H_2O/NaOH$[a]	T.F.A.	H_2O/HCl[b]
$^1J_{C2H2}$	201.7	203.5	219.1	217.3
$^3J_{C2H6}$	10.3	10.4	7.6	8.0
$^3J_{C4H2}$ / $^3J_{C4H6}$ / $^2J_{C5H6}$	} c	} c	} c	} c
$^1J_{C6H6}$	177.6	180.3	191.0	189.8
$^2J_{C6H2}$	9.5	8.8	6.7	6.3
$^1J_{CH(CH_2)}$	126.9	128.2	130.6	130.0
$^1J_{CC(CH_3)}$	127.6	128.2	130.6	130.3
$^1J_{CH(5\text{-}CH_3)}$	127.6	127.6	130.0	129.4

(a) pH ~ 9.

(b) 50% conc. HCl.

(c) Complex multiplet, couplings not resolved.

TABLE 45

25MHz ^{13}C n.m.r. spectral data

4-tert-Butyl-5-methylpyrimidine

Chemical shifts/p.p.m.

Solvent:	$CDCl_3$	T.F.A.	H_2O/HCl[a]
C2	88.25	80.88	80.88
C4	106.63	115.21	113.73
C5	61.20	67.62	66.77
C6	91.79	87.62	87.30
C	-27.84	-25.58	-26.26
$(CH_3)_3$	-38.01	-39.50	-38.81
5-CH_3	-48.07	-48.62	-48.00

Coupling constants/Hz

Solvent:	$CDCl_3$	T.F.A.	H_2O/HCl[a]
$^1J_{C2H2}$	201.7	220.0	217.9
$^3J_{C2H6}$	10.9	7.8	8.0
$^3J_{C4H2}$	} b	} b	} b
$^3J_{C4H6}$			
$^2J_{C5H6}$	b	b	b
$^1J_{C6H6}$	177.0	190.4	189.8
$^3J_{C6H2}$	9.2	6.7	6.4
$^1J_{CH(CH_3)_3}$	126.3	128.5	127.8
$^1J_{CH(5\text{-}CH_3)}$	127.0	130.9	130.6

(a) 50% conc. HCl.

(b) Complex multiplet, couplings not resolved.

TABLE 46

25MHz ^{13}C n.m.r. spectral data
5-Methyl-4-phenylpyrimidine

Chemical shifts/p.p.m.

Solvent:	$CDCl_3$	T.F.A.	H_2O/HCl[a]
C2	89.47	81.90	81.68
C4	97.82	104.29	103.17
C5	61.15	67.31	66.72
C6	91.64	86.88	85.83
CH_3	-49.87	-50.04	-49.46
C1'	70.78	65.51	65.99
C2'	61.35	62.66	62.28
C3'	61.79	62.66	62.78
C4'	62.34	66.65	65.51

Coupling constants/Hz

Solvent:	$CDCl_3$	T.F.A.	H_2O/HCl[a]
$^1J_{C2H2}$	202.6	220.3	217.9
$^3J_{C2H6}$	10.8	7.6	7.3
$^3J_{C4H2}$	} b	} b	} b
$^3J_{C4H6}$			
$^2J_{C5H6}$	b	b	b
$^1J_{C6H6}$	178.2	191.7	191.6
$^3J_{C6H2}$	9.2	6.3	6.2
$^1J_{CH(5\text{-}CH_3)}$	128.2	130.9	130.9

(a) 50% conc. HCl.
(b) Complex multiplet, couplings not resolved.

TABLE 47

25MHz ^{13}C n.m.r. spectral data

1,5-Dimethylpyrimidinium iodide

Chemical shifts/p.p.m.

Solvent:	D.M.S.O.
C2	84.57
C4	97.17
C5	66.89
C6	85.59
CH_3	-50.75
$N\text{-}CH_3$	-21.20

Coupling constants/Hz

Solvent:	D.M.S.O.	
$^1J_{C2H2}$	220.9	(218.5)
$^3J_{C2H4}$	} a	(11·3)
$^3J_{C2H6}$		(4·9)
$^1J_{C4H4}$	189.8	(188.0)
$^3J_{C4H2}$	} a	} a
$^3J_{C4H6}$		
$^2J_{C5H4}$	} a	} a
$^2J_{C5H6}$		
$^1J_{C6H6}$	192.8	(191.6)
$^3J_{C6H2}$	} a	(~5)
$^3J_{C6H4}$		(~5)
$^1J_{CH(CH_3)}$	130.0	(-)
$^1J_{CH(N\text{-}CH_3)}$	145.3	(-)

Values in parenthesise are those obtained when selectively decoupling N-methyl protons with low power irradiation.

(a) Complex multiplet, couplings not resolved.

TABLE 48

25MHz ^{13}C n.m.r. spectral data
1,5-Dimethyl-4-ethylpyrimidinium iodide

Chemical shifts/p.p.m.

Solvent:	D.M.S.O
C2	84.43
C4	111.44
C5	65.17
C6	83.31
5-CH_3	-50.92
CH_2	-37.84
CH_3	-55.88
N-CH_3	-22.25

Coupling constants/Hz

Solvent:	D.M.S.O.	
$^1J_{C2H2}$	219.1	(216.7)
$^3J_{C2H6}$	a	(4.8)
$^3J_{C6H2}$	} a	} a
$^3J_{C4H6}$		
$^2J_{C5H6}$	a	a
$^1J_{C6H6}$	192.9	(189.8)
$^3J_{C6H2}$	a	(5)
$^1J_{CH(5\text{-}CH_3)}$	129.4	(-)
$^1J_{CH(CH_2)}$	128.2	(-)
$^1J_{CH(CH_3)}$	128.2	(-)
$^1J_{CH(N\text{-}CH_3)}$	145.3	(-)

Values in parenthesise are those obtained when selectively decoupling N-methyl protons with low power irradiation.

(a) Complex multiplet, couplings not resolved.

TABLE 49

25MHz ^{13}C n.m.r. spectral data
1,5-Dimethyl-4-phenylpyrimidinium iodide

Chemical shifts/p.p.m.

Solvent:	D.M.S.O.
C2	84.39
C4	102.83
C5	64.11
C6	86.53
5-CH_3	-48.75
C1'	65.36
C2'	62.35
C3'	63.25
C4'	68.30
N-CH_3	-22.23

Coupling constants/Hz

Solvent:	D.M.S.O	
$^1J_{C2H2}$	220.9	(218.5)
$^3J_{C2H6}$	a	(4.9)
$^3J_{C4H2}$ } $^3J_{C4H6}$	a	a
$^2J_{C5H6}$	a	a
$^1J_{C6H6}$	192.9	(191.0)
$^3J_{C6H2}$	a	(~5)
$^1J_{CH(5\text{-}CH_3)}$	143.4	(-)
$^1J_{CH(N\text{-}CH_3)}$	130.6	(-)

Values in parenthesise are those obtained when selectively decoupling N-methyl protons with low power irradiation.

(a) Complex multiplet, couplings not resolved.

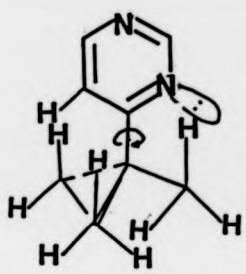
methylpyrimidine and 5-methyl-4-phenylpyrimidine were also in good agreement with the parent system. It was therefore felt appropriate to use the $^{3}JC2H6$ protonation parameters from 1-methylpyrimidinium iodide to estimate the N-1:N-3 protonation ratios in the 4-substituted 5-methyl pyrimidines. Interestingly, it can be seen from the data in Table 28 that the N-1:N-3 protonation ratios become less extreme when the 5-methyl group is introduced into the ring system. We must therefore conclude that the 5-methyl group is indeed affecting the preferred conformation of the 4-substituent.

While this reduction in the N-1:N-3 protonation ratio was to be expected for the case of 5-methyl-4-phenylpyrimidine (see earlier discussions) it is not immediately apparent why this should also occur with the 4-alkyl substituted systems. In particular we have previously argued a case for the 4-tert-butylpyrimidine to suggest that the N-1:N-3 protonation ratio in this case might become more extreme as a result of the introduction of the 5-methyl substituent.

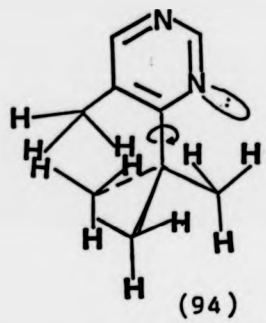
One possible explanation for the reduction in steric hindrance could involve a change in the preferred orientations of the protons on the tert-butyl group. In the absence of the 5-methyl group there are no major barriers to the rotation of the tert-butyl group around the C - C bond to C-4. On the other hand, in the 5-methylated system there is a considerable interaction between the rotating tert-butyl group and the methyl group at C-5. To minimise the interactions it might be argued that the protons on the tert-butyl group would take up

the preferred conformations as shown in (94). If this conformation is still present as the methyl protons of the tert-butyl group rotate past the N-3 position then steric hindrance at N-3 would be reduced from the situation in (93).

A similar line of argument could be applied to the 4-ethyl and 4-isopropyl substituted systems.



(93)



(94)

AN ASSESSMENT OF THE POSSIBLE USE OF $^{13}C - {}^{1}H$ COUPLING CONSTANTS OTHER THAN $^{3}JC2H6$ FOR THE DETERMINATION OF N-1:N-3 PROTONATION RATIOS

(i) One bond $^{13}C - {}^{1}H$ coupling constants (^{1}JCH)

On protonation it was observed that all the ^{1}JCH couplings increased markedly from the non-protonated state. It is interesting to note that in aqueous base all the ^{1}JCH values are slightly larger than the values in $CDCl_3$. This is probably due to H-bonding between the water and the ring nitrogens which would have an effect similar to protonation in that all the ^{1}JCH values would increase but to a much lesser extent. For the unsubstituted pyrimidine system the effects of N-methylation are shown in Table 50. As suspected the

value of all the ^{1}JCH values increased on methylation, the largest effects being observed on the carbon atoms adjacent to the sites of methylation.

TABLE 50

	$CDCl_3$	D.M.S.O.
$^{1}JC2H2$	203.2	220.9
$^{1}JC4H4$	181.0	191.6
$^{1}JC5H5$	168.1	179.4
$^{1}JC6H6$	181.0	195.9

A summary of the $^{1}JC6H6$ values for all the 4-substituted pyrimidines and 4-substituted-5-methyl-pyrimidines studied is given in Table 51.

It is difficult to ascertain whether or not the $^{1}JC4H4$ and $^{1}JC6H6$ from 1-methylpyrimidinium iodide can be considered as parameters for the two extreme protonated species since it was necessary to use D.M.S.O. as the solvent for the methiodide and T.F.A. and water for mono-protonated pyrimidine and it has previously been established that solvent has a considerable effect on the ^{1}JCH parameters. The corrected $^{1}JC6H6$ value for pyrimidine in aqueous acid 194.3 Hz therefore is in reasonably good agreement with the average value of $^{1}JC4H4$ and $^{1}JC6H6$ from 1-methylpyrimidinium iodide 193.7 Hz, particularly when considering the different solvents and the accuracy to which these couplings were determined ($\pm$ 0.3 Hz).

TABLE 51

R	R'		$CDCl_3$	H_2O/OH^-	T.F.A.	H_2O/HCl
H	H	$^1JC2H2$	203.2		220.9	219.1
		$^1JC5H5$	168.1		181.9	180.3
		$^1JC6H6$	181.0		195.3	193.7
Me	H	$^1JC2H2$	202.0	203.8	219.1	217.3
		$^1JC5H5$	165.4	167.8	177.9	177.9
		$^1JC6H6$	180.3	183.1	193.5	191.6
Et	H	$^1JC2H2$	202.0	203.9	219.1	217.3
		$^1JC5H5$	167.3	169.1	177.6	177.0
		$^1JC6H6$	180.7	183.1	193.5	191.7
iPr	H	$^1JC2H2$	201.7	203.2	219.1	217.0
		$^1JC5H5$	164.8	168.5	177.0	176.7
		$^1JC6H6$	180.1	182.8	192.9	191.3
t-But	H	$^1JC2H2$	201.7	202.9	219.7	217.3
		$^1JC5H5$	164.2	168.5	-	-
		$^1JC6H6$	179.7	182.5	-	-
Ph	H	$^1JC2H2$	202.6		219.1	217.3
		$^1JC5H5$	165.4		177·0	176·7
		$^1JC6H6$	180.7		194·7	193·8

TABLE 51 (continued)

R	R'		$CDCl_3$	H_2O/OH^-	T.F.A.	H_2O/HCl
2-methyl-phenyl	H	$^1JC2H2$	202.9		219.4	219.1
		$^1JC5H5$	166.6		177·8	177·6
		$^1JC6H6$	181.0		194·3	193·4
2,4,6-trimethyl-phenyl	H	$^1JC2H2$	202.6		220.0	217.6
		$^1JC5H5$	166.6		179.7	178.5
		$^1JC6H6$	180.9		194.7	193.5
H	Me	$^1JC2H2$	203.9	205.7	220.9	219.7
		$^1JC6H6$	178.8	181.9	191.6	191.0
Me	Me	$^1JC2H2$	201.1		217.6	217.3
		$^1JC6H6$	177.3		189.8	189.8
Et	Me	$^1JC2H2$	201.7	203.5	219.1	217.3
		$^1JC6H6$	177.6	180.3	191.0	189.8
t-But	Me	$^1JC2H2$	201.7		220.0	217.0
		$^1JC6H6$	177.0		190.4	189.8
Ph	Me	$^1JC2H2$	202.6		220.3	217.9
		$^1JC6H6$	178.2		191.7	191.6

The nature of the substituent at C-4 also has an effect on $^{1}JC6H6$. This can be seen by considering the variation of $^{1}JC6H6$ values in $CDCl_3$ (179·7 - 181.0 Hz) for the 4-substituted system (Table 51). Although this difference is relatively small it is a significant variation when considering the difference in the $^{1}JC6H6$ protonation parameters (4.3 Hz) from 1-methylpyrimidinium iodide.

It was not possible therefore, to make any reliable assessment of the N-1:N-3 protonation ratios of the 4-substituted and 4-substituted-5-methylpyrimidines based on ^{1}JCH .

(ii) Two bond ^{13}C - ^{1}H coupling constants (^{2}JCH)

(a) $^{2}JC5H6$

Unfortunately this particular coupling was not well resolved in many of the systems studied due to the presence of additional long-range couplings from the protons of the alkyl substituents at C-4.

For the 4-substituted pyrimidines the value of $^{2}JC5H6$ in deuterochloroform was approximately 8 Hz. However, due to complexity of the C-5 resonance for the 4-substituted 5-methylpyrimidines this coupling was not resolved. In T.F.A. and hydrochloric acid only a few values of $^{2}JC5H6$ were resolved but in general it appeared that this coupling decreases on protonation and this decrease is greater as the percentage N-1 protonation increases.

Assuming that 1-methylpyrimidine was once again a suitable model for the effects of protonation in these systems it was possible to obtain $^{2}JC5H6$ protonation

parameters of 3.7 and 8.0 Hz for N-1 and N-3 protonation respectively. Within the limits of experimental error the average of these values is consistent with the $^{2}JC5H6$ value of 5.5 Hz observed for pyrimidine and 5-methylpyrimidine where the protonation ratio would be 50:50.

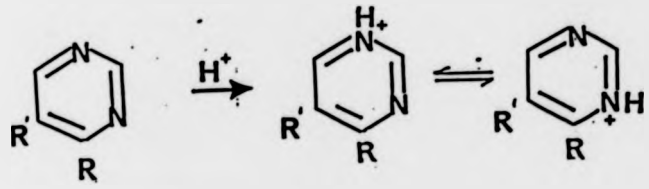
Although $^{2}JC5H6$ does provide a potential means of assessing the N-1:N-3 protonation ratio, for the reasons previously stated it is not easy to measure the $^{2}JC5H6$ couplings in these systems and hence this parameter is not as useful as the $^{3}JC2H6$ coupling in assessing the N-1:N-3 protonation ratio.

(b) $^{2}JC6H5$

The observed values for this coupling in $CDCl_3$, T.F.A. and aqueous base are given in Table 52. It can be seen that a small increase in this coupling is observed on protonation.

Attempts to obtain protonation parameters for this coupling from 1-methylpyrimidinium iodide were unsuccessful due to the complexity of the C-6 resonance However, from a consideration of the N-1 and N-3 methiodides of 4-methylpyrimidine and 4-(2,4,6-trimethylphenyl)pyrimidine it is possible to conclude that $^{2}JC6H5$ increases to approximately 4.7 Hz on protonation of N-1, while protonation at N-3 brings about a modest increase in $^{2}JC6H5$ to give a value in the region of 3 Hz. The $^{2}JC6H5$ coupling therefore appears to be relatively insensitive to changes in the N-1:N-3 protonation ratio.

TABLE 52



R	R'	$^2J_{C6H5}$[a] / Hz		
		$CDCl_3$	T.F.A.	H_2O/HCl[b]
H	H	2.7	3.7	3.7
Me	H	2.8	4.2	4.2
Et	H	2.7	4.2	4.3
i-Pr	H	2.8	4.3	4.3
t-But	H	2.4	-	-
Ph	H	2.4	4·1	4·2
2-methylphenyl	H	2.7	-	-
2,4,6-trimethylphenyl	H	3.0	3.3	3.3

(a) Accurate to ± 0.3 Hz.

(b) Values corrected for incomplete protonation.

Considering the limited accuracy of the couplings measured in the protonated systems (± 0.3 Hz) it can be seen that $^{2}JC6H5$ can only be used to detect major changes in the N-1:N-3 protonation ratio.

(c) $^{2}JC4H5$

Due to the complexity of the C-4 resonance it was not possible to measure this coupling without selectively irradiating the alkyl group protons for each pyrimidine in each of the solvents. In view of the limited use of the $^{2}JC6H5$ coupling this was not considered to be necessary.

(ii) Three bond $^{13}C - {}^{1}H$ couplings constants (^{3}JCH)

(a) $^{3}JC4H2$ and $^{3}JC4H6$

Once again due to the complexity of the C-4 resonance it was not possible to measure these couplings.

(b) $^{3}JC6H2$

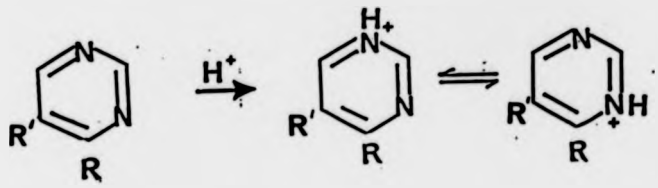
This coupling is of particular interest since like $^{3}JC2H6$ it is a vicinal coupling through the N-1 nitrogen atom. Once again, however, the complexity of the C-6 resonance in 1-methylpyrimidinium iodide prevented us from obtaining $^{3}JC6H2$ protonation parameters. Nevertheless, the other methiodides prepared indicated that the $^{3}JC6H2$ protonation parameters would have values of about 4.7 and 9.4 Hz corresponding to the effects of N-1 and N-3 protonation respectively.

The values of $^{3}JC6H2$ for the pyrimidines studied are given in Table 53, both for the non-protonated species in $COCl_3$ and the mono-protonated species in T.F.A. and aqueous hydrochloric acid. For the two symmetrical pyrimidines, namely pyrimidine and 5-methylpyrimidine, the observed value of $^{3}JC6H2$ in acid (~7 Hz) is in good agreement with the average value of the protonation parameters (4.7 and 9.4 Hz) thus providing support for the use of these parameters. The $^{3}JC6H2$ values observed for the other pyrimidines in acid can, therefore, be interpreted in terms of N-1:N-3 protonation ratios and clearly show the expected increased preference for N-1 protonation with increasing bulk of the 4-substituent. However, the $^{3}JC6H2$ couplings in acid could not be measured to the same degree of accuracy as the $^{3}JC2H6$ couplings and this together with the smaller difference in $^{3}JC6H2$ protonation parameters (4.7 Hz) compared to the difference in $^{3}JC2H6$ protonation parameters (6.5 Hz) inevitably means that this coupling is inferior to the $^{3}JC2H6$ coupling in assessing accurate protonation ratios.

Conclusions

Although in theory it would appear that couplings other than $^{3}JC2H6$ can be used to study the N-1:N-3 protonation ratio in those types of pyrimidine included in our study, in practical terms they offer no advantages over the use of $^{3}JC2H6$. We have found that the values

TABLE 53



R	R	$^3JC6H2$ / Hz[a]		
		$CDCl_3$	T.F.A.	H_2O[b]/HCl
H	H	8.9	7	7.0
Me	H	9.3	6.1	6.7
Et	H	9.0	6.1	6.7
i-Pr	H	9.2	6.0	6.7
t-But	H	9.1	-	-
Ph	H	9.1	4·9	5·2
2-methylphenyl	H	9.1	-	-
2,4,6-trimethylphenyl	H	9.1	7·0	6·7
H	Me	9.2		~7
Me	Me	9.2	6.8	6.7
Et	Me	9.1	6.7	6.7
t-But	Me	9.2	6.7	6.6
Ph	Me	9.1	6.2	6.4

(a) Accurate to ± 0.3 Hz.

(b) Values corrected for incomplete protonation.

of $^{3}JC2H6$ can be determined with a sufficiently high degree of accuracy ($\pm$ 0.2 Hz) to enable the extent of protonation at N-1 to be determined with an accuracy of about $\pm$ 5% .

CHAPTER 4

An assessment of ^{13}C chemical shift parameters to assess the N-1:N-3 protonation ratio of 4-substituted pyrimidines.

We have previously described our investigations of the N-1:N-3 protonation ratios of a number of 4-substituted pyrimidines. Although these studies were primarily concerned with the use of ^{13}C - ^{1}H coupling constants as a means of assessing the protonation ratio, a considerable quantity of chemical shift data was also acquired. Since chemical shift changes resulting from protonation have been used [30] as a means of determining the N-1:N-3 protonation ratios in a number of substituted pyrimidines it was of interest to see if this approach could be used for the pyrimidines included in our study.

^{1}H n.m.r. studies

In general the use of ^{1}H n.m.r. chemical shift data for studying protonation ratios in pyrimidines has not proved to be very satisfactory since only small changes in shift are usually observed on protonation. The protonation ratios derived by this method are thus inevitably imprecise and conclusions are generally of a qualitative nature. The method developed by Gil [28] for 4-phenylpyrimidine serves as a good example for the ^{1}H n.m.r. approach. The observed changes in chemical shifts and couplings constants of 4-phenylpyrimidine on protonation and the analagous changes for pyridine were reported (Table 54). Carbon tetrachloride and T.F.A. were chosen as the solvents for the non-protonated and protonated species respectively. On the basis of the pyridine parameters the expected changes in the ^{1}H

TABLE 54

4-PHENYLPYRIMIDINE

Observed change in chemical shift/p.p.m.		Observed change in ^{1}H-^{1}H coupling constants	
H2	+0.35	$^{4}J_{H2H6}$	+1.14
H5	+0.87	$^{5}J_{H2H5}$	-0.3
H6	+0.36	$^{3}J_{H5H5}$	+1.3

PYRIDINE

Observed change in chemical shift/p.p.m.		Observed change in ^{1}H-^{1}H coupling constants	
H2	+0.27	$^{3}J_{H2H5}$	-1.1
H3	+1.16	$^{4}J_{H2H4}$	-0.3
H4	+1.30	$^{5}J_{H2H5}$	-0.2
		$^{4}J_{H2H6}$	+1.1
		$^{3}J_{H3H4}$	+0.3

CALCULATED CHEMICAL SHIFT CHANGES FOR 4-PHENYLPYRIMIDE

	N-1 Protonation	N-3 Protonation	N-1:N-3 50:50
H2	+0.27	+0·27	+0.27
H5	+1.16	+1.16	+1.16
H6	+0.27	+1.30	+0.79

TABLE 54

4-PHENYLPYRIMIDINE

Observed change in chemical shift/p.p.m.		Observed change in ^1H-^1H coupling constants	
H2	+0.35	$^4J_{H2H6}$	+1.14
H5	+0.87	$^5J_{H2H5}$	-0.3
H6	+0.36	$^3J_{H5H5}$	+1.3

PYRIDINE

Observed change in chemical shift/p.p.m.		Observed change in ^1H-^1H coupling constants	
H2	+0.27	$^3J_{H2H5}$	-1.1
H3	+1.16	$^4J_{H2H4}$	-0.3
H4	+1.30	$^5J_{H2H5}$	-0.2
		$^4J_{H2H6}$	+1.1
		$^3J_{H3H4}$	+0.3

CALCULATED CHEMICAL SHIFT CHANGES FOR 4-PHENYLPYRIMIDE

	N-1 Protonation	N-3 Protonation	N-1:N-3 50:50
H2	+0.27	+0·27	+0.27
H5	+1.16	+1.16	+1.16
H6	+0.27	+1.30	+0.79

chemical shifts of 4-phenylpyrimidine were calculated for N-1 and N-3 protonation and the case where a 50:50 ratio exists (Table 54). From a comparison of this data it was concluded that protonation essentially occured at N-1. The close similarity of the J values for both 4-phenylpyrimidine and pyridine was considered to be corroborative evidence that N-1 was the site of protonation.

^{13}C n.m.r. studies

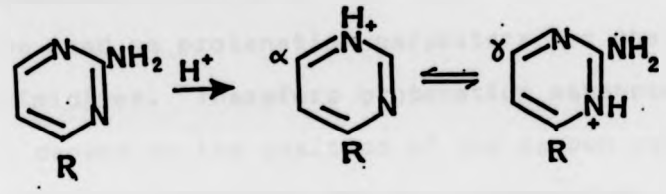
In contrast to the ^{1}H n.m.r. studies the much larger protonation shifts observed in ^{13}C n.m.r spectroscopy can potentially offer a more accurate means of determining the protonation ratios. We have therefore attempted to assess the suitability of using ^{13}C n.m.r. chemical shift data for determining the N-1:N-3 protonation ratio of the pyrimidines included in this study.

Before considering this work, however, it is important to note that ^{13}C n.m.r. chemical shifts are sensitive to solvent and concentration changes. During this study therefore efforts were made to minimise these effects. Samples were prepared at the same concentrations and where possible comparisons between compounds were made between spectra of the protonated and non-protonated species in the same solvents. The same reference compound 1,4-dioxan could be used for all the solvents and since it had previously been reported that the chemical shift of 1,4-dioxan was relatively insensitive to solvent effects[30] a possible source of error was eliminated.

The 2-aminopyrimidines and the alkyl/aryl pyrimidines required separate treatment of these results. Since the 2-aminopyrimidines were the first to be prepared this system will be considered first.

(i) <u>2-Aminopyrimidines with substituents at C-4</u>

Let us consider a 4-substituted 2-amino-pyrimidine (95) where on protonation X% of the molecules are protonated at N-1 (96) and (100-X)% of the molecules are protonated at N-3 (97).



(95) X% (96) (100-X) % (97)

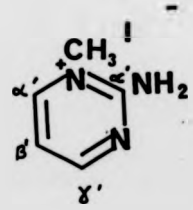
Let us assume that the change in chemical shift on protonation ($\Delta\delta$) of a carbon atom in the pyrimidine ring depends both on the position of the carbon atom relative to the site of protonation and also the nature of the substituent on that carbon (R). These parameters are given the notation α_R, β_R and γ_R. It can be seen therefore that the chemical shift change of C-6 will depend on α_H, γ_H and X. If the α_H and γ_H parameters can be determined the it is possible to use the expression below to calculate X (% N-1 protonation).

$$\Delta\delta \text{ C-6} = \frac{X\alpha_H + (100-X)\gamma_H}{100}$$

Unfortunately the α_H and γ_H for the two protonated forms (96) and (97) cannot be determined directly since

on the n.m.r. time scale only an average spectrum is observed. In order to obtain these values it is necessary to consider the symmetrical pyrimidine (95, R = H) where it is known that X = 50%. For 2-amino-pyrimidine the observed chemical shift change for C-6 on protonation was-0.42 p.p.m. This is significantly different from the chemical shift change of C-6 on protonation of pyrimidine (+1.21 p.p.m.). It is clear therefore that the α_H and γ_H parameters determined from pyrimidine (-7.16 and +9.79 p.p.m. respectively) cannot be used as protonation parameters for the 2-aminopyrimidines. Therefore protonation parameters not only depend on the position of the carbon relative to the site of protonation and the nature of the substituent on that carbon but also on the nature of other substituents on the ring. Previous workers have suggested that substituents which are capable of conjugating with the ring have a considerable impact on the protonation parameters [30].

It is interesting to note that for 2-amino-1-methyl-pyrimidinium iodide (98) methylation has a similar effect on both the C-2 and C-6 chemical shifts (-6.78 and-6.80 p.p.m. respectively).



(98)

Thus if the methylation parameters α'_H and γ'_{NH2} are very similar it is not an unreasonable assumption that the protonation parameters α_H and γ_{NH_2} are similar. For 2-aminopyrimidine therefore it is possible to suggest the α_H and γ_H protonation parameters would be -7.40 and +6.54 p.p.m. respectively. It is interesting to note that the α_H parameter is very similar to that found for pyrimidine but the γ_H parameter is significantly different.

2-Aminopyrimidines substituted at C-4

For 2-amino-4-methylpyrimidine (95, R = Me) the observed change in chemical shift of C-2 was -7.22 p.p.m. which is in reasonable agreement with 2-aminopyrimidine (95, R = H) It is possible to suggest that the α_H and γ_H parameters derived from the latter can be used to assess the protonation ratio in the 2-amino-4-methylpyrimidine system. The observed change in chemical shift of C-6 in 2-amino-4-methylpyrimidine (+4.33 p.p.m.) can thus be interpreted as indicating a N-1:N-3 protonation ratio of 78:22. This result is much more extreme than would be expected and thus suggests that the assumptions made in determining the protonation parameters may not be valid.

As was previously discussed the introduction of groups capable of conjugating with the pyrimidine ring can have considerable effect on the chemical shift protonation parameters. Thus it is clear that for pyrimidines with substituents at C-4 capable of exerting such effects a reliable assessment of the protonation ratio cannot be made using the previously derived α_H and γ_H parameters.

For example the observed change in the C-6 chemical shift for 2,4-diaminopyrimidine is -13.95 p.p.m. Clearly this far exceeds the α_H parameter (-7.40 p.p.m.) derived from 2-aminopyrimidine. Similarly for the other 2-aminopyrimidines studied the effects of conjugation made it impossible to make an estimate of the protonation ratio.

(ii) 4-Alkyl and 4-aryl substituted pyrimidines

For pyrimidines bearing only an alkyl or aryl substituent on the 4 position of the ring the assumptions made to establish the protonation parameters are fewer. Namely, that the α_H value for C-2 is equal to the α_H value for C-6. Thus the α_H and γ_H parameters for this system were assessed as -7.16 and +9.98 p.p.m. From Table 55 it can be seen that the choice of solvents for the non-protonated and protonated pyrimidines is particularly important in determining the protonation parameters. As can be seen the chemical shifts in aqueous base show characteristic shifts which are similar to those for protonation but of a smaller magnitude. These effects can be attributed to H-bonding of the nitrogen atoms to the water in aqueous base. Similar effects were noted for ^1JCH values which were also consistent with H-bonding in aqueous base.

The α_H and γ_H parameters derived by ourselves are in excellent agreement with the reported values by Pugmire and Grant[29] (-7.30 and +9.95 p.p.m. respectively) but significantly different from the values determined by Riand (-6.1 and 7.3 p.p.m. respectively). Riand [30]

TABLE 55

^{13}C chemical shifts of pyrimidine in various solvents

	$CDCl_3$	H_2O/OH^-	T.F.A.	H_2O/HCl[a]
C2	91.72	90.8	84.74	84.56
C4,6	89.78	90.60	91.24	91.19
C5	54.61	55.80	57.63	57.81

PROTONATION PARAMETERS/P.P.M.

-6.98	-7.16
+3.02	+3.20
+9.70	+9.98

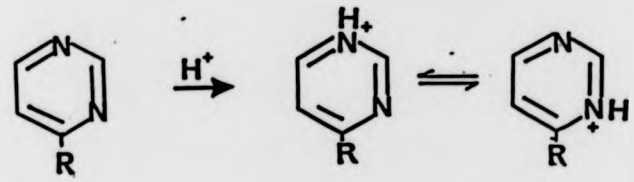
(a) Corrected for incomplete protonation.

suggested the difference between their values and those of Pugmire and Grant were due to the difference in solvent and the choice of reference. As can be seen from Table 55 Riand's values are consistent with the protonation parameters derived from aqueous acid and aqueous base. Since as previously explained H-bonding occurs in aqueous base the values used by Rianddo not take this factor into account.

The observed changes in the C-6 chemical shift of the 4-alkyl and aryl pyrimidines studied and the calculated N-1:N-3 protonation ratios are given in Table 56. In both T.F.A. and aqueous acid these ratios appear to be much more extreme than would be expected. In the case of 4-phenylpyrimidine the calculated percentage N-1 protonation exceeds 100%. As previously discussed substituents capable of conjugating with the pyrimidine ring can affect the protonation parameters therefore this value is unreliable.

It is possible that alkyl substituents on the pyrimidine ring may also affect the protonation parameters. It is interesting for this purpose to compare 5-methyl-pyrimidine. The α_H and γ_H parameters for this pyrimidine were determined as -6.72 and +8.46 p.p.m. respectively. Although the α_H parameter is once again very similar to that determined for pyrimidine (7.16 p.p.m.), the γ_H parameter is 1.5 Hz smaller than for pyrimidine (9.98 p.p.m.). Therefore it is likely that a methyl group in the 4 position of the ring will also affect the protonation parameters. Furthermore, other alkyl groups capable of

TABLE 56



R	$CDCl_3$	T.F.A.	N-1:N-3	H_2O/HCl[a]	N-1:N-3
Me	89.24	86.64	76:24	88.12	65:35
Et	89.57	86.05	80:20	81.32	71:29
i-Pr	89.61	85.03	87:13	86.47	77:23
t-But	89.61	83.54	96:40	84.11	90:10
Ph	90.17	81.38	112:	81.90	106:
2-methylphenyl	89.56	83.07	98:2	83.30	95:5
2,4,6-trimethyl-phenyl	89.72	88.56	66:34	86.28	78:22

PROTONATION PARAMETERS/P.P.M.

-6·78	-7·16
+3·02	+3·20
+9·70	+9·98

(a) Corrected for incomplete protonation.

exerting a stronger +I effect may affect the protonation parameters to a greater extent.

We therefore conclude that we can have little confidence in the protonation ratios determined by the chemical shift method although the values suggest a trend which is consistent with the method based on the $^{3}JC2H6$ coupling.

The chemical shift method involves making a number of assumptions which are difficult to assess. Furthermore, since chemical shifts are known to be sensitive to concentrations, solvent and choice of reference compound, there are a number of sources of potential error. In contrast to this our method based on the $^{3}JC2H6$ coupling constant is very much more readily assessed since it is based on only one assumption, namely that the effects of protonation and methylation are similar. Evidence to support the latter assumption has already been discussed.

We feel therefore that more confidence can be placed in those N-1:N-3 protonation ratios determined by the coupling constant approach than in the chemical shift approach with its inherent assumptions and complexities.

EXPERIMENTAL

2-AMINOPYRIMIDINE

This was available from Sigma Chemicals and was used for ^{1}H and ^{13}C n.m.r. spectroscopy without further purification, m.p. 126-8°C.

δ ^{1}H ($CDCl_3$), 6.51 (1H, t, 5Hz), 8.19 (2H, d, 5Hz).

δ ^{1}H (T.F.A.), 7.15 (1H, t, 6Hz), 8.68 (2H, d, 6Hz).

2-AMINO-1-METHYLPYRIMIDINIUM IODIDE

2-Aminopyrimidine (2g) was dissolved in a minimum amount of acetone in a small conical flask. Iodomethane (10g) was added, the flask stoppered and stored for 3 days. The resulting white needles (43g, 86%) were filtered off and recrystallised from ethanol to give the desired product, m.p. 255-6°C (. decomposes). Microanalysis found: C, 25·14; H, 3·38; N, 17·87%. Calc. for $C_5H_8N_3I$: C, 25·33; H, 3·40; N, 17·73%.

δ ^{1}H (D_2O), 3.95 (3H, s), 7.18 (1H, d of d, 4, 6Hz), 8.45 (1H, d of d, 3, 6Hz), 8.85 (1H, d of d, 3, 4Hz)

2-AMINO-4-METHYLPYRIMIDINE

This was available from Sigma Chemicals and was used without further purification, m.p. 150-60°C.

δ ^{1}H ($CDCl_3$), 2.30 (3H, s), 6.36 (1H, d, 5Hz), 8.01 (1H, d, 5Hz)

δ ^{1}H (T.F.A.), 2.71 (3H, s), 6.99 (1H, d, 6Hz), 8.37 (1H, d, 6Hz)

2-AMINO-1,4-DIMETHYLPYRIMIDINIUM IODIDE

To a solution of 2-amino-4-methylpyrimidine (2g) dissolved in a minimum of acetone was added iodomethane (10g) and the mixture was stored in a stoppered flask for 3 days.

The resultant cream crystals were filtered off and recrystallised from ethanol to give the desired product (4·2g, 91%), m.p. 258-9°C.

Microanalysis found: C, 28·68; H, 4·01; N, 16·87%.

Calc. for $C_6H_{10}N_3I$: C, 28·70; H, 4·01; N, 16·74%.

δ ^1H (D_2O), 2.60 (3H, s), 3.83 (3H, s), 7.01 (1H, d, 6Hz) 8.26 (1H,d,6Hz)

2-AMINO-6-METHYL-4(3H)-PYRIMIDINONE

Ethyl acetoacetate (43g) was added dropwise over 1 hour to a mechanically stirred mixture of guanidine carbonate (20g), potassium hydroxide (37g) and water (100ml). The solution was then heated under reflux for 3 hours, cooled and then concentrated hydrochloric acid was added until the solution had a pH of 5. The resultant white precipitate of 2-amino-4-methyl-4(3H)-pyrimidinone was filtered off and dried (25g, 90%), m.p. 298-300°C (Lit. m.p. 297°C[49]). δ ^1H (D_2O), 3·20 (3H, s), 5·90 (1H, s).

2-AMINO-4-CHLORO-6-METHYLPYRIMIDINE

Phosphoryl chloride (50ml) was added to 2-amino-6-methyl-4(3H)-pyrimidinone (25g) and the mixture was heated in an oil bath maintained at 100°C for 3 hours. The excess phosphoryl chloride was removed under reduced pressure and the residue carefully poured into ice. The resultant solution was carefully neutralised with sodium hydroxide solution (30%) whilst keeping the temperature below 60°C. After cooling, the precipitate of 2-amino-4-chloro-6-methylpyrimidine was filtered off and recrystallised

from ethanol (20g, 70%), m.p. 184-6°C (Lit. m.p. 186°C[50]). Microanalysis found: C, 41·70; H, 4·00; N, 29·05%. Calc. for $C_5H_6N_3Cl$: C, 41·83; H, 4·21; N, 29·27%.

2-AMINO-4-CHLORO-1.6-DIMETHYLPYRIMIDINUM METHOSULPHATE

2-Amino-4-chloro-6-methylpyrimidine (10g), nitrobenzene (15ml) and methyl sulphate (7ml) were heated to 75-80°C using a thermostatically controlled oil bath. The solid gradually dissolved over a period of one hour and then the mixture was heated for a further 30 minutes during which time a cream precipitate of 2-amino-4-chloro-1,4-dimethyl-pyrimidinium methosulphate began to form.. After cooling, the product was filtered off under suction and then washed with portions of ice cold acetone until the product (7g, 34%) was white and powdery, m.p. 145-153°C (Lit. m.p 155-7°C[37] δ^1H (D_2O), 2·72 (3H, s), 3·77 (6H, s), 7·15 (1H, s). A small quantity (<5%) of the isomeric 2-amino-4-chloro-3,6-dimethylpyrimidinium methosulphate was also present. δ ^{1}H(D_2O), 2·61 (3Hs), 3·96 (6H, s), 7·26 (1H, s).

2-AMINO-4-CHLORO-1.6-DIMETHYLPYRIMIDINIUM IODIDE

2-amino-6-chloro-1,6-dimethylpyrimidinium methosulphate (7g) was dissolved in water (5ml) and treated with a molar equivalent of saturated sodium iodide solution. A white precipitate of 2-amino-4-chloro-1,6-dimethylpyrimidinium iodide formed immediately. This was filtered off and recrystallised from ethanol (6g, 90%), m.p. 260-2°C (Lit. m.p. 261-2°C[37]). δ ^{1}H(D_2O), 2·63(3H, s), 3·75(3H, s), 7·25(1H, s).

CATALYTIC REDUCTION OF 2-AMINO-4-CHLORO-6-METHYLPYRIMIDINE WITH 10% PALLADIUM ON CHARCOAL

A mixture of 2-amino-4-chloro-6-methylpyrimidine (2g), magnesium oxide (2g), 10% palladium on charcoal (0·2g) and ethanol (25ml) was hydrogenated with vigerous stirring at atmospheric pressure. The uptake of hydrogen was recorded at regular intervals. After 45 minutes the uptake ceased and 323ml of hydrogen had been used. The solution was filtered through celite filter aid and the filtrate was evaporated to dryness under reduced pressure. ^{1}H n.m.r. spectroscopy showed the product to be 2-amino-4-methylpyrimidine (1·5g, 98%). δ ^{1}H($CDCl_3$), 2·18 (3H, s), 6·35 (1H, d, 6Hz), 7·97 (1H, d, 6Hz). This was confirmed by comparison with an authentic sample.

CATALYTIC REDUCTIONS OF 2-AMINO-4-CHLORO-1,6-DIMETHYLPYRIMIDINIUM METHOSULPHATE

(i) 10% Palladium on charcoal

A mixture of 2-amino-4-chloro-1,6-dimethylpyrimidinium methosulphate (1g), 10% palladium on charcoal (0·2g) and water (20ml) was hydrogenated with vigorous stirring at atmospheric pressure. The uptake of hydrogen continued at a steady rate for 1½ hours and 515ml hydrogen had been used. The solution was filtered through celite and the filtrate evaporated to dryness under reduced pressure. The ^{1}H n.m.r. spectrum was consistent with a tetra-hydro derivative of 2-amino-3,4-dimethylpyrimidinium methosulphate. δ ^{1}H(D_2O), 1·0 (3H, d, 7Hz), 1·6 (2H, broad multiplet), 2·8 (1H, broad multiplet), 2·9 (3H, s), 3·1 (2H, t, 8Hz), 3·8 (3H, s).

The experiment was repeated but the uptake of hydrogen was stopped after 170ml had been taken up. ^1H n.m.r. spectroscopy showed the product to be a mixture of starting material (~60%), 2-amino-1,4-dimethyl pyrimidinum methosulphate (~10%) and the tetra-hydro derivative of the latter (~30%).

(ii) Raney Nickel

A mixture of 2-amino-4-chloro-1,6-dimethylpyrimidinum methosulphate (2g), freshly prepared Raney nickel (0·5g) and water was hydrogenated at atmospheric pressure. The uptake of hydrogen continued for 2 hours and 495ml of hydrogen was taken up. The solution was filtered through celite and the solvent was removed under reduced pressure. The ^1H n.m.r. spectrum was very broad but indicated the tetra-hydro derivative of 2-amino-3,4-dimethylpyrimidinium methosulphate.

(iii) Lindlar's catalyst

A mixture of 2-amino-4-chloro-1,6-dimethylpyrimidinium methosulphate (2g), Lindlar's catalyst (0·2g) and water (25ml) was hydrogenated at atmospheric pressure. The uptake of hydrogen ceased after 1 hour when 352ml of hydrogen had been taken up. ^1H n.m.r. spectroscopy showed the product to be starting material (~20%), 2-amino-3,4-dimethylpyrimidinum methosulphate (~30%) and the tetra-hydro derivative of the latter (~50%).

(iv) Copper Chromite

A mixture of 2-amino-4-chloro-1,6-dimethylpyrimidinium methosulphate (2g), copper chromite (0·5g) and water (25ml) was hydrogenated at atmospheric pressure. After 4 hours no hydrogen had been taken up. The solution was

filtered through celite and the solvent removed under reduced pressure. ^{1}H n.m.r. spectroscopy showed the product to be a mixture of starting material (~60%) and 2-amino-3,4-dimethyl-6-hydroxypyrimidinum methosulphate (~40%). δ ^{1}H (D_2O), 2·4 (3H, s), 3·6 (3H, s), 3·8 (3H, s), 5·9 (1H, s).

CATALYTIC REDUCTION OF 2-AMINO-4-CHLORO-6-METHYLPYRIMIDINIUM IODIDE WITH 10% PALLADIUM ON CHARCOAL

A mixture of 2-amino-4-chloro-1,6-dimethyl pyrimidinium iodide, 10% palladium on charcoal (0·2g) and water (25ml) was hydrogenated at atmospheric pressure. The uptake of hydrogen was stopped after 48 hours when 160ml of hydrogen had been taken up. The solution was filtered through celite and the solvent removed under reduced pressure. ^{1}H n.m.r. spectroscopy showed the product to be a mixture of 2-amino-3,4-dimethylpyrimidinium iodide (~40%), the tetra-hydro derivative (~20%), 2-amino-3,4-dimethyl-6-hydroxypyrimidinium iodide (~20%) and other decomposition products.

REACTION OF 2-AMINO-4-CHLORO-1,6-DIMETHYLPYRIMIDINIUM METHOSULPHATE WITH ZINC AND BOILING WATER

2-Amino-4-chloro-1,6-dimethylpyrimidinium methosulphate (5g), zinc powder (5g) and water (50ml) were heated under reflux for ½ hour . The solution was filtered whilst hot and the filtrate evaporated to dryness under reduced pressure. ^{1}H n.m.r. spectroscopy showed that the product was a mixture of 2-amino-3,4-dimethylpyrimidinium methosulphate (~55%) and 2-amino-3,4-dimethyl-6-hydroxy-

pyrimidinium methosulphate (~45%).

REACTION OF 2-AMINO-4-CHLORO-1,6-DIMETHYLPYRIMIDINIUM IODIDE WITH ZINC AND BOILING WATER

Zinc powder (5g) and water (50ml) were heated to boiling under reflux. 2-Amino-4-chloro-1,6-dimethylpyrimidinium iodide (6g) was quickly added and the mixture boiled for precisely 5 minutes. The hot solution was quickly filtered and then the water was removed from the filtrate under reduced pressure. The yellow/brown residue (4·3g) was shown by ^{1}H n.m.r. spectroscopy to consist of mainly 2-amino-3,4-dimethylpyrimidinium iodide (~90%). The product was purified by chromatography on a cellulose column using methanol as elutant. Although 2-amino-3,4-dimethylpyrimidinium iodide was obtained as a cream crystalline solid on removing the methanol under reduced pressure, it was found to be extremely hygroscopic and changed to an oil on exposure to the atmosphere. Furthermore, in the moist state decomposition began to occur. For these reasons it was not possible to obtain a satisfactory elemental analysis. However, ^{1}H and ^{13}C n.m.r. spectroscopy unambiguously identified the product.

δ ^{1}H (D_2O), 2.70 (3H, s), 3.81 (3H, s), 7.12 (1H, d, 5Hz), 8.64 (1H, d, 5Hz)

2-AMINO-4-PHENYLPYRIMIDINE

Bromobenzene (16ml) was added dropwise to lithium shot (2·1g) in dry ether (100ml) so as to maintain a gentle reflux. The mixture was then heated under reflux until all the lithium had dissolved (1-1½hours). Toluene (200ml) was added and the ether distilled out of the reaction mixture. 2-Aminopyrimidine (95g) dissolved in hot toluene (500ml) was quickly added and the mixture heated under reflux for 20 hours. The reaction mixture was then cooled in ice and hydrochloric acid solution (400ml, 50%) was cautiously added. The aqueous extract was separated from the organic layer and the pH adjusted to 9 with sodium hydroxide solution (30%). The light tan precipitate (7g) was filtered off and recrystallised from ethanol to give an off-white precipitate of 2-amino-4-phenylpyrimidine (4·5g, 26%), m.p. 162-4°C (Lit. m.p. 162-4°C [4]). Microanalysis found: C, 70·05; H, 5·22; N, 24·54%. Calc. for $C_{10}H_9N_3$: C, 70·16; H, 5·30; N, 24·54%.

δ ^{1}H ($CDCl_3$), 7.13 (1H, d, 5Hz), 7.5 - 7.7, 8.0 - 8.3 (5H, complex), 8.45 (1H, d, 5Hz)

δ ^{1}H (T.F.A.), 7.28 - 8.20 (complex envelope of signals)

2-AMINO-1-METHYL-4-PHENYLPYRIMIDINIUM IODIDE

2-Amino-4-phenylpyrimidine (1g), dry iodomethane (5g) and ethanol (10ml) were heated under reflux for 4 hours. After cooling, the precipitated product was filtered off and recrystallised from ethanol to give off-white leaflets of 2-amino-1-methyl-4-phenylpyrimidinium iodide (1·2g, 66%), m.p. 254-5°C. Microanalysis found: C, 41·96; H, 3·78; N, 13·42%. $C_{11}H_{12}N_3I$ requires: C, 42·19; H, 3·86; N, 13·42%.

δ ^{1}H (d_6D.M.S.O.), 3.88 (3H, s), 7.5 - 8.4 (complex envelope of signals)

2-AMINO-4-(3H)-PYRIMIDINONE

Dry methanol (50ml) was added to a 100ml round bottomed flask fitted with a reflux condenser and drying tube. Small pieces of sodium (12g in all) were gradually added to the methanol so that a gentle reflux occured. The mixture was then heated under reflux until the last remnants of sodium dissolved. The excess methanol was removed under reduced pressure to yield dry powdery sodium methoxide (27g). This was added to a mixture of ethyl acetate (49ml) and methyl formate (31ml) contained in a P.T.F.E.-lined autoclave which was cooled in a crushed ice and salt bath. The autoclave was quickly sealed and heated to 60°C over a period of 1 hour during which time the contents were efficiently stirred. The stirring was continued and the mixture was maintained at 60°C for a further 3 hours. When cool, the contents of the autoclave were discharged into a slurry of guanidine carbonate (45g) and sodium hydroxide (20g) in crushed ice and water. The mixture was then heated under reflux for 3 hours. The resultant solution was neutralised with concentrated hydrochloric acid and the white precipitate filtered off. Recrystallisation from water and treatment with decolourising charcoal gave 2-amino-4(3H)-pyrimidinone (30g, 54%), m.p. 265-7°C (Lit. m.p. 280°C[14]). δ ^{1}H (D.M.S.O.), 5·60 (1H, d, 7Hz), 6-7 (2H, b.s.), 7·70 (1H, d, 7Hz).

2-AMINO-4-CHLOROPYRIMIDINE

2-Amino-4(3H)-pyrimidinone (10g) and phosphoryl chloride (50ml) were heated together at 100°C for 4 hours. After cooling, the excess phosphoryl chloride was removed under reduced pressure and the residue cautiously poured onto ice. The resultant solution was neutralised with sodium hydroxide solution (30%) whilst cooling in an ice bath. The light yellow precipitate which formed was filtered off and recrystallised from ethanol to give 2-amino-4-chloropyrimidine (8g, 68%). A precise melting point was not observed but the product became increasingly dark in colour above 140°C (Lit. decomposes at 168°C [14]). Microanalysis found: C, 36·94; H, 3·05; N, 32·48%. Calc. for $C_4H_4N_3Cl$: C, 37·08; H, 3·11; N, 32·44%.

δ ^1H (d_6D.M.S.O.), 6.65 (1H, d, 6Hz), 8.22 (1H, d, 6Hz)

δ ^1H (T.F.A.), 6.99 (1H, d, 6Hz), 8.15 (1H, d, 6Hz)

2-AMINO-4-METHOXYPYRIMIDINE

Small pieces of sodium (0·35g in all) were added to dry methanol (25ml) in a 50ml round bottomed flask fitted with a reflux condenser and drying tube. When all the sodium had dissolved 2-amino-4-chloropyrimidine (2g) was added and the mixture heated under reflux for 2 hours. After cooling, the solution was filtered and the filtrate evaporated to dryness under reduced pressure. The residue was recrystallised from water to give white prisms of 2-amino-4-methoxypyrimidine (1·5g, 77%) m.p. 119-21°C (Lit. m.p. 125-6°C [14]). Microanalysis found: C, 47·82; H, 5·67; N, 33·87%. Calc. for $C_5H_7N_3O$: C, 47·99; H, 5·64; N, 33·58%.

δ ^{1}H ($CDCl_3$), 3.86 (3H, s), 6.07 (1H, d, 6Hz), 8.01 (1H, d, 6Hz)

δ ^{1}H (T.F.A.), 3.71 (3H, s), 6.49 (1H, d 6Hz) 8.32 (1H, d, 6Hz)

2,4-DIAMINOPYRIMIDINE

Ammonia was bubbled through ethanol (100ml), contained in a P.T.F.E.-lined autoclave, until the solution was saturated. 2,4-dichloropyrimidine (5g) was added and the autoclave sealed. The mixture was heated to 180°C for 20 hours. After cooling, sodium hydroxide solution (33ml, 2M) was added and then the resultant solution evaporated to dryness under reduced pressure. The residue was boiled with 1,4-dioxan (25ml). The insoluble inorganic salts were filtered from the hot solvent and the filtrate was allowed to cool. The resultant white crystals of 2,4-diaminopyrimidine were filtered off and dried (2·5g, 68%), m.p. 149-50°C (Lit. m.p. 146°C[51]) Microanalysis found: C, 43·35; H, 5·44; N, 51·08%. Calc. for $C_4H_6N_4$: C, 43·63; H, 5·49; N, 50·88%.

δ ^{1}H (D_2O/OH^-), 5.92 (1H, d, 6Hz), 7.64 (1H, d, 6Hz)

δ ^{1}H (T.F.A.), 6.09 (1H, d, 7Hz), 7.09 (1H, d, 7Hz)

2,4-DIAMINO-1-METHYLPYRIMIDINIUM IODIDE

2,4-diaminopyrimidine (1g), iodomethane (5g) and ethanol (5ml) were heated under reflux for one hour. The resultant precipitate was filtered off and recrystallised from ethanol/ethyl acetate to give 2,4-diamino-1-methylpyrimidinium iodide (1·6g, 70%), m.p. 273-4°C (Lit. m.p. 274°C[39]). Microanalysis found: C, 24·01; H, 3·47; N, 22·39%. Calc. for $C_5H_9N_4I$: C, 23·83; H, 3·60; N, 22·23%.

δ ^1H (D_2O), 3.57 (3H, s), 6.20 (1H, 6.5Hz), 7.63 (1H, d, 6.5Hz)

2,4-DIAMINO-6-CHLOROPYRIMIDINE

2,4,6-Trichloropyrimidine (10g) was heated in a P.T.F.E.lined-autoclave at 140°C for 18 hours with ethanol (100ml) saturated with ammonia. After cooling, the solvent was removed under reduced pressure and the residue recrystallised from water to give 2,4-diamino-6-chloropyrimidine (7g, 88%), m.p. 197-8°C (Lit. m.p. 196·5-197·5°C[52]). Microanalysis found: C, 32·95; H, 3·47; N, 38·61%. Calc. for $C_4H_5N_4Cl$: C, 33·23; H, 3·49; N, 38·76%.

2,4-DIAMINO-6-CHLORO-1-METHYLPYRIMIDINIUM IODIDE AND
2,4-DIAMINO-6-CHLORO-3-METHYLPYRIMIDINIUM IODIDE

2,4-Diamino-6-chloropyrimidine (5g), iodomethane (25g) and ethanol (50ml) were heated under reflux for 24 hours. After cooling, the cream precipitate was filtered off, washed with a little cold ethanol and dried. ^1H n.m.r. spectroscopy showed the product (7g, 71%) to be an isomeric mixture of 2,4-diamino-6-chloro-1-methylpyrimidinium iodide (70%). δ ^1H (D.M.S.O.), 3·55 (3H, s), 6·39 (1H, s) and 2,4-diamino-6-chloro-3-methylpyrimidinium iodide (30%). δ ^1H (D.M.S.O.), 3·43 (3H, s), 6·23 (1H, s).

2,4-DIAMINO-3-METHYLPYRIMIDINIUM IODIDE

A mixture of 2,4-diamino-6-chloro-1-methylpyrimidinium iodide and 2,4-diamino-6-chloro-3-methylpyrimidinium iodide (5g) was added to a boiling mixture of zinc powder (10g) and water (50ml) and then heated under reflux for 2 hours.

The solution was then filtered under vacuum whilst hot and the filtrate evaporated to dryness under reduced pressure. Several fractional crystallisations of the residue from water enabled a less soluble component to be isolated which was shown to be 2,4-diamino-3-methylpyrimidinium iodide (1·7g), m.p. 258-9°C. Microanalysis found: C, 23·89; H, 3·50; N, 22·31%. $C_5H_9N_4I$ requires: C, 23·83; H, 3·60; N, 22·23%. Evaporation of the aqueous filtrate gave 2,4-diamino-1-methylpyrimidinium iodide (3·9g) which was identified by comparison with an authentic sample, m.p. 273-4°C.

δ ^{1}H (D_2O), 3.55 (3H, s), 6.32 (1H, d, 6Hz), 7.90 (1H, d, 6Hz)

2-AMINO-4-DIMETHYLAMINOPYRIMIDINE

2-Amino-4-chloropyrimidine (5g), dimethylamine (7g) and ethanol (50ml) were heated in a P.T.F.E. autoclave at 160°C for 18 hours. After cooling, sodium hydroxide solution (20ml, 2M) was added and the solution evaporated to dryness under reduced pressure. The residue was boiled with ethyl acetate, decolourised with charcoal and then filtered whilst still hot. On cooling the filtrate white glistening needles of 2-amino-4-dimethylaminopyrimidine formed (3·9g, 73%), m.p. 156-7°C (Lit. m.p. 156°C[53]). Microanalysis found: C, 51·88; H, 7·43; N, 40·52%. Calc. for $C_6H_{10}N_4$: C, 52·16; H, 7·30; N, 40·55%.

δ ^{1}H ($CDCl_3$), 3.02 (3H, s), 5.84 (1H, d, 6Hz), 7.82 (1H,d, 6Hz)

δ ^{1}H (T.F.A.), 3.40 (6H, s), 6.47 (1H, d, 7Hz), 7.66 (1H, d, 7Hz)

2-AMINO-4-DIMETHYLAMINO-1-METHYLPYRIMIDINIUM IODIDE

2-Amino-4-dimethylaminopyrimidine (1g), iodomethane (5g) and ethanol (10ml) were heated under reflux for 4 hours. After cooling, the light yellow precipitate was filtered off and recrystallised from ethanol to give white crystals of 2-amino-4-dimethylamino-1-methylpyrimidinium iodide (1·3g, 65%), m.p. 283-4°C. Microanalysis found: C, 30·04; H, 4·67; N, 20·09%. $C_7N_4H_{13}I$ requires: C, 30·01; H, 4·68; N, 20·00%.

δ ^1H (D.M.S.O.), 3.17 (3H, s), 3.28 (3H, s), 3.47 (3H, s), 6.44 (1H, d, 7Hz), 7.84 (1H, d, 7Hz)

2-AMINO-4-(4-METHOXYPHENYLAMINO)PYRIMIDINE

2-Amino-4-chloropyrimidine (5g), 4-methoxyaniline (15g) and ethanol (100ml) were heated to 180°C for 18 hours in an autoclave. After cooling, sodium hydroxide solution (20ml, 2M) was added to the deep purple solution and then the solvent was removed under reduced pressure. The excess 4-methoxyaniline was dissolved from the residue with cold ether. The remaining solid was dissolved in hydrochloric acid solution (2M) then heated to boiling and neutralised with sodium hydroxide solution (2M). After cooling, the resultant purple precipitate was filtered off and dried to give 2-amino-4-(4-methoxyphenylamino)pyrimidine (6·2g, 74%), m.p. 142-3°C. Microanalysis found: C, 60·94; H, 5·53; N, 26·09%. $C_{11}H_{12}N_4O$ requires: C, 61·10; H, 5·59; N, 25·91%.

δ ^1H ($CDCl_3$), 3.70 (3H, s), 5.89 (1H, d, 6Hz), 6.78 (2H, d, 8Hz), 7.42 (2H, d, 8Hz), 7.61 (1H, d, 6Hz)

2-AMINO-4-(4-METHOXYPHENYLAMINO)-1-METHYLPYRIMIDINIUM IODIDE

2-Amino-4-(4-methoxyphenylamino)pyrimidine (1g), iodomethane (5g) and ethanol (10ml) were heated under reflux for 4 hours. On cooling, silver grey crystals precipitated from the black solution. Recrystallisation from ethanol gave 2-amino-4-(4-methoxyphenylamino)pyrimidinium iodide (0·9g, 54%), m.p. 238-9°C. Microanalysis found: C, 30·04; H, 4·67; N, 20·09%. $C_7N_4H_{13}I$ requires: C, 30·01; H, 4·68; N, 20·00%.

δ ^1H (d_6D.M.S.O.) 3.50 (3H, s), 3.72 (3H, s), 6.29 (1H, d, 7Hz) 6.89 (2H, d, 9Hz), 7.61 (2H, d, 9Hz), 7.85 (1H, d, 7Hz)

2-AMINO-4-STYRYLPYRIMIDINE

2-Amino-4-methylpyrimidine (10g), benzaldehyde (10g) and formic acid (25ml) were heated under reflux for 48 hours. The resultant deep red solution was poured into ice (300g) and acidified with concentrated hydrochloric acid (40ml). The solution was cooled overnight and the precipitate of 2-benzylamino-4-styrylpyrimidine filtered off (7g), m.p. 160-2°C (Lit. m.p. 156-9°C[54]). Microanalysis found: C, 79·21; H, 5·96; N, 14·73%. Calc. for $C_{19}H_{15}N_3O$: C, 79·41; H, 5·96; N, 14·62%. The filtrate was made alkaline with sodium hydroxide solution (2M). A sticky red/yellow mass resulted which was extracted with chloroform. After drying the extracts over anhydrous magnesium sulphate and removing the solvent under reduced pressure a mustard yellow solid of 2-amino-4-styrylpyrimidine remained (10g, 55%), m.p. 140-5°C (Lit. m.p. 147-8°C[54]). Recrystallisation from ethanol gave white crystals (7·5g, 42%), m.p. 152-3°C.

Microanalysis found: C, 72·77; H, 5·67; N, 21·20%. Calc. for $C_{12}H_{11}N_3$: C, 73·07; H, 5·62; N, 21·30%.

δ ^1H ($CDCl_3$), 6.59 (1H, d, 6Hz), 6.90 (1H, d, 16Hz), 7.2 - 7.5 (5H, b.s), 7.77 (1H, d, 16Hz), 8.20 (1H, d, 6Hz)

δ ^1H (T.F.A.)7.0 - 8.4 (complex envelope of resonances)

2-AMINO-1-METHYL-4-STYRYLPYRIMIDINIUM IODIDE

2-Amino-4-styrylpyrimidine (1g), iodomethane (5g) and ethanol (10ml) were heated under reflux for 4 hours. After cooling, the yellow precipitate was filtered off and recrystallised from ethanol to give yellow needles of 2-amino-1-methyl-4-styrylpyrimidinium iodide (1·3g, 76%), m.p. 247-8°C. Microanalysis found: C, 45·80; H, 4·01; N, 12·33%. $C_{13}H_{14}N_3I$ requires: C, 46·04; H, 4·16; N, 12·39%.

δ ^1H (d_6D.M.S.O.), 3.74 (3H, s), 7.05 - 8.54 (complex envelope of signals).

PYRIMIDINE

This was prepared in a one step synthesis by a modification of the method of Bredereck, Gompper and Morlock [44].

A three neck round bottomed flask was equipped with a magnetic stirrer, thermometer, addition funnel and reflux condenser. A second condenser, set downward for distillation, was connected to the top of the reflux condenser by means of a still head fitted with a thermometer. Formamide (200g), ammonium formate (20g) and water (2ml) were added to the flask and heated in an oil bath at 180-190°C. 1,1,3,3-Tetramethoxypropane (100g) was added dropwise over 1 hour during which time the internal temperature fell to 120°C. The water flow in the reflux condenser was regulated so that the methanol and methyl formate produced in the reaction distilled out as they formed. Gradually the internal temperature increased to 140-150°C, then the mixture was heated under reflux for a further 2 hours. After cooling, the mixture was poured into sodium hydroxide solution (200ml, 1M) and extracted with chloroform. After drying the extracts over anhydrous magnesium sulphate the chloroform was removed under reduced pressure and the residue (35g) distilled at atmospheric pressure, b.p. 123-6°C at 755mm Hg (Lit. b.p. 123-4°) to give pyrimidine (24g, 50%).

δ ^{1}H ($CDCl_3$), 7.30 (1H, d of t, 5, 1Hz), 8.72 (2H, d, 5Hz), 9.19 (1H, b.s)

1-METHYLPYRIMIDINIUM IODIDE

Pyrimidine (1g) and iodomethane (5g) were left for 3 days in a stoppered tube. The precipitated solid was recrystallised from absolute ethanol to give pale yellow leaflets (2·2g, 70%) of 1-methylpyrimidinium iodide, m.p. 134-5°C, begins to decompose above 120°C (Lit. m.p. 136°C[55]). Microanalysis found: C, 26·81; H, 3·08; N, 12·69%. Calc. for $C_5H_7N_2I$: C, 27·05; H, 3·18; N, 12·62%.

δ ^{1}H (D.M.S.O), 4.19 (3H, s), 8.21 (1H, t, 6Hz), 9.2 - 9.5 (2H, complex), 9.77 (1H, b.s)

4-METHYLPYRIMIDINE

This was prepared using the same apparatus as described for pyrimidine. Formamide (95ml), ammonium chloride (6g) and water (3ml) were heated in an oil bath maintained at 180-190°C. 4,4-Dimethoxybutan-2-one (50g) was added over 1 hour and the methanol and methyl formate produced in the reaction were distilled out as they were formed. The mixture was then heated under reflux for a further 2 hours. After cooling, the mixture was poured into sodium hydroxide solution (125ml, 1M) and extracted with chloroform. The chloroform solution was dried over anhydrous magnesium sulphate and then the solvent was removed under reduced pressure. The resultant dark brown liquid was distilled firstly under vacuum, b.p. 22°C at 0·15mm Hg, and then at atmospheric pressure, b.p. 141-2°C (Lit. m.p. 140-2°C[44]) to yield 4-methylpyrimidine (19g, 55%).

The picrate was recrystallised from ethanol to give bright yellow crystals, m.p. 131-2°C (Lit. m.p. 130-132°C[44]).

Microanalysis found: C, 40·81; H, 2·70; N, 21·47%. Calc. for $C_{11}H_9N_5O_7$: C, 40·86; H, 2·80; N, 21·66%.

δ ^1H ($COCl_3$), 2.56 (3H, s), 7.23 (1H, d, 5Hz), 8.63 (1H, d, 5Hz), 9.17 (1H, b.s)

1.4-DIMETHYLPYRIMIDINIUM IODIDE AND 3,4-DIMETHYLPYRIMIDINIUM IODIDE

4-Methylpyrimidine (1g), iodomethane (5g) and dry ethanol (5ml) were heated under reflux for 4 hours under anhydrous conditions. After cooling and storing overnight, yellow/orange needles precipitated from the dark red solution. The solvent was decanted off and the needles were washed five times with dry ethanol then dried under high vacuum (0·1mm Hg) at room temperature (2·3g, 92%). ^1H and ^{13}C n.m.r. spectroscopy showed the product to be a 7:3 mixture of 1,4-dimethylpyrimidinium iodide and 3,4-dimethylpyrimidinium iodide respectively. Attempts to recrystallise the mixture were unsuccessfull. The products decomposed to a dark red oil when heated in a solvent. The crystals were highly hygroscopic and unstable decomposing slowly in the absence of moisture and air.

Microanalysis found: C, 30·29; H, 3·85; N, 11·65%.

$C_6H_9N_2I$ requires: C, 30·53; H, 3·84; N, 11·87%.

δ ^1H (D.M.S.O.), 2.78 (3H, s), 4.24 (3H, s), 8.04 (1H, d, 6Hz), 9.18 (1H, d of q, 6, 2Hz), 9.62 (1H, b.s)

δ ^1H (d_6D.M.S.O), 2.87 (3H, s), 4.20 (3H, s), 8.06 (1H, d, 6Hz), 9.25 (1H, d, 6Hz), 9.72 (1H, b.s)

TRANS-1-CHLOROPENTEN-3-ONE

Propionyl chloride (35g) was added to an ice cold suspension of aluminium chloride (55g) in carbon tetrachloride (150ml) in a round bottomed flask. A rapid stream

of dry acetylene was bubbled through the mixture for 3 hours whilst maintaining the reaction temperature between 0-10°C. The resultant product was carefully poured into a slurry of crushed ice and salt. When cool, the mixture was extracted with ether. The extracts were dried over magnesium sulphate then the solvent was removed under reduced pressure to yield the desired product (30g, 67%). ^1H n.m.r. spectroscopy showed the purity of the product to be greater than 95%. δ ^1H ($COCl_3$), 1·09 (3H, t, 7Hz), 2·52 (2H, q, 7Hz), 6·41 (1H, d, 13Hz), 7·17 (1H, d, 13Hz).

The product was reported to be lachrymatory, vesicant and liable to spontaneous violent decomposition so it was used in the preparation of 4-ethylpyrimidine without further purification.

4-ETHYLPYRIMIDINE

A round bottomed flask containing formamide (200ml) and water (2ml) was heated under reflux in an oil bath at 180-190°C. The previously prepared trans-1-chloropenten-3-one (30g) was added dropwise over 1 hour then the mixture was heated under reflux for a further 2 hours. Care was taken to ensure that the ammonium salts which sublimed from the reaction did not block the condenser. After cooling, the mixture was poured into sodium hydroxide solution (200ml, 1M) and extracted with chloroform. The extracts were dried over anhydrous magnesium sulphate and then the chloroform was removed under reduced pressure to yield a viscous dark brown liquid. Distillation under vacuum gave 4-ethylpyrimidine (7g, 25%) b.p. 35-40°C at

0·4mm Hg. A considerable quantity of a dark brown polymeric residue remained. The product redistilled cleanly at atmospheric pressure, b.p. 158-60°C at 750mm Hg (Lit. b.p. 159-60°C[44]).

δ ^{1}H ($CDCl_3$), 1.33 (3H, t, 7Hz), 2.81 (2H, q, 7Hz), 7.22 (1H, d, 5Hz), 8.67 (1H, d, 5Hz), 9.20 (1H, b.s)

TRANS-1-CHLORO-4-METHYLPENTEN-3-ONE

2-Methylpropionyl chloride (35g) was added to an ice cold suspension of aluminium chloride (48g) in carbon tetrachloride (150ml). A rapid stream of dry acetylene was bubbled through the mixture for 3 hours whilst the reaction temperature was maintained between 0-10°C. The resultant mixture was carefully discharged into a slurry of crushed ice and salt and then extracted, when cool, with ether. After drying the extracts over magnesium sulphate the solvent was removed under reduced pressure to yield the desired product (33g, 76%). The ^{1}H n.m.r. spectrum showed the product to be at least 90% pure. δ ^{1}H ($CDCl_3$), 1·10 (6H, d, 6Hz), 2·70 (1H, heptet, 6Hz), 6·53 (1H, d, 13Hz), 7·25 (1H, d, 13Hz).

This product was used in the preparation of 4-iso-propylpyrimidine without further purification.

4-ISO-PROPYLPYRIMIDINE

Formamide (200ml) and water (2ml) were heated in an oil bath maintained at 180-190°C and then the previously prepared trans-1-chloro-4-methylpenten-3-one (33g) was added dropwise over 1 hour. The mixture was heated under

reflux for a further 2 hours, cooled, poured into sodium hydroxide solution (200ml, 1M) and extracted with chloroform. After drying the combined extracts over anhydrous magnesium sulphate, the chloroform was removed under reduced pressure and the residue distilled under vacuum, b.p. 38-42°C at 0·4mm Hg. A second distillation at atmospheric pressure, b.p. 170-2°C at 755 mm, gave 4-iso-propylpyrimidine (14g, 51%).

The picrate was recrystallised from ethanol to give yellow needles (m.p. 88-9°C). Microanalysis found: C, 44·36; H, 3·67; N, 19·92%. $C_{13}H_{13}N_5O_7$ requires: C, 44·45; H, 3·73; N, 19·94%.

δ ^1H ($CDCl_3$), 1.30 (6H, d, 6Hz), 2.99 (1H, hept., 6Hz), 7.14 (1H, d, 5Hz), 8.54 (1H, d, 5Hz), 9.06 (1H, b.s)

3-CHLORO-4,4-DIMETHYLPENT-2-ENAL (Z)

Phosphoryl chloride (56ml) was added dropwise to dimethyl formamide (46ml) whilst stirring efficiently. The highly exothermic reaction temperature was not allowed to exceed 50°C. The resultant adduct was heated to 60°C using an oil bath and 3,3-dimethylbutan-2-one (30g) in 1,2-dichloroethane (75ml) was then added slowly so that the reaction temperature did not exceed 65°C. The mixture was kept at 60° for a further four hours, then cooled, poured into ice, neutralised with sodium acetate and finally extracted with 1,2-dichloroethane. After drying the combined extracts over anhydrous magnesium sulphate and removing the solvent under reduced pressure a pale yellow liquid remained (35g). The ^1H n.m.r. spectrum showed the product to contain approximately

80% 3-chloro-4,4-dimethylpent-2-enal (28g, 64%). δ ^{1}H ($CDCl_3$), 1·26 (9H, s), 6·09 (1H, d, 6Hz), 9·96 (1H, d, 6Hz). The remaining 20% consisted mainly of dimethyl formamide. In view of the difficulties in removing the dimethyl formamide the crude product was used in the preparation of 4-tert-butylpyrimidine without further purification.

4-TERT-BUTYLPYRIMIDINE

The previously prepared 3-chloro-4,4-dimethylpent-2-enal (35g, 80% pure) was added dropwise to a mixture of formamide (150ml) and water (2ml) in a round bottomed flask in an oil bath maintained at 180-190°C. After the addition was complete the mixture was heated under reflux for a further 3 hours, cooled, poured into sodium hydroxide solution (150ml, 1M) and then extracted with chloroform. The combined extracts were dried over anhydrous magnesium sulphate and then the solvent was removed under reduced pressure. The residue was distilled firstly under vacuum, b.p. 40-45°C at 0·2mm Hg, then at atmospheric pressure, b.p. 174-5°C at 742mm Hg, to yield 4-tert-butylpyrimidine (14g, 54%).

The picrate was recrystallised from ethanol to give yellow leaflets, m.p. 142-3°C (Lit. m.p. 142-3°C [44]). Microanalysis found: C, 45·81; H, 4·06; N, 19·15%. $C_{14}H_{15}N_5O_7$ requires: C, 46·03; H, 4·24; N, 19·17%.

δ ^{1}H ($CDCl_3$), 1.29 (9H, s), 7.42 (1H, d, 5Hz), 8.66 (1H, d, 5Hz), 9.19 (1H, b.s)

4-TERT-BUTYL-1-METHYLPYRIMIDINIUM IODIDE

4-tert-Butylpyrimidine (1g), iodomethane (5g) and ethanol (5ml) were heated under reflux for 4 hours. The resultant precipitate was filtered off and recrystallised from ethanol to give cream coloured needles of 4-tert-butyl-1-methylpyrimidinium iodide (2g, 98%), m.p. 216-8°C. Microanalysis found: C, 38·72; H, 5·41; N, 10·07%. $C_9H_{15}N_2I$ requires: C, 38·87; H, 5·44; N, 10·07%. δ ^1H (D_2O), 1.45 (9H, s), 4.31 (3H, s), 8.20 (1H, d, 6Hz), 9.04 (1H, d of d, 6, 1Hz), 9.46 (1H, b.s)

3-CHLORO-3-PHENYLPROP-2-ENAL (Z)

Phosphoryl chloride (52ml) was added dropwise to dimethyl formamide (38ml) in a round bottomed flask at such a rate that the reaction temperature did not exceed 50°C. The resultant adduct was heated to 40-45°C using an oil bath and then acetophenone (33g) in 1,2-dichloroethane (65ml) was carefully added dropwise so that the internal temperature did not exceed 50°C. The mixture was maintained at 40-45°C for a further 2 hours, cooled, poured into ice, neutralised with sodium acetate and finally extracted with 1,2-dichloroethane. The combined extracts were dried over magnesium sulphate and then the 1,2 dichloroethane was removed under reduced pressure. ^1H n.m.r. spectroscopy showed the residue (40g) to contain approximately 75% of the desired product (30g, 67%). δ ^1H ($CDCl_3$), 6·51 (1H, d, 6Hz), 7·41 (5H, broad multiplet), 10·06 (1H, d, 6Hz). The remaining 25% consisted of mainly dimethyl formamide. This product was used in the preparation of 4-phenylpyrimidine without further purification.

4-PHENYLPYRIMIDINE

The previously prepared 3-chloro-3-phenylpent-2-enal (40g, 75% pure) was added dropwise to a mixture of formamide (150ml) and water (2ml) in a round bottomed flask heated in an oil bath maintained at 180-190°C. When the addition was complete the mixture was heated under reflux for 3 hours, cooled, poured into sodium hydroxide solution (150ml, 1M) and extracted with chloroform. The extracts were dried over magnesium sulphate and the solvent removed under reduced pressure. The residue was distilled under vacuum b.p. 96-100°C at 0·1mm Hg, to give a crystalline product (11g, 40%). The product was recrystallised from petroleum ether to give off white leaflets of 4-phenylpyrimidine, m.p. 59-60°C (Lit. m.p. 60-61°C[44]). Microanalysis found: C, 76·75; H, 5·10; N, 17·64%. Calc. for $C_{10}H_8N_2$: C, 76·89; H, 5·16; N, 17·94%.

δ ^1H ($CDCl_3$), 7.1 - 8.0 (envelope of overlapping signals), 8.60 (1H. b.d, 5Hz), 9.12 (1H, b.s)

1-METHYL-4-PHENYLPYRIMIDINIUM METHOSULPHATE

A mixture of 4-phenylpyrimidine (1g), dimethyl sulphate (3ml) and nitrobenzene (5ml) was heated to 80°C using an oil bath. The solid gradually dissolved and after 10 more minutes a precipitate formed. After cooling, the solid was filtered off and washed several times with ether.

δ ^1H (d_6D.M.S.O.), 4.22 (3H, s), 7.6 - 7.8, 7.3 - 8.5 (5H complex), 8.85 (1H, d, 6Hz), 9.37 (1H, d of d, 6, 1Hz), 9.59 (1H, b.s).

4-(2-METHYLPHENYL) PYRIMIDINE

Bromotoluene (13·8g) was added dropwise to lithium leaflets (1·05g) in dry ether (100ml) under a dry argon atmosphere at such a rate to ensure a gentle reflux was maintained. The mixture was then heated under reflux for a further hour, after which time most of the lithium had reacted. After cooling, pyrimidine (6g) was added dropwise so that a gentle reflux once again occurred. The mixture was heated under reflux for a further hour, cooled, poured into water and extracted with chloroform. After drying the extracts over anhydrous magnesium sulphate and removal of the solvent under reduced pressure a dark brown residue remained. This was dissolved in acetone and oxidised by adding portions of a saturated solution of potassium permanganate in acetone until a pale pink colour persisted. The solution was filtered and the filtrate retained. The acetone was removed under reduced pressure and the residue distilled under vacuum, b.p. 100-110°C at 0·1mm Hg, to yield 4-(2-methyphenyl) pyrimidine (3·2g, 25%).

The picrate was recrystallised from ethanol, m.p. 134-5°C. Microanalysis found: C, 50·83; H, 2·98; N, 17·75%. $C_{17}H_{13}N_5O_7$ requires: C, 51·13; H, 3·28; N, 17·54%.

δ ^1H ($CDCl_3$), 2.46 (3H, s), 7.1 - 7.6 (envelope of overlapping signals), 8.79 (1H, d, 5Hz), 9.30 (1H, b.s)

REACTION OF 2,4,6-TRIMETHYLPHENYLETHANONE WITH A VILSMEIER REAGEANT (CHLOROFORMYLATION OF KETONES)

Phosphoryl chloride (36ml) was added dropwise to dimethyl formamide (30ml) over 2 hours at such a rate that the internal temperature of the reaction did not exceed 50°C. The resultant adduct was heated in an oil bath maintained at 60°C and 2,4,6-trimethylphenylethanone (25g) in 1,2-dichloroethane (50ml) was added dropwise over 4 hours at such a rate that the internal temperature of the reaction did not exceed 70°C. The mixture was heated for a further 2 hours, cooled, poured into ice, neutralised with sodium acetate and finally extracted with 1,2-dichloroethane. The combined extracts were dried over magnesium sulphate and then the solvent was removed under reduced pressure. ^1H n.m.r. spectroscopy indicated that the major product (~70%) was 2,4,6-trimethylbenzoic acid. δ ^1H ($CDCl_3$), 2·30 (dH, s), 2·42 (6H, s), 6·93 (2H, s), 12·02 (1H, bs, exchanges with D_2O). The remaining 30% consisted mainly of dimethyl formamide and starting material. The products were dissolved in ether and extracted with sodium bicarbonate solution. The combined bicarbonate extracts were acidified with hydrochloric acid and re-extracted with ether. The ether extracts were dried over anhydrous magnesium sulphate and the solvent was removed under reduced pressure to yield a white solid (18g), m.p. 153-5°C. This was confirmed to

be 2,4,6-trimethylbenzoic acid by comparison with an authentic sample, m.p. 154-5°C.

2,4,6-TRIMETHYLBENZOYL CHLORIDE

A mixture of 2,4,6-trimethylbenzoic acid (18g) and thionyl chloride (12ml) in a 50ml round bottomed flask fitted with a reflux condenser and drying tube was heated under reflux on a boiling water bath for one hour. The mixture was cooled and the flask was fitted with a still head and condenser. The excess thionyl chloride was distilled off at atmospheric pressure, b.p. 78-80°C and then the residue was distilled under vacuum, b.p. 86°C at 1mm Hg, to yield 2,4,6-trimethylbenzoyl chloride (17g, 85%). δ ^{1}H ($COCl_3$), 2·29 (3H, s), 2·34 (6H, s), 6·88 (2H, s).

REACTION OF 2.4.6-TRIMETHYLBENZOYL CHLORIDE WITH ACETYLENE IN THE PRESENCE OF A FRIEDEL-CRAFTS CATALYST

2,4,6-Trimethylbenzoyl chloride (17g) was added to an ice cold suspension of aluminium chloride (15g) in carbon tetrachloride (100ml). The mixture was heated in an oil bath maintained at 50°C and then a rapid stream of dry acetylene was bubbled through the mixture for 8 hours. The resultant mixture was carefully discharged into a slurry of crushed ice and salt and when cool extracted with ether. After drying the combined ether extracts over anhydrous magnesium sulphate the solvent was removed under reduced pressure. ^{1}H n.m.r. spectroscopy showed that the residue was a mixture of 2,4,6-trimethylbenzoyl chloride (~50%) and 2,4,6-trimethylbenzoic acid.

4-(2.4.6-TRIMETHYLPHENYL) PYRIMIDINE

Mesityl bromide (25g) was added to lithium leaflets (1·8g) in dry ether (100ml) and then the mixture was heated under reflux for 72 hours under anhydrous conditions after which time most of the lithium had reacted. When cool, pyrimidine (8g) was added dropwise so that a gentle reflux occurred. The mixture was heated under reflux for a further hour, cooled, poured into water and finally extracted with chloroform. The combined extracts were dried over anhydrous magnesium sulphate and the solvent removed under reduced pressure. The residue was dissolved in acetone and oxidised by adding small portions of a saturated solution of potassium permanganate in acetone until a pale pink colour persisted. The solution was filtered and the acetone removed from the filtrate under reduced pressure. Ther residue was distilled under vacuum, b.p. 124-32°C at 0·7mm Hg, to yield a white crystalline product (7·5g, 30%). The product was recrystallised from petroleum ether to give 4-(2,4,6-trimethylphenyl)pyrimidine, m.p. 66-7°C (Lit. m.p. 67°C [43]). Microanalysis found: C, 78·63; H, 7·29; N, 14·12%. Calc. for $C_{13}H_{14}N_2$: C, 78·75; H, 7·12; N, 14·13%.

δ ^{1}H ($CDCl_3$), 2.02 (6H, s), 2.32 (3H, s), 6.97 (2H, b.s), 7.25 (1H, d of d, 5Hz, 1Hz), 8 .78 (1H, d, 5Hz), 9.34 (1H, b.s)

1-METHYL-4-(2,4,6-TRIMETHYLPHENYL)PYRIMIDINIUM IODIDE AND 3-METHYL-4-(2,4,6-TRIMETHYLPHENYL)PYRIMIDINIUM IODIDE

4-(2,4,6-Trimethylphenyl)pyrimidine (1g), dry iodomethane (5g) and absolute ethanol (5ml) were heated under reflux for 4 hours under anhydrous conditions. After cooling, the dark yellow solution was evaporated to dryness under reduced pressure. The crystalline residue was washed twice with a little cold absolute ethanol and then dried under vacuum (0.1mm Hg) to yield light yellow crystals which were extremely hygroscopic. ^{1}H and ^{13}C n.m.r. spectroscopy showed the product to be a 2:1 mixture of 1-methyl-4-(2,4,6-trimethylphenyl)pyrimidinium iodide and 3-methyl-4-(2,4,6-timethylphenyl)pyrimidinium iodide respectively. Microanalysis found: C, 49·65; H, 5·07; N, 8·23%. $C_{14}H_{17}N_2I$ requires: C, 49·43; H, 5·04; N, 8·23%.

δ ^{1}H (d_6D.M.S.O.), 1.96 (6H, s), 2.02 (3H, s), 3.81 (3H, s), 7.04 (2H, b.s), (H5 and H6 resonances obscured) 9.92 (1H,b.s)

δ ^{1}H (d_6D.M.S.O.), 2.09 (6H, s), 2.30 (3H, s), 3.24 (3H, s), 6.95 (2H, b.s), (H5 and H6 resonances obscured), 9.78 (1H, b.s)

5-METHYLPYRIMIDINE

This was available from Sigma Chemicals and was used without further purification.

δ ^{1}H ($CDCl_3$), 2.29 (3H, s), 8.46 (2H, b.s), 8.93 (1H, b.s)

1,5-DIMETHYLPYRIMIDINIUM IODIDE

5-Methylpyrimidine (2g), iodomethane (10g) and ethanol (10ml) were heated under reflux for 4 hours. The solvent was removed under reduced pressure to yield a dark red viscous oil. ^{13}C and ^{1}H n.m.r. spectroscopy unambiguously identified the product as 1,5-dimethyl

pyrimidinium iodide contaminated with ethanol. The product was dried under vacuum (0·1mm Hg) at 55°C for 4 hours to yield a red/brown solid (2·4g, 96%) which was extremely hygroscopic. Microanalysis found: C, 28·98; H, 3·74; N, 11·59%. $C_6H_9N_2I$ requires: C, 30·53; H, 3·84; N, 11·87:.

δ ^{1}H (d_6D.M.S.O.), 2.40 (3H, s), 4.13 (3H, s), 9.04 (1H, b.s), 9.30 (1H, b.s)

3-CHLORO-2-METHYLBUT-2-ENAL(ISOMERIC MIXTURE)

Phosphoryl chloride (53ml) was carefully added dropwise to dimethyl formamide (44ml) so that the highly exothermic reaction was kept under control and the internal temperature of the reaction did not exceed 50°C. The resultant adduct was heated in an oil bath maintained at 30-35°C and butan-2-one (20g) was added dropwise to the mixture at such a rate that the internal temperature of the reaction did not exceed 50°C. The mixture was maintained at 30-35°C for a further 2 hours, cooled, poured into ice, neutralised with sodium acetate and extracted with 1,2-dichloroethane. The combined extracts were dried over magnesium sulphate and the solvent was removed under reduced pressure. ^{1}H n.m.r. spectroscopy showed the residue (28g) to be approximately 80% 3-chloro-2-methylbut-2-enal (22g, 67%) present as a mixture of the Z and E isomers. δ^1H, E isomer (70%), ($CDCl_3$), 1·9 (3H, q, 2Hz), 2·6 (3H, q, 2Hz), 10·1 (1H, s), δ^1H, Z isomer (30%), ($CDCl_3$), 1·8 (3H, s), 2·4 (3H, s), 10·3 (1H, s). The remaining 20% consisted mainly of dimethyl formamide. The crude product was used in the preparation of 4,5-dimethylpyrimidine without further purification.

4,5-DIMETHYLPYRIMIDINE

The previously prepared 3-chloro-2-methylbut-2-enal (28g, 80% pure) was added dropwise to a mixture of formamide (150ml) and water (2ml) in a round bottomed flask in an oil bath maintained at 180-90°C. When the addition had been completed the mixture was heated under reflux for 3 hours, then cooled, poured into sodium hydroxide solution (150ml, 1M) and finally extracted with chloroform. The combined extracts were dried over anhydrous magnesium sulphate and the solvent was removed under reduced pressure. The residue was distilled firstly under vacuum, b.p. 40-42° at 0·4mm Hg, then at atmospheric pressure, b.p. 172-4° at 742mm Hg (Lit. b.p. 175°C at 744mm [56]) to give 4,5-dimethylpyrimidine (7g, 35%).

The picrate was recrystallised from ethanol to give yellow needles, m.p. 161-3°C (Lit. m.p. 162°C). Microanalysis found: C, 42·67; H, 3·02; N, 21·05%. Calc. for $C_{12}H_{11}N_5O_7$: C, 42·74; H, 3·29; N, 20·77%.

^1H ($CDCl_3$), 2·25 (3H, s), 2·46 (3H, s), 8·41 (1H, b.s.), 8·92 (1H, b.s.)

3-CHLORO-2-METHYLPENT-2-ENAL (ISOMERIC MIXTURE)

Phosphoryl chloride (46ml) was carefully added dropwise to dimethyl formamide (39ml). The resultant solution was heated in an oil bath maintained at 40-45°C and then pentan-3-one (20g) in 1,2-dichloroethane (100ml) was added dropwise at such a rate that the internal temperature of the reaction did not exceed 50°C. After the addition had been completed the mixture was heated for a

further hour, cooled, poured into ice, neutralised with sodium acetate and finally extracted with 1,2-dichloroethane. The combined extracts were dried over anhydrous magnesium sulphate and the solvent was removed under reduced pressure. ^1H n.m.r. spectroscopy showed the residue (32g) to be approximately 70% 3-chloro-3-ethyl-2-methylpent-2-enal (22g, 71%) present as a mixture of the Z and E isomers. δ ^1H, E isomer (55%), ($CDCl_3$), 1·3(3H, t, 6Hz), 1·9 (3H, s), 2·6 (2H, q, 6Hz), 10·0 (1H, s). δ ^1H, Z isomer (45%), ($CDCl_3$), 1·2 (3H, t, 6Hz), 1·8 (3H, s), 2·8 (2H, q, 6Hz), 10·2 (1H, s). The remaining 30% consisted mainly of dimethylformamide and so the crude product was used in the preparation of 4-ethyl-5-methylpyrimidine without further purification.

4-ETHYL-5-METHYLPYRIMIDINE

The crude 3-chloro-2-methylpent-2-enal (32g, 70% pure) was added dropwise to a mixture of formamide (150ml) and water (2ml) contained in a round bottomed flask heated in an oil bath maintained at 180-90°C. The mixture was heated under reflux for a further 2 hours, cooled, poured into sodium hydroxide solution (150ml, 1M) and extracted with chloroform. The combined extracts were dried over magnesium sulphate and the solvent was then removed under reduced pressure. The residue was distilled firstly under vacuum, b.p. 32-34°C at 0·1mm Hg then at atmospheric pressure, b.p. 179-81°C at 743mm Hg to give 4-ethyl-5-methyl pyrimidine (9g, 44%).

The picrate was recrystallised from ethanol to give yellow plates, m.p. 111-3°C (Lit. m.p. 111°C[56]). Micro-

analysis found: C, 44·25; H, 3·72; N,19·68%. Calc. for $C_7H_{10}N_2$: C, 44·44; H, 3·70; N, 19·94%.

δ ^1H ($CDCl_3$), 1.28 (3H, t, 7Hz), 2.23 (3H, s), 2.75 (2H, q, 7Hz), 8.30 (1H, b.s), 8.85 (1H, b.s)

1,5-DIMETHYL-4-ETHYLPYRIMIDINIUM IODIDE

4-Ethyl-5-methylpyrimidine (2g), iodomethane (10g) and ethanol (10ml) were heated under reflux for 4 hours. The solvent was removed under reduced pressure to yield orange/yellow crystals. ^1H and ^{13}C n.m.r. spectroscopy unambiguously identified the product as 1,5-dimethyl-4-ethylpyrimidinium iodide (3·9g, 91%). However, it was not possible to obtain a satisfactory elemental analysis since the product was extremely hygroscopic and unstable. Some decomposition had occurred before the microanalysis could be carried out.

δ ^1H (D_2O), 1.27 (3H, t, 7Hz), 2.40 (3H, s), 3.05 (2H, q, 7Hz), 9.01 (1H, b.s), 9.40 (1H, b.s)

4-TERT-BUTYL-5-METHYLPYRIMIDINE

A solution of 5-methylpyrimidine (8g) in dry pentane was added dropwise to a solution of tert-butyllithium in pentane (40ml, 1·4M) which was cooled in an ice bath and maintained under a dry argon atmosphere. During the addition a yellow precipitate was formed. The mixture was stirred at 0^oC for a further 30 minutes, heated under reflux for 1 hour, cooled, poured into water and

then extracted with chloroform. The combined chloroform extracts were dried over anhydrous magnesium sulphate and the chloroform was then removed under reduced pressure. The residue was dissolved in acetone and oxidised with small portions of a saturated solution of potassium permanganate in acetone until a pale pink colour persisted. The solution was filtered and the acetone removed from the filtrate under reduced pressure. The residue was distilled under vacuum to give 4-tert-butyl-5-methylpyrimidine (25g, 19%), b.p. 52-55°C at 0·1mm Hg. Some unreacted 5-methylpyrimidine (1·5g), b.p. 30-34°C at 0·2mm Hg was also recovered.

The picrate of 4-tert-butyl-5-methylpyrimidine was recrystallised from ethanol to give yellow crystals, m.p. 137-8°C. Microanalysis found: C, 47·22; H, 4·33; N, 18·74%. $C_9H_{14}N_2$ requires: C, 47·49, H, 4·52; N, 18·46%.

^{1}H($CDCl_3$), 1·25 (9H, s), 2·30 (3H, s), 8·25 (1H, b.s.), 8·85 (1H, b.s.)

3-CHLORO-2-METHYL-3-PHENYLPROP-2-ENAL (ISOMERIC MIXTURE)

Phosphoryl chloride (46ml) was carefully added dropwise to dimethylformamide (38ml) over 1-2 hours. The resultant solution was heated in an oil bath maintained at 55-60°C and then 1-phenylpropanone (33g) in 1,2-dichloroethane (100ml) was added dropwise at such a rate that the reaction temperature did not exceed 65°C. The mixture was heated for a further hour, cooled, poured into ice, neutralised with sodium acetate and then extracted with 1,2-dichloroethane. The combined extracts were dried over anhydrous magnesium sulphate

and the solvent was then removed under reduced pressure. ^1H n.m.r. spectroscopy showed the residue (52g) to be approximately 70% 3-chloro-2-methyl-3-phenylprop-2-enal (36g, 81%) present as a mixture of the Z and E isomers. δ ^1H, E isomer (90%), ($CDCl_3$), 2·0 (3H, s), 7·3 (5H, bs), 9·4 (1H, s). δ^1H, Z isomer (10%), ($CDCl_3$), 1·8 (3H, s), 7·3 (5H, b.s.), 10·3 (1H, s). The remaining 30% consisted of mainly dimethyl formamide so the crude product was used in the preparation of 5-methyl-4-phenylpyrimidine without further purification.

5-METHYL-4-PHENYLPYRIMIDINE

The previously prepared 3-chloro-2-methyl-3-phenylprop-2-enal (52g, 70% pure) was added dropwise to a preheated mixture of formamide (200ml) and water (3ml) using an oil bath maintained at 180-190°C. The resultant mixture was heated under reflux for a further 3 hours, cooled, poured into sodium hydroxide solution (200ml, 2M) and finally extracted with chloroform. The combined chloroform extracts were dried over anhydrous magnesium sulphate and the solvent was then removed under reduced pressure. The residue was distilled under vacuum, b.p. 92-94°C at 0·1mm Hg (Lit. b.p. 80-85° at 0·001mm [56])to give 5-methyl-4-phenylpyrimidine, m.p. 28-30°C (Lit. m.p. 31-32°C [56]).

The picrate was recrystallised from ethanol to give yellow needles, m.p. 139-40°C (Lit. m.p. 139°C [56]). Microanalysis found: C, 50·97; H, 3·18; N, 17·59%. Calc. for $C_{17}H_{13}N_5O_7$: C, 51·13; H, 3·28; N, 17·54%.

δ ^{1}H ($CDCl_3$), 2.30 (3H, s), 7.1 - 7.6 (5H, complex), 8.41 (1H, b.s), 8.92 (1H, b.s)

1,5-DIMETHYL-4-PHENYLPYRIMIDINIUM IODIDE

5-Methyl-4-phenylpyrimidine (2g), iodomethane (10ml) and ethanol (10ml) were heated under reflux for 4 hours. The solvent was removed under reduced pressure to yield dark yellow hygroscopic crystals of 1,5-dimethyl-4-phenylpyrimidinium iodide (3·2g, 88%). Microanalysis found: C, 45·34; H, 4·09; N, 8·94%. $C_{12}H_{13}N_2I$ requires: C, 46·17; H, 4·20; N, 8·97%.

δ ^{1}H (d_6D.M.S.O.), 2.50 (3H, s), 4.20 (3H, s), 7.4 - 7.8 (5H, complex), 9.25 (1H, b.s), 9.50 (1H, b.s)

N.M.R. SPECTROSCOPY

^{1}H n.m.r. Spectra

^{1}H n.m.r. spectra were determined on Hitachi Perkin-Elmer R.24 and R24B n.m.r. spectrometers.

^{1}H chemical shifts are in p.p.m. relative to T.M.S. and are accurate to $\pm$ 0.05 p.p.m. ^{1}H - ^{1}H coupling constants are in Hz and are accurate to $\pm$ 1Hz.

^{13}C n.m.r. spectra

^{13}C n.m.r. spectra were determined on a Jeol JNM-FX100 Fourier Transform n.m.r. spectrometer. All ^{13}C chemical shifts are relative to 1,4-dioxan and are accurate to 0.02 p.p.m. ^{13}C - ^{1}H coupling constants are accurate to $\pm$ 0.3 Hz with the exception of those for $^{3}JC2H6$ which are accurate to $\pm$ 0.2 Hz. Spectrum simulation was used to check if a first order analysis was appropriate.

^{13}C n.m.r. samples were prepared to a standard concentration (approximately 1.8 mol. dm^{-3} with respect to the pyrimidine).

^{13}C - ^{1}H coupling information was obtained where possible from the nuclear Overhausser enhanced fully coupled spectrum. Where strong acids were used the standard conditions could not be used due to overheating of the sample. However this problem was overcome by using very low irradiation power levels.

A typical fully coupled spectrum required 14,000 pulses (approximately 15 hours) to obtain a satisfactory signal to noise ratio.

For the N-methylpyrimidinium iodides it was necessary to use low power irradiation of the N-methyl protons to expose the other ^{13}C - ^{1}H couplings.

pKa DETERMINATIONS

These determinations were carried out by U.V. spectroscopy by a standard method [23].

A Pye-Unicam SP8-100 ultra-violet spectrometer was used for this purpose since it had a fixed wavelength facility and an accurate digital readout.

The pH measurements were made with a Radiometer PHM82 pH meter (resolution $\pm$ 0.01 p.H).

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