An N.M.R. Method for the Quantitative Determination of the N-1:N-3 Protonation Ratio in 2,4-Diaminopyrimidine

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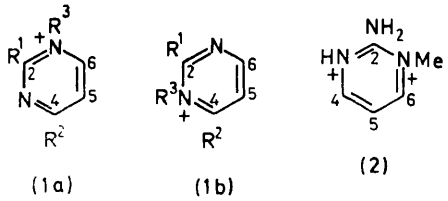
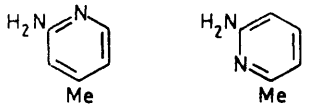
Summary The N-1:N-3 protonation ratio in monoprotonated 2,4-diaminopyrimidine has been determined from $^3J_{C\text{-}2,H\text{-}6}$ by using the N-1- and N-3-methylpyrimidinium iodides as model systems.

2,4-DIAMINOPYRIMIDINES are important in antibacterial chemotherapy and are known to bind to their intracellular receptor, dihydrofolate reductase, more effectively when in a protonated state.[1] A method which provides information about the basicities of the potential protonation sites within this system is thus of considerable value. To determine pK_a values for both N-1 and N-3 in these systems requires a knowledge not only of the pK_a value for the whole molecule but also of the relative proportions of the protonated forms (**1a**) and (**1b**) ($R^1 = R^2 = NH_2$, $R^3 = H$). This latter information has not been previously available because of the rapid exchange which occurs between the two protonated forms. We now report a method for determining the relative proportions of the protonated forms (**1a**) and (**1b**) ($R^1 = R^2 = NH_2$, $R^3 = H$) based on a consideration of the value of $^3J_{C\text{-}2,H\text{-}6}$ in the ^{13}C n.m.r. spectrum.

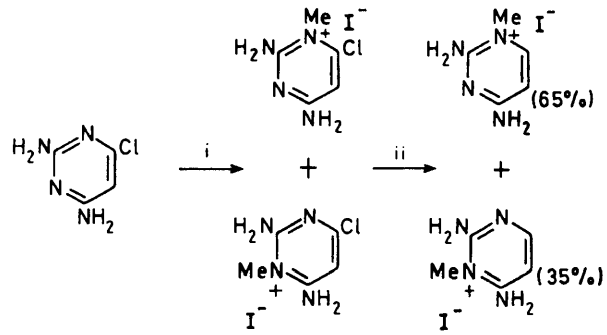


(1a) (1b) (2)



(3) (4)

Although protonation is known to affect the 'through nitrogen' $^3J_{CH}$ vicinal couplings in a variety of nitrogen heterocycles[2–4] coupling measurements have not been used for the quantitative determination of the relative proportions of the protonated forms (**1a**) and (**1b**) ($R^3 = H$) in substituted pyrimidines. Such information is directly available from $^3J_{C\text{-}2,H\text{-}6}$ measurements in the protonated system [(**1a**) ⇌ (**1b**), $R^3 = H$] if the values in the two individual protonated forms can be determined. Although these values cannot be determined from the rapidly exchanging protonated system we believe that the methiodides (**1a**) and (**1b**) ($R^3 = Me$) can, in many cases, provide suitable values. For this reason we have developed a method for preparing the previously unreported N-3 methiodides [(**1b**), $R^3 = Me$]. A typical route is shown in the Scheme.



SCHEME. Reagents and conditions: i, MeI, EtOH, reflux; ii, Zn–H_2O, 100 °C.

The resulting mixture of products was separated either by chromatography on cellulose or by fractional crystallisation. Thus, for example, in the 2,4-diaminopyrimidine system the N-1 and N-3 methiodides [(**1a**) and (**1b**), $R^1 = R^2 = NH_2$, $R^3 = Me$] gave values for $^3J_{C\text{-}2,H\text{-}6}$ of 5·8 Hz† and 13·4 Hz respectively enabling the value of $^3J_{C\text{-}2,H\text{-}6} = 7{\cdot}2$ Hz in the protonated 2,4-diaminopyrimidine system [(**1a**) ⇌ (**1b**), $R^1 = R^2 = NH_2$, $R^3 = H$] to be interpreted in terms of an N-1:N-3 protonation ratio of 82:18. This ratio is less extreme than generally assumed and enables the pK_a values for N-1 and N-3 to be calculated to be 7·31 and 6·66 respectively, based on data previously reported for the whole molecule (pK_a 7·40 ± 0·03).[5]

† Couplings were determined by comparison with simulated spectra and are accurate to ±0·2 Hz.

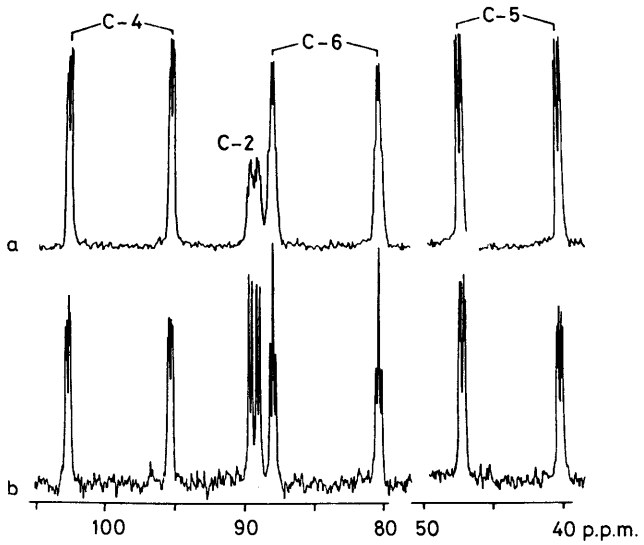


FIGURE. ^{13}C N.m.r. spectrum of (**1**) ($R^1 = NH_2$, $R^2 = H$, $R^3 = Me$) in [$^2H_6$]dimethyl sulphoxide (a) without proton decoupling, (b) with very low power irradiation of methyl protons to expose $^3J_{C\text{-}2,H\text{-}6}$ and $^3J_{C\text{-}2,H\text{-}4}$. Chemical shifts are in p.p.m. from dioxan.

Support for the use of methiodides as model compounds for these studies comes from a consideration of the 2-amino- and 2-amino-4-methyl-substituted systems. In the case of the 2-aminopyrimidine the methiodide (**1**) ($R^1 = NH_2$, $R^2 = H$, $R^3 = Me$) in both water and dimethyl sulphoxide (Figure) gave $\frac{1}{2}(^3J_{C\text{-}2,H\text{-}6} + {}^3J_{C\text{-}2,H\text{-}4}) = 9{\cdot}85$ Hz, in good agreement with the 10·0 Hz observed in the protonated system (**1**; $R^1 = NH_2$, $R^2 = R^3 = H$). Furthermore, the ^{13}C n.m.r. spectrum of (**2**), obtained by dissolving the methiodide (**1**) ($R^1 = NH_2$, $R^2 = H$, $R^3 = Me$) in concentrated sulphuric acid, showed $^3J_{C\text{-}2,H\text{-}6} = {}^3J_{C\text{-}2,H\text{-}4} = 7{\cdot}3$ Hz indicating the similarity in the effects of protonation and methylation.

For the 2-amino-4-methylpyrimidine system the N-1 and N-3 methiodides were found to give vicinal couplings similar to those observed in the 2-amino- and 2,4-diamino-substituted systems. Thus $^3J_{C\text{-}2,H\text{-}6} = 6{\cdot}0$ Hz for (**1a**) ($R^1 = NH_2$, $R^2 = R^3 = Me$) and 13·6 Mz for (**1b**) ($R^1 = NH_2$, $R^2 = R^3 = Me$). In contrast with the 2,4-diamino-system, however, $^3J_{C\text{-}2,H\text{-}6} = 9{\cdot}5$ Hz for the protonated 2-amino-4-methylpyrimidine system (**1a**) $\rightleftharpoons$ (**1b**) ($R^1 = NH_2$, $R^2 = Me$, $R^3 = H$) in water indicating an N-1 : N-3 protonation ratio of 54 : 46. This in turn implies that $pK_{N\text{-}1} - pK_{N\text{-}3} = 0{\cdot}07$, in excellent agreement with the 0·07 predicted from a consideration of the model compounds (**3**) (pK_a 7·48)[6] and (**4**) (pK_a 7·41)[6] and providing further support for the use of methiodides as model compounds in these studies.

Our method for the determination of protonation ratios should be applicable to a wide range of substituted pyrimidines and avoids the assumptions and complexities inherent in the chemical shift approach.[7] We are currently assessing the relative contributions of the steric and electronic effects of substituents in determining the basicities of the ring nitrogens in a range of substituted pyrimidines.

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