Creatinine: an Examination of its Structure and Some of its Reactions by Synergistic Use of MNDO Calculations and Nuclear Magnetic Resonance Spectroscopy

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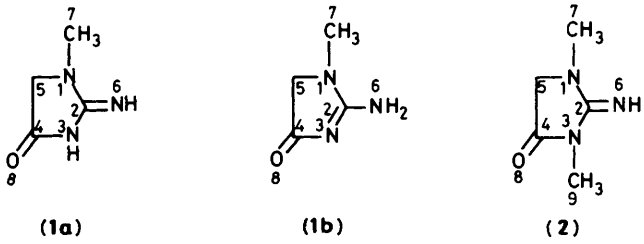
MNDO calculations do not predict correctly the tautomer of creatinine (**1**) present in aqueous solution as deduced from n.m.r. evidence. The reason is substantial hydrogen bonding to the solvent. However, calculations do illuminate the form of protonated creatinine and lead to a semi-quantitative explanation of the higher basicity of glycocyamidines with exocyclic C=N, *e.g.* methylcreatinine (**2**). Both calculations and spectroscopy show the ring-opening reaction of creatinine in alkaline solution to give creatine (**5**). The significance of this to the clinical assay of creatinine (the Jaffé reaction) is discussed. In acid solution creatinine undergoes slow ring closure.

2-Amino-1,5-dihydro-1-methylimidazol-4-one (creatinine) (**1**) is of considerable biological importance; it is an end product of nitrogen metabolism in vertebrates.[1] Abnormal creatinine levels in urine and plasma are important indications in the diagnosis of kidney dysfunction.[2] In spite of the simplicity of both the creatinine molecule and its method of analysis (the Jaffé reaction) there are a number of, as yet, unanswered questions. We now report an attempt to resolve some of these questions by the concurrent application of carbon-13 n.m.r. spectroscopy and MNDO calculations.[3]



Results and Discussion

The are two possible tautomers of creatinine (**1a** and **b**). The molecule is normally written as (**1a**) and there is evidence from an *X*-ray crystallographic study[4] to show that this is correct for the solid state. However, it is by no means certain that this is true of the molecule in solution. Kenyon and Rowley,[5] from a study of pK_a values, hydrolytic stabilities, deuterium exchange rates, and spectral properties, came out tentatively in favour of the tautomer containing an endocyclic C=N [*i.e.* (**1b**)]. Application of the MNDO method allowed us to calculate the enthalpies of formation of the two creatinine isomers: (**1a**) −71.0 and (**1b**) −61.1 kJ mol^{-1}. These values apply to isolated, gas-phase molecules and the fact that the favoured tautomer (**1a**) is that observed in the crystalline state shows that hydrogen bonding within the crystal does not change the situation. The complete neglect of solvation effects in the MNDO calculation, with a molecule as highly functionalised as creatinine, coupled with the small enthalpy difference between the two isomers (9.9 kJ mol^{-1}), means that the calculations give no sure indication of the state of molecules in aqueous solution.

Carbon-13 n.m.r. spectra of creatinine and its derivatives in acid solution have been reported[6] but not, to our knowledge, spectra of the neutral species. For creatinine there are resonances at 32.9, 59.1, 172.1, and 191.5 p.p.m. These figures do not allow us to distinguish between (**1a**) and (**1b**). However, it is instructive to compare the figures with those of an analogue of creatinine where tautomerism is not possible, *viz.* methylcreatinine, 1,5-dihydro-2-imino-1,3-dimethylimidazol-4-one (**2**). This molecule must have an exocyclic C=N. The hydriodide of this compound was prepared by methylation of creatinine (there is no doubt about the position of methylation[5]) and an aqueous solution was exactly neutralised with NaOH. Resonances were obtained at 31.0, 39.6, 56.6, 159.6, and 177.3 p.p.m. Comparison of the resonances at low field with those of creatinine suggest that the compounds exist as different tautomers, *i.e.* creatinine must be present in solution as (**1b**), thus confirming the conclusion of Kenyon and Rowley[5] and confounding the MNDO calculations. This exercise highlights the danger of taking any quantum mechanical calculations relevant to isolated molecules in the gas phase and assuming that matters will be unchanged for the same molecule in aqueous solution where hydrogen bonding is an important factor. This caveat applies less if the solvent is nonpolar. It was found, in a study of tautomerism amongst the thiadiazoles (Hector's Base and related compounds), that structures deduced from carbon-13 and nitrogen-15 n.m.r. spectra in nonpolar solvents[7] were accurately predicted by MNDO calculations.[8]

There is an effect applying to all the glycocyamidines, highlighted by Kenyon and Rowley:[5] those with an exocyclic C=N are stronger bases than those with an endocyclic C=N. For example, the pK_a of (**1**) is 4.78,[9] whereas that of (**2**) is 8.07.[5] In principle both (**1**) and (**2**) may be protonated at four positions (three nitrogens and an oxygen) but both MNDO calculations and primitive valence bond theory indicate that two of the four positions are much more basic than the other two. Calculated enthalpies of formation (in kJ mol^{-1}) for the various protonated forms of (**1**) and (**2**) are shown in Table 1. These figures suggest that methylcreatinine (**2**) is protonated on the exocyclic nitrogen (*i.e.* position 6) and that, irrespective of the tautomeric form of free creatinine, the most stable tautomer on protonation is (**3**), having a structure analogous to that of protonated methylcreatinine. Formation of (**3**) could occur by protonation of the exocyclic nitrogen of (**1a**) or a ring nitrogen of (**1b**) and the latter process must be energetically equivalent to conversion of solvated (**1b**) into solvated (**1a**) and subsequent protonation of the exocyclic C=N. The unfavourable enthalpy change to convert the endocyclic C=N into an exocyclic C=N preceding protonation may explain, in a qualitative way, the greater basicity of glycocyamidines with exocyclic C=N bonds. However, the situation is slightly complicated by the datum in Table 1 which shows that protonation of the oxygen of (**1b**) is

Table 1. Enthalpies of formation ($\Delta H_f°$/kJ mol^{-1}) of protonated forms of creatinine and methylcreatinine

	Species		
Position of protonation	(1a)	(1b)	(2)
1	726.2	733.4	
3	706.3	557.6	
6	557.6	721.3	558.1
8	644.4	567.9	636.9

Table 2. Chemical shifts in n.m.r. spectra of protonated creatinine and methylcreatinine

Carbon atom	(1)	(2)
2	159.3	160.2
4	174.7	173.5
5	56.7	56.1
7	33.8	28.8
9		34.2

also energetically favourable. Thus it is impossible to predict with certainty the structure of protonated creatinine in a hydrogen-bonding solvent. The carbon-13 chemical shifts of protonated creatinine and methylcreatinine are displayed in Table 2 and our values agree well with those of Kenyon *et al.*[6] The similarity of the shifts at C-2 and C-4 for the two compounds indicates unambiguously that the protonated forms are analogues and that *O*-protonation of (**1**) is not significant. The argument used above to explain the greater basicity of (**2**) assumed that protonation of (**1**) and (**2**) occurred at the same site. This is now established experimentally.

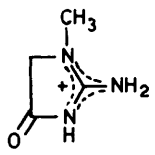
The sensitivity of chemical shifts to the site of protonation is worthy of examination and illustrates a further use of the MNDO method. Values for the charge on C-2 and C-4 in the *N*-protonated species (**3**) are +0.501 and +0.334 and in protonated methylcreatinine the corresponding values are +0.501 and +0.332. Thus similar chemical shifts are to be expected. For *O*-protonation of (**1b**) the values are +0.428 and +0.255; very different chemical shifts would be expected. This is further evidence for *N*-protonation of both (**1**) and (**2**).

We must now face the objection that all the recorded enthalpy calculations on isolated molecules are rendered valueless by the energy changes involved in solvation, as we suggested is the case in determining the favoured tautomer of (**1**). However, the energy differences for the different sites of protonation are much greater (100 rather than 10 kJ mol^{-1}). Also, all cationic species will be solvated and the differences will be of degree rather than kind. Thus the *ordering* of the enthalpies of protonation in the gas phase should reflect the same order for the solvated molecule in solution. The absolute values are probably without significance. A final defence of MNDO is that we have used the calculations only to illuminate experimental data rather than to replace them.

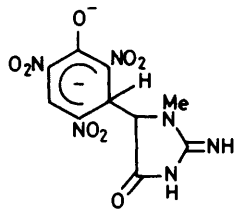
Creatinine levels in biological fluids are determined colorimetrically by reaction with picric acid (2,4,6-trinitrophenol) in alkaline solution (the Jaffé reaction). The nature of the chromogen has been under discussion for some years. Greenwald and Gross[10] isolated a red compound from the reaction mixture but could not identify it with certainty. Seelig and Würst[11] suggested that the chromogen contains the enolate anion of creatinine and this view was revived by Vasiliades[12] following an extensive n.m.r. study of the reaction mixture. Butler[13] and Kovar *et al.*[14] suggested that the carbanion of creatinine forms a Janovsky complex with picrate (**4**). However,

Table 3. Enthalpies of formation ($\Delta H_f°$/kJ mol^{-1}) of anions of creatinine

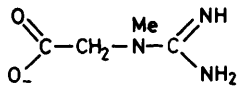
Position of deprotonation	(1a)	(1b)
3	−205.1	
5	−127.5	+48.9
6	−71.1	



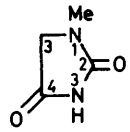
(**3**)



(**4**)



(**5**)



(**6**)

the reported n.m.r. spectra[12] are not consistent with formation of a Janovsky complex.[15] To help resolve this inconsistency we thought it might be of value to examine the behaviour of creatinine in alkaline solution in the absence of an excess of picrate.

Creatinine is not very soluble in 2M-NaOH. The carbon-13 n.m.r. spectrum of the resulting solution had all the peaks corresponding to neutral creatinine (asterisked) but each one was doubled, *viz.* 32.9*, 39.1, 56.4, 58.9*, 163.0, 172.1*, 179.1, and 191.5*. The unmarked peaks correspond exactly to those of the ring-opened compound creatine (**5**). An equilibrium had been reached by the time (1 day) the first spectrum had been accumulated; there was no further change in the spectrum over the next 7 days. Spectra in 1M- and 3M-NaOH suggest that the position of equilibrium is pH-dependent. This conclusion is somewhat tentative as, because of the low solubility of creatinine, each spectrum took a long time to acquire and side reactions occurred.

MNDO calculations of the deprotonated forms of creatinine indicate how complex the situation is. The enthalpies of formation (in kJ mol^{-1}) of various deprotonated forms are shown in Table 3. The rather unexpected result is the ready deprotonation of N-3. There is the possibility of delocalisation of the negative charge over the proximate groups and so the situation is not unlike that of Meldrum's Acid.[16] However, from the carbon-13 n.m.r. spectra there is no evidence for formation of this species in significant quantity. Ionisation of the methylene group does occur, as evidenced by hydrogen exchange at that position.[17] From the MNDO calculations it is clear that the carbanion is formed by deprotonation of (**1a**) rather than (**1b**); location of the negative charge on oxygen in the latter case destroys conjugation and so delocalisation is reduced.

The only reaction of creatinine in alkaline solution observable by n.m.r. is ring opening. In a previous study of the formation of xanthane hydride[18] we found the application of MNDO methods to possible intermediates on the reaction path to be of value. The same approach in the present situation

indicates that addition of HO^- to C-4, in either neutral (**1a**) or (**1a**) deprotonated at N-3, gives stable adducts with weakening of the C(4)–N(3) bond. Transfer of a proton from the added HO group to N-3 and re-optimisation results in ring-opening, the observed effect. The spectrum of methylcreatinine (**2**) in alkaline solution (31.0*, 32.1, 39.2*, 56.3*, 58.9, 159.6*, 161.3, 171.2, and 177.3* p.p.m.) provides evidence for the same ring-opening process to give methylcreatine. MNDO calculations on this compound give results which parallel exactly those for creatinine, except that the C(4)–N(3) bond is more weakened on adduct formation. Although the calculations indicate that ring-opening could occur with the anion it seems more likely that reaction occurs *via* the neutral species. This must be the course of the reaction with methylcreatinine.

A solution of 1-methylhydantoin (**6**) in alkali exhibited no evidence of ring-opening. MNDO calculations showed that C-2 is much more electrophilic than C-4, in contrast to creatinine and methylcreatinine, and that accordingly C-2, rather than C-4, is the favoured site for addition of hydroxide. There is no weakening of the C(4)–N(3) bond upon addition of HO^- at C-2. However, a pH-dependent equilibrium for the cyclisation of 2,2,3,5-tetramethylhydantoic acid to the corresponding hydantoin was observed by Kirby *et al.*[19] Cyclisation in this reaction is favoured by the *gem*-dimethyl effect.

We must now consider the relevance of the n.m.r. studies to previous work on the chromogen of the Jaffé reaction. In Vasiliades' work, where creatinine was dissolved in alkaline Me_2SO, ring-opening must occur and the n.m.r. spectra need re-examination with this in mind. In any event, the concentrations are too high for the results to be relevant to the coloured species formed during the analytical procedure. We believe that the evidence[13] in favour of a Janovsky complex still stands but the MNDO calculations do raise other questions. Creatine does not give an immediate positive Jaffé reaction and so reaction must involve an anionic species of creatinine. Methylcreatinine gives a positive Jaffé reaction and so the anionic species cannot be that formed by deprotonation of N-3. It must, therefore, be the carbanion. It is well known that formation of a C–C bond in a Janovsky complex does give the most stable form of σ-complex.

Acid is known to effect ring-closure of creatine to creatinine but the conditions required for this reaction appear to be in some doubt. A solution of creatine in 5M-HCl gave a ^{13}C n.m.r. spectrum with eight peaks (33.7, 39.7, 54.1, 56.6, 159.3, 159.8, 173.5, and 174.7 p.p.m.). From our data, and those of Kenyon *et al.*,[6] it is clear that the solution contains both creatine and creatinine. However, ring-closure at ambient temperature is a slow process and the values of [creatine]/[creatinine] after 0, 3, 5, and 7 days were 3.1, 0.9, 0.5, and 0.3. It is clear that quantitative conversion of creatine into creatinine will not occur under the mild conditions (picric acid for 1 h) given by Varley *et al.*[20] The acid-catalysed cyclisation of hydantoic acid to hydantoin appears to occur more readily.[21]

Experimental

All reagents were the purest commercially available. Methylcreatinine hydriodide was prepared by the method of Kenyon and Rowley.[5] For the n.m.r. spectra 3-trimethylsilylpropane-1-sulphonic acid was used as the standard.

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