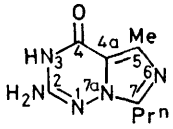
On the Site of Protonation of the 2-Aminoimidazo[5,1-*f*][1,2,4]triazine System: *X*-Ray, ^{13}C Nuclear Magnetic Resonance, and CNDO/2 Studies

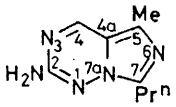
By **J. Patrick Riley, Frank Heatley,** and **Ian H. Hillier,*** Chemistry Department, University of Manchester, Manchester M13 9PL
Peter Murray-Rust and Judith Murray-Rust, Chemistry Department, University of Stirling, Stirling FK9 4LA

The preferred site of protonation of the 2-aminoimidazo[5,1-*f*][1,2,4]triazine system has been studied by crystallographic, ^{13}C n.m.r., and semi-empirical molecular orbital methods. In each case it is found that protonation occurs at N(6).

THE syntheses of 2-amino-5-methyl-7-propylimidazo-[5,1-*f*][1,2,4]triazin-4(3*H*)-one (1) and the corresponding triazine (2) have been recently reported.[1] Both compounds form a monobasic salt but it is not obvious which nitrogen atom is protonated. The position of protonation is relevant to an understanding of the molecular features which contribute to the biological activity of these substances and also to the design of related drugs. This paper describes *X*-ray and ^{13}C n.m.r. results together with semi-empirical molecular orbital calculations which provide strong evidence that it is the nitrogen atom at position 6 which is protonated in both molecules.



(1)



(2)

EXPERIMENTAL

Crystallography.—After considerable searching it was possible to find a good single crystal of the hydrated methanesulphonate of (1), but because this lost water very easily it was sealed in a capilliary tube. Weissenberg photography showed the data to be good and the crystal system to be triclinic. Unit-cell data were determined from precession photographs. The density and structure analysis confirmed the crystal form to be a monohydrate.

Crystal data. $C_9H_{14}N_5O^+ \cdot CH_3O_3S^- \cdot H_2O$, $M = 321.2$. Triclinic, $a = 9.65(1)$, $b = 9.25(1)$, $c = 9.78(1)$ Å, $\alpha = 101.32(3)$, $\beta = 102.47(3)$, $\gamma = 109.94(3)°$, $U = 766$ Å^3, $D_m = 1.387$, $Z = 2$, $D_c = 1.392$ g cm^{-3}, $F(000) = 320$. Mo-K_α radiation, $\lambda = 0.710\,7$ Å; μ(Mo-K_α) = 1.88 cm^{-1}. Space group $P\bar{1}$. Crystal dimensions *ca.* 0.4 × 0.4 mm.

Data were collected for *hk*0—6 with $\theta_{max.}$ 27.5° on a Stoe Stadi 2 two-circle diffractometer (graphite monochromated Mo-$K_{\bar{\alpha}}$ radiation). This gave 2 856 data of which 2 072 unique reflexions had $I > 4\sigma(I)$. Lorentz and polarisation corrections (but none for absorption) were applied and the data scaled by a Wilson plot. The structure was solved by Patterson and Fourier methods with the SHELX system of crystallographic programs [2] which was used for all calculations on the UMRCC CDC 7600. Scattering factors were taken from ref. 3. All hydrogen atoms except one in the water molecule were unambiguously located from difference maps which also showed that the methanesulphonate group was rotationally disordered about the C–S axis. Fractional occupancies summing to unity were introduced for two independent SO_3 groups and the structure refined by full-matrix least-squares in two blocks with unit weights. All heavier atoms were allowed anisotropic temperature factors and hydrogen atoms were given individual isotropic temperature factors and their positions allowed to vary freely. Refinement converged with a conventional *R* of 0.044 when all shifts in parameters were $<\sigma$. Positional parameters are given in Table 1.†

TABLE 1

Fractional atomic co-ordinates (× 10^4) with estimated standard deviations in parentheses

	x/a	*y/b*	*z/c*	Occupancy
C(2)	6 258(3)	4 588(3)	1 644(3)	
C(4)	8 009(3)	5 528(3)	0 241(3)	
C(5)	6 767(3)	3 529(3)	−2 384(3)	
C(7)	4 646(3)	1 989(3)	−1 949(3)	
C(10)	7 822(5)	4 059(5)	−3 267(5)	
C(11)	3 119(3)	0 694(3)	−2 161(4)	
C(12)	3 203(4)	−0 859(4)	−2 041(7)	
C(13)	1 622(5)	−2 157(5)	−2 271(6)	
C(20)	2 180(6)	0 500(7)	1 614(5)	
C(4a)	6 879(3)	4 152(3)	−0 964(3)	
N(1)	5 172(3)	3 358(2)	0 576(2)	
N(2)	6 046(4)	4 884(4)	2 959(3)	
N(3)	7 599(3)	5 622(3)	1 504(3)	
N(6)	5 393(3)	2 205(3)	−2 944(3)	
N(7a)	5 552(2)	3 165(2)	−0 718(2)	
O(1)	9 200(3)	6 499(2)	0 174(2)	
O(2)	1 353(4)	0 697(5)	3 889(4)	8 087(67)
O(3)	3 849(5)	0 601(5)	4 055(4)	8 087(67)
O(4)	3 449(5)	2 990(3)	3 875(3)	8 087(67)
O(5)	9 669(3)	7 802(3)	4 086(3)	
O(21)	2 775(25)	0 046(16)	4 314(14)	1 913(67)
O(31)	2 039(17)	2 220(19)	4 160(15)	1 913(67)
O(41)	4 463(15)	2 375(24)	3 885(14)	1 913(67)
S(1)	2 791(1)	1 270(1)	3 524(1)	
H(3)	8 162(33)	6 286(34)	2 217(33)	
H(5)	0 602(43)	1 933(45)	5 419(42)	
H(6)	5 019(34)	1 636(36)	−3 819(36)	
H(2a)	5 161(40)	4 286(39)	3 083(35)	
H(2b)	6 699(40)	5 733(43)	3 617(40)	
H(10a)	7 984(54)	3 227(62)	−3 800(55)	
H(10b)	8 792(63)	4 632(62)	−2 757(57)	
H(10c)	7 542(67)	4 692(72)	−3 682(67)	
H(11a)	2 628(39)	1 083(41)	−1 465(40)	
H(11b)	2 489(44)	0 491(44)	−3 187(46)	
H(12a)	3 437(52)	−1 160(53)	−3 080(54)	
H(12b)	3 766(90)	−0 487(92)	−1 118(95)	
H(13a)	1 695(55)	−2 730(58)	−1 862(55)	
H(13b)	0 858(52)	−2 381(50)	−3 193(52)	
H(13c)	0 946(56)	−1 640(57)	−1 614(55)	
H(20a)	1 539(74)	0 715(74)	1 283(69)	
H(20b)	1 680(54)	−0 584(61)	1 417(49)	
H(20c)	3 245(71)	0 773(65)	1 247(61)	

† Lists of structure factors and thermal parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 22514 (18 pp., 1 microfiche). See Notice to Authors No. 7 in *J.C.S. Perkin II*, 1978, Index issue.

^{13}C *N.m.r. Results.*—The proton noise-decoupled ^{13}C n.m.r. spectrum of (2) and for the hydrochloride (both in dimethyl sulphoxide) were obtained by use of a Varian SC 300 spectrometer operating at 75.5 MHz. The assignment of the spectrum of (2), summarised in Table 2, was based on a comparison with previously assigned spectra,[4-6] particularly those of 2,4-diazaindene and purine. Table 3 shows a corresponding assignment of the ^{13}C n.m.r. spectrum of the hydrochloride of (2), arrived at by assuming that the peaks do not change their relative positions upon protonation. To use these data to determine the site of protonation we refer to the work of Pugmire and Grant [7] who studied the ^{13}C n.m.r. spectrum of purine, and its anion and cation. To interpret such spectra they quote the following shifts arising from protonation of the nitrogen atom of pyridine: these are (compared with those for the free base) 7.8 (α), −4.4 (β), and −12.7 (γ) p.p.m. They then used these rules that the shielding at a carbon atom α to the site of protonation is increased, whilst the shieldings of the β and γ carbon atoms are decreased. Application of these empirical rules to our data in Table 3 suggests that the predominant site of protonation is nitrogen atom 6, the shieldings at the α carbon atoms (5, 7) being increased, whilst that at the γ carbon atom (4) is decreased. However, the small increase in shielding at carbon atom (4a) [β to nitrogen atom (6)] may indicate that protonation at the bridgehead nitrogen atom (7a) occurs to some small extent.

Molecular Orbital Results.—Semi-empirical MO calculations by use of the CNDO/2 method of Pople [8] were used to calculate the total energy of (2) and its various protonated species. The bicyclic system was taken to be planar and the bond lengths were, in the main, taken from our structure for (1). The relative energies listed in Table 4 lead to the conclusion that protonation is at N-6. The magnitude of these energy differences is in line with the results of Neiman [9] who performed a similar theoretical study of the site of protonation of purine.

TABLE 2

Assignment of ^{13}C n.m.r. spectrum of (2)

Exp. peak posn.	Assignment	
δ_C *	Carbon atom	Reason
157.2	2	Bonded to $-NH_2$, downfield shift
117.2	4a	High-field, slightly br perhaps unresolved m
153.4	4	Large −CH splitting
134.8	5	Quartet splitting from −Me
140.6	7	Elimination

* Chemical shift in p.p.m. downfield from tetramethylsilane as internal standard. Derived assuming δ_C of dimethyl sulphoxide to be 39.6.

TABLE 3

^{13}C N.m.r. spectrum of hydrochloride of (2)

Exptl. peak posn. δ_C	C atom	Δ/p.p.m.*
158.3	2	−1.1
115.8	4a	1.4
155.7	4	−2.3
127.8	5	7.0
136.5	7	4.1

* Positive value indicates upfield shift (increased shielding).

TABLE 4

Relative energies (eV) calculated by the CNDO method for (2) and its protonated species

Protonation site of parent molecule	Energy
(2)	0
N(7a)	−7.2
N(1)	−14.0
N(3)	−14.3
N(6)	−14.8

RESULTS AND DISCUSSION

Crystallographic Results.—*X*-Ray crystallographic investigations were confined to the hydrated methanesulphonate of (1), since suitable single crystals of salts of (2) could not be obtained. All atoms in the structure were located except one hydrogen atom in the water molecule. The methanesulphonate ion is rotationally disordered but apart from this there is no evidence of fractional occupancy for any other atoms. In particular the proton attached to N(6) is especially well defined having one of the lowest temperature factors of the hydrogen atoms and showing no signs of less than unit occupancy. This is strong evidence that protonation occurs at this point in the crystal form studied. A final difference map showed no evidence for protonation near any of the other potentially basic atoms, although of course some residual electron density could be seen which was due to the lone pairs of electrons. Confirmation of the site of protonation comes from the N(6) · · · O(3) distance (2.81 Å) which can only be explained by a hydrogen bond between these atoms. There were no other interatom contacts (see later) which suggested alternative sites of protonation. This bond is somewhat destroyed by the rotation of the SO_3^- group and becomes bifurcated with N(6) · · · O(21) 2.99 and N(6) · · · O(41) 3.07 Å (Table 5).

TABLE 5

Some intermolecular contacts (Å) <3.0 Å

Hydrogen bonds			
N(3) · · · O(5)	2.74	N(2) · · · O(41)	2.76
N(6) · · · O(3^{I})	2.81	O(5) · · · O(2^{II})	2.69
N(6) · · · O(21^{I})	2.99	O(5) · · · O(21^{II})	2.96
N(6) · · · O(41^{I})	3.07	O(5) · · · O(2^{III})	2.74
N(2) · · · O(4)	2.96	O(5) · · · O(31^{III})	2.60
Others			
N(6) · · · O(3^{IV})	2.99		

Roman numeral superscripts denote the following equivalent positions relative to the reference molecule at x, y, z:

I $x, y, 1 + z$ III $1 - x, 1 - y, 1 - z$
II $x - 1, y - 1, z$ IV $1 - x, -y, -z$

Bond lengths and angles in the ions are given in Table 6 along with the estimated standard deviations calculated from the normal matrix. There is nothing unexpected in the geometry and since the ring system has not been studied previously it is not possible to see if protonation affects the bonds and angles at N(6). There is extensive hydrogen bonding in the structure but it is not very easy to interpret since the disordered SO_3 group plays a major part in it. It is probable that one of the hydrogens on the water molecule is also disordered because of this, and a rather complex pattern of inter-

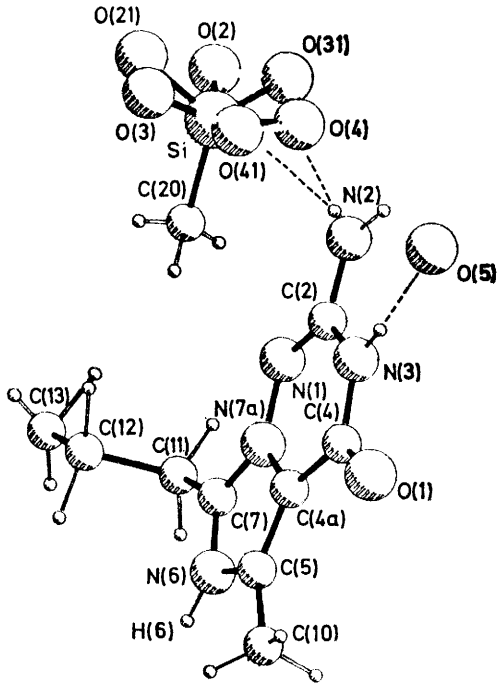
molecular contacts is found for this molecule. The crystallochemical unit is shown in the Figure.

Conclusion.—The preferred site of protonation of the aminoimidazotriazine system has been studied by three methods, corresponding to the gas, solution, and solid states. There are reservations associated with each method and it is therefore very gratifying to see that in each case the same answer has been convincingly given. Crystal packing forces are well known to be able to cause quite large distortions in molecules and are certainly capable of affecting the site of protonation if the enthalpy difference is not too large. In particular, the detailed arrangement of hydrogen bonding could critically affect the approach of the cations and anions and alter the electrostatic potentials near atoms able to be protonated. Similarly, in solution the site of protonation might depend on the nature of the solvent. However, the CNDO/2 calculations suggest that protonation at N(6) is favoured over other positions by *ca.* 10 kcal mol^{-1}, and it is gratifying to see that this is confirmed by the unambiguous nature of the results from solution and solid phases. We can fairly confidently predict therefore that regardless of the environment N(6) is considerably more basic than any other site in the molecule and that this will probably apply when (2) interacts with biological molecules.

TABLE 6

Bond angles (°) and distances (Å) with estimated standard deviations in parentheses

C(2)–N(1)	1.316(3)	C(20)–S(1)	1.750(4)
C(2)–N(2)	1.337(4)	C(4a)–N(7a)	1.398(3)
C(2)–N(3)	1.375(4)	N(1)–N(7a)	1.388(3)
C(4)–C(4a)	1.452(4)	O(2)–O(31)	1.278(15)
C(4)–N(3)	1.373(4)	O(2)–S(1)	1.457(3)
C(4)–O(1)	1.218(3)	O(3)–O(21)	1.097(18)
C(5)–C(10)	1.493(5)	O(3)–S(1)	1.423(3)
C(5)–C(4a)	1.361(4)	O(4)–O(31)	1.420(15)
C(5)–N(6)	1.368(3)	O(4)–O(41)	1.288(21)
C(7)–C(11)	1.488(4)	O(4)–S(1)	1.433(3)
C(7)–N(6)	1.337(4)	O(21)–S(1)	1.489(13)
C(7)–N(7a)	1.334(3)	O(31)–S(1)	1.450(11)
C(11)–C(12)	1.491(4)	O(41)–S(1)	1.503(11)
C(12)–C(13)	1.522(5)		
C(10)–H(10a)	0.921(52)	C(13)–H(13c)	1.158(53)
C(10)–H(10b)	0.873(53)	C(20)–H(20a)	0.742(63)
C(10)–H(10c)	0.858(63)	C(20)–H(20b)	0.910(48)
C(11)–H(11a)	0.988(36)	C(20)–H(20c)	1.125(62)
C(11)–H(11b)	0.999(40)	N(2)–H(2a)	0.894(33)
C(12)–H(12a)	1.092(47)	N(2)–H(2b)	0.847(36)
C(12)–H(12b)	0.876(82)	N(3)–H(3)	0.774(28)
C(13)–H(13a)	0.737(50)	N(6)–H(6)	0.837(31)
C(13)–H(13b)	0.967(46)		
N(2)–C(2)–N(1)	118.3(3)	O(31)–S(1)–O(3)	136.3(6)
N(3)–C(2)–N(1)	124.8(3)	O(31)–S(1)–O(4)	59.0(6)
N(3)–C(2)–N(2)	116.9(3)	O(31)–S(1)–O(21)	107.5(9)
N(3)–C(4)–C(4a)	112.3(2)	O(41)–S(1)–C(20)	103.7(5)
O(1)–C(4)–C(4a)	125.1(3)	O(41)–S(1)–O(2)	152.0(6)
O(1)–C(4)–N(3)	122.6(3)	O(41)–S(1)–O(3)	66.2(9)
C(4a)–C(5)–C(10)	131.5(3)	O(41)–S(1)–O(4)	52.0(9)
N(6)–C(5)–C(10)	123.0(3)	O(41)–S(1)–O(21)	106.2(10)
N(6)–C(5)–C(4a)	105.5(2)	O(41)–S(1)–O(31)	106.8(10)
N(6)–C(7)–C(11)	127.2(2)	H(10a)–C(10)–C(5)	113.0(29)
N(7a)–C(7)–C(11)	126.7(3)	H(10b)–C(10)–C(5)	114.9(33)
N(7a)–C(7)–N(6)	106.1(2)	H(10b)–C(10)–H(10a)	96.2(39)
C(12)–C(11)–C(7)	113.9(3)	H(10c)–C(10)–C(5)	107.3(39)
C(13)–C(12)–C(11)	112.5(3)	H(10c)–C(10)–H(10a)	120.5(49)
C(5)–C(4a)–C(4)	134.8(3)	H(10c)–C(10)–H(10b)	104.5(44)
N(7a)–C(4a)–C(4)	118.2(2)	H(11a)–C(11)–C(7)	109.2(19)
N(7a)–C(4a)–C(5)	107.0(2)	H(11a)–C(11)–C(12)	111.2(21)
N(7a)–N(1)–C(2)	112.4(2)	H(11b)–C(11)–C(7)	105.4(22)
C(4)–N(3)–C(2)	125.4(2)	H(11b)–C(11)–C(12)	107.0(22)
C(7)–N(6)–C(5)	111.9(2)	H(11b)–C(11)–H(11a)	109.9(30)
C(4a)–N(7a)–C(7)	109.5(2)	H(12a)–C(12)–C(11)	95.3(24)
N(1)–N(7a)–C(7)	123.6(2)	H(12a)–C(12)–C(13)	104.2(24)
N(1)–N(7a)–C(4a)	126.8(2)	H(12b)–C(12)–C(11)	95.7(52)
S(1)–O(2)–O(31)	63.6(6)	H(12b)–C(12)–C(13)	111.6(52)
S(1)–O(3)–O(21)	71.1(8)	H(12b)–C(12)–H(12a)	134.7(60)
O(41)–O(4)–O(31)	122.1(9)	H(13a)–C(13)–C(12)	110.8(37)
S(1)–O(4)–O(31)	61.1(5)	H(13b)–C(13)–C(12)	114.6(26)
S(1)–O(4)–O(41)	66.8(6)	H(13b)–C(13)–H(13a)	127.2(45)
S(1)–O(21)–O(3)	64.7(7)	H(13c)–C(13)–C(12)	110.3(23)
O(4)–O(31)–O(2)	124.1(9)	H(13c)–C(13)–H(13a)	96.4(44)
S(1)–O(31)–O(2)	64.2(6)	H(13c)–C(13)–H(13b)	91.9(33)
S(1)–O(31)–O(4)	59.9(5)	H(20a)–C(20)–S(1)	110.5(50)
S(1)–O(41)–O(4)	61.2(6)	H(20b)–C(20)–S(1)	105.5(29)
O(2)–S(1)–C(20)	103.0(2)	H(20b)–C(20)–H(20a)	101.4(52)
O(3)–S(1)–C(20)	105.9(3)	H(20c)–C(20)–S(1)	107.6(28)
O(3)–S(1)–O(2)	113.8(3)	H(20c)–C(20)–H(20a)	121.1(59)
O(4)–S(1)–C(20)	107.3(2)	H(20c)–C(20)–H(20b)	109.6(39)
O(4)–S(1)–O(2)	111.2(2)	H(2a)–N(2)–C(2)	119.9(21)
O(4)–S(1)–O(3)	114.5(2)	H(2b)–N(2)–C(2)	118.0(24)
O(21)–S(1)–C(20)	114.4(5)	H(2b)–N(2)–H(2a)	121.4(33)
O(21)–S(1)–O(2)	69.7(8)	H(3)–N(3)–C(2)	115.2(22)
O(21)–S(1)–O(3)	44.2(7)	H(3)–N(3)–C(4)	119.4(22)
O(21)–S(1)–O(4)	137.1(5)	H(6)–N(6)–C(5)	124.8(21)
O(31)–S(1)–C(20)	117.4(6)	H(6)–N(6)–C(7)	123.2(21)
O(31)–S(1)–O(2)	52.2(6)		



A view of the cation with the crystallographic atom numbering system

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