Purine-6,8-diones: a Study of their Ionisation and their Methylation Reactions

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Purine-6,8-diones are divided by their physical properties into three classes: those of class (a) carry a hydrogen atom or a methyl group at position 1, and those of class (b) bear a 3-methyl substituent. The members of class (c), bearing both 1- and 3-methyl groups, exist either as betaines or, after protonation, as the conjugate acids, with the positive charge confined to the pyrimidine ring. Anions are formed predominantly by proton loss from position 9. Consistent with this, methylation of monoanions proceeds at position 9, except in the case of members of class (b), where alkylation at N-9 is sterically hindered by the 3-methyl substituent. Methylation of dianions follows in general the sequence of proton attachment, unless steric factors prevent this.

Protonation and alkylation of neutral molecules proceeds predominantly in the pyrimidine ring. However in the case of 9-methyl derivatives, methylation at N-3 is sterically hindered.

THE behaviour of purines towards nucleophilic and electrophilic reagents depends on the molecular form involved (neutral form, anion, or cation). This is exemplified in the present study of purine-6,8-diones. We have established the predominant tautomeric forms of these compounds in aqueous solution; we have also attempted to define the sites associated with the various ionisation processes and have determined the course of electrophilic methylation.

TABLE 1

U.v. absorption spectra of purine-6,8-diones * ($\lambda_{max.}$ in nm)

		N		A_1			A_2		C_1			C_2	
No.	Posns. of Me groups	$\lambda_{max.}$	log ε	$\lambda_{max.}$	log ε	$\Delta(A_1 - N)$	$\lambda_{max.}$	$\Delta(A_2 - A_1)$	$\lambda_{max.}$	log ε	$\Delta(C_1 - N)$	$\lambda_{max.}$	$\Delta(C_2 - C_1)$
(1)		256	3·98	267	3·97	+11	272	+5	>271	3·99	>+15		
(2)	1	257	3·96	274	3·97	+17	>279	>+5	>268	3·98	>+11		
(3)	3	275	4·06	284	4·14	+9	>285	>+1	273	4·06	−2	271	−2
(4)	7	258	4·06	270	4·06	+12	273	+3	>270	4·09	>+12		
(5)	9	258	4·07	263	4·08	+5	>273	>+10	>270	4·09	>+12		
(6)	1,3	285 [a]	4·01	285 [b]	3·81	0			272	4·05	−13	<270	>−2
(7)	1,7	261	3·97	275	3·99	+14			>272	4·01	>+11		
(8)	1,9	258	4·04	272	4·03	+14			>268	4·06	>+10		
(9)	3,7	277	4·13	285	4·15	+8			273	4·07	−4		
(10)	3,9	278	4·24	290	4·16	+12			278	4·19	0		
(11)	7,9	260	4·02	267	4·05	+7			>271	4·07	>+11		
(12)	1,3,7	287 [a,c]							274		−13		
(13)	1,3,9	269 [a,c]							273		+4	>278	>+5
(14)	1,7,9	262	3·93						>272	3·96	>+10		
(15)	3,7,9	280	4·17						277	4·10	−3		

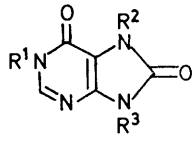
* N = neutral molecule, A_1 = monoanion, A_2 = dianion, C_1 = monocation, C_2 = dication.

[a] Zwitterion. [b] Zwitterion–anion. [c] These measurements were carried out with the crude iodides. The pure compounds were isolated as picrates.

Tautomerism.—As shown in Table 1, the neutral forms of the parent compound (1) and of the *N*-methyl derivatives (2), (4), (5), (7), (8), (11), and (14) exhibit similar u.v. absorption maxima ($\lambda_{max.}$ 259 ± 3 nm) and extinction coefficients (log $\varepsilon_{max.}$ 4·00 ± 0·07). Compound (14) has a fixed structure; hence all the derivatives just mentioned share structure (I), and these are designated class (a). Thus all those members of class (a) which contain NH groups are present in aqueous solution predominantly in the lactam form.

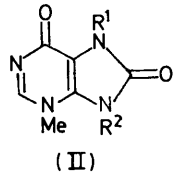
Introduction of a 3-methyl substituent [class (b)] shifts the value of $\lambda_{max.}$ to 277·5 ± 2·5 nm and raises the extinction (log ε) to 4·15 ± 0·09. Hence compounds (3), (9), (10), and (15) are in the same tautomeric form (II), which is fixed in compound (15).

Class (c) comprises the 1,3-dimethyl derivatives (6), (12), and (13). These compounds, whether present as betaines [(IIIA and B) and (IVA)] or their conjugate acids [(IIIC) and (IVB)], always bear a positive charge in the pyrimidine ring. The betaine forms of compounds (6) and (12) show $\lambda_{max.}$ 286 ± 1 nm, but the absorption maximum of the betaine (IVA) is at 269 nm. Presumably this difference is due to spreading of the negative charge over both rings in (IIIA and B). Indeed when



(I)

(1) $R^1 = R^2 = R^3 = H$
(2) $R^1 = Me$, $R^2 = R^3 = H$
(4) $R^2 = Me$, $R^1 = R^3 = H$
(5) $R^3 = Me$, $R^1 = R^2 = H$
(7) $R^1 = R^2 = Me$, $R^3 = H$
(8) $R^1 = R^3 = Me$, $R^2 = H$
(11) $R^2 = R^3 = Me$, $R^1 = H$
(14) $R^1 = R^2 = R^3 = Me$



(II)

(3) $R^1 = R^2 = H$
(9) $R^1 = Me$, $R^2 = H$
(10) $R^2 = Me$, $R^1 = H$
(15) $R^1 = R^2 = Me$

TABLE 2

pK Values and R_F values of purines-6,8-diones

No.	Posns. of Me groups	pK: Anion formation N-1	Anion formation N-7	Anion formation N-9	pK: Cation formation	R_F in solvent [a] (A)	(B)	(C)
(1)		(>14	11·4	8·2) [b]	<−1·9	0·24	0·27	0·61
(2)	1		>13	8·2	<−1·8	0·31	0·48	0·63
(3)	3		>13	5·5	*ca.* +1·5	0·22	0·26	0·44
(4)	7	10·5		7·8	<−2	0·44	0·43	0·66
(5)	9	(8·4	>13) [b]		<−2	0·40	0·36	0·63
(6)	1,3		5·3 [c]	2·8 [d]	<−2 [e]	0·18		0·50
(7)	1,7			8·5	<−2	0·55	0·49	0·67
(8)	1,9		8·5		<−1·5	0·56	0·61	0·67
(9)	3,7			6·0	+1·5	0·36	0·24	0·51
(10)	3,9		9·0		+1·3 [f]	0·22	0·33	0·56
(11)	7,9	9·2			<−1·5	0·67	0·51	0·66
(12)	1,3,7			2·9 [d]	[g]	0·21		0·54
(13)	1,3,9		2·4 [h]		−1·5 [e]	0·16		0·78
(14)	1,7,9				<−2	0·88	0·71	0·72
(15)	3,7,9				+1	0·44	0·50	0·63

[a] All compounds show violet fluorescence. For composition of solvents see Experimental section. [b] pK Values in parentheses indicate that dissociation of particular NH groups cannot be determined unequivocally because of formation of tautomeric anions. [c] Transition from zwitterion (IIIA and B) to zwitterion–anion (VII). [d] Transition from cation (IIIC) to zwitterion (IIIA and B). [e] For dication. [f] Since no change of $\lambda_{max.}$ was observed for this transition, the pK was determined by measuring $\delta_{2\text{-H}}$ as function of pH. [g] No change of $\lambda_{max.}$, extinction, or n.m.r. signals was observed even at pH −3. Apparently compound (12) does not form a dication. [h] Transition from cation (IVB) to zwitterion (IVA).

TABLE 3

N.m.r. spectra of purine-6,8-diones in D_2O at 70 °C

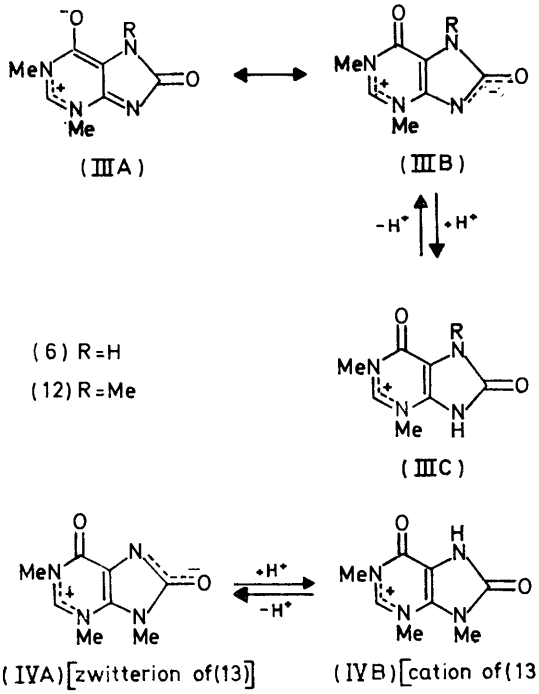
No.	Posns. of Me groups	$\delta_{2\text{-H}}$ N	A	Δ(N − A)	C	(Δ(N − C)	δ_{NMe} [a] N	A	Δ(N − A)	C	Δ(N − C)
(1)		8·24	8·17 [b]	+0·07	8·91	−0·67					
(2)	1	8·37	8·20 [c]	+0·17	9·05	−0·68	3·73	3·70	+0·03	3·86	−0·13
(3)	3	8·37	8·25 [d]	+0·12	9·16	−0·79	3·99	3·82	+0·17	4·19	−0·20
(4)	7	8·23	8·11	+0·12	8·94	−0·71	3·65	3·66	−0·01	3·70	−0·05
(5)	9	8·26	8·15 [e]	+0·11	8·67	−0·41	3·44	3·43	+0·01	3·66	−0·22
(6)	1,3	9·21 [f,g]			9·47 [h]	−0·26	(1) 3·82			3·89	−0·07
							(3) 4·06			4·17	−0·11
(7)	1,7	8·29	8·10	+0·19	8·98	−0·69	(1) 3·68	3·62	+0·06	3·83	−0·15
							(7) 3·62	3·59	+0·03	3·65	−0·03
(8)	1,9	8·40	8·17	+0·23	8·95	−0·55	(1) 3·71	3·65	+0·06	3·89	−0·18
							(9) 3·45	3·40	+0·05	3·58	−0·13
(9)	3,7	8·33	8·11	+0·22	9·03	−0·70	(3) 3·94	3·81	+0·13	4·09	−0·15
							(7) 3·63	3·58	+0·05	3·60	+0·03
(10)	3,9	8·24	8·01	+0·23	9·05	−0·81	(3) 4·21	4·11	+0·10	4·38	−0·17
							(9) 3·79	3·67	+0·12	3·78	+0·01
(11)	7,9	8·18	8·07	+0·11	8·31	−0·13	(7) 3·63	3·60	+0·03	3·67	−0·04
							(9) 3·40	3·37	+0·03	3·50	−0·10
(12)	1,3,7 [i]	9·19 [f]			9·54 [h]	−0·35	(1) 3·75			3·80	−0·05
							(3) 3·97			4·12	−0·15
							(7) 3·60			3·63	−0·03
(13)	1,3,9 [i]	9·48 [f]			9·49 [k]	−0·01	(1) 3·81			3·80	+0·01
							(3) 4·39			4·36	−0·01
							(9) 3·74			3·75	−0·01
(14)	1,7,9	8·38			8·47	−0·09	(1) 3·67			3·70	−0·03
							(7) 3·62			3·62	0
							(9) 3·43			3·44	−0·01
(15)	3,7,9	8·26			9·07	−0·81	(3) 4·16			4·35	−0·19
							(7) 3·65			3·67	−0·02
							(9) 3·77			3·80	−0·03

[*] N = neutral molecule, A = anion, C = cation.

[a] Figures in parentheses indicate the *N*-methyl group involved. [b] The dianion of (1) shows $\delta_{2\text{-H}}$ 8·09. [c] The dianion of (2) shows $\delta_{2\text{-H}}$ 8·11; $\delta_{1\text{-Me}}$ 3·64. [d] Dianion of (3): $\delta_{2\text{-H}}$ 7·93; $\delta_{3\text{-Me}}$ 3·79; measurements at room temp., because the compound tended to decompose at higher temperatures. [e] The dianion shows $\delta_{2\text{-H}}$ 8·02; $\delta_{9\text{-Me}}$ 3·40. [f] The values under N refer to the zwitterion (IIIA and B). [g] Because of rapid H–D exchange, this 2-H signal was determined in H_2O solution. [h] The values under C refer to the cationic salt (IIIC). [i] These measurements were carried out at room temp., because the compound decomposes at 70° [k] The values under C refer to the cationic salt (IVB).

this resonance possibility is eliminated by protonation, a hypsochromic shift of 13 nm is observed, and the $\lambda_{max.}$ values of the cations (IIIC) are almost identical with that of (IVB) (Table 1).

The n.m.r. spectra (Table 3) reveal a significant influence of *N*-methyl substituents in the pyrimidine ring on the 2-proton. When positions 1 and 3 lack an alkyl group, the 2-H signal is at highest field [δ 8·22 $\pm$ 0·04 in compounds (1), (4), (5), and (11)]. Introduction of a 1- or a 3-methyl group deshields the 2-H by 0·06—0·20 p.p.m.



In members of class (c), the 2-H signal is at δ 9·2—9·5, *i.e. ca.* 1 p.p.m. downfield of the average value for classes (a) and (b) (see Table 3). This is characteristic of purine cations bearing an amidinium-like structure.[1] Furthermore, only in the members of class (c) does the 2-H undergo rapid exchange in D_2O at 70°, in accord with earlier observations in other series of purines.[2]

Anion Formation.—Class (a). In compounds (7) and (8) anion formation is characterised by identical p*K* values (8·2; Table 2) and bathochromic displacements of $\lambda_{max.}$ (14 nm), and by similar upfield shifts of the 2-H n.m.r. signals. In other words, dissociations of the 7- and of the 9-protons are not clearly distinguishable, in spite of the fact that resonance stabilisation of the two anions involves quite different structures [(V) and (VI)]. In contrast, dissociation of the 1-proton in compound (11) is characterised by a p*K* value of 9·2 and by a much smaller displacement of $\lambda_{max.}$ (7 nm). On this basis, it is possible to establish the ionisation sequence for certain members of the present series.

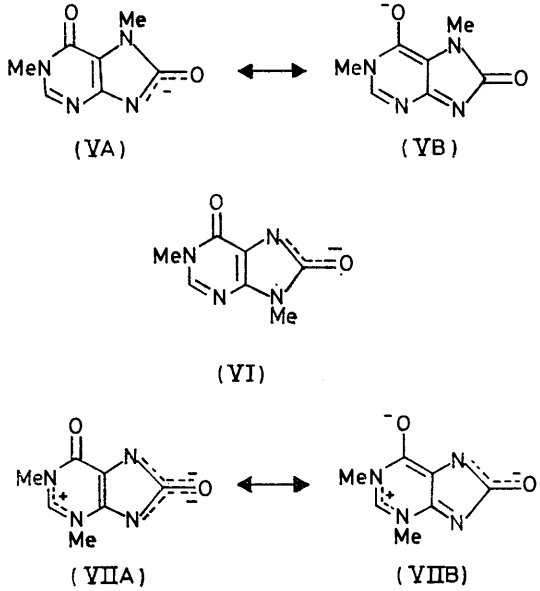
In the 7-methyl derivative (4) monoanion formation is characterised by p*K* 7·8, $\Delta\lambda_{max.}$ +12 nm, and $\delta_{2\text{-H}}$ 8·11, and thus closely resembles the behaviour of (7). Therefore the main dissociation sequence is assumed to be 9-H, 1-H.

The first p*K* value of the 9-methyl derivative (5) (8·4) is close to that of (8); also the $\delta_{2\text{-H}}$ values of the anions of (5) and (8) are very similar. However the bathochromic shift of $\lambda_{max.}$ accompanying anion formation in (5) is only 5 nm, but in (8) it is 14 nm. Thus the monoanion of (5) may be a mixture of tautomers, both the 7- and the 1-proton participating in dissociation.

For the 1-methyl derivative (2) the first p*K* value (8·2) is in the same range as for (4), (5), (7), and (8). Likewise, $\lambda_{max.}$ of the monoanion of (2) resembles those of the anionic forms of (7) and (8). Therefore in (2), dissociation of both the 7- and the 9-proton may contribute to monoanion formation; no inference about the preference for one or the other of the tautomeric anions can be drawn from the physical data available.

The first p*K* value of compound (1) (8·2) is the same as for (2), but $\lambda_{max.}$ of the anion is 7 nm lower than for the monoanion of (2). A similar difference (9 nm) was observed for the absorption maxima of (5) and (8) (see above). Therefore it appears that all three NH groups in (1) may contribute to monoanion formation.

Class (b). The 3-methyl derivatives (3) and (9) have exceptionally low p*K* values (5·5 and 6). In (9), anion formation can involve only the 9-proton. On the other hand, the p*K* value for dissociation of the 7-proton in the 3,9-dimethyl derivative (10) (9·0) resembles that of the 1,9-dimethyl isomer (8) (8·5). Thus in class (b) p*K* values for dissociation of the 7-proton are about 3 units higher than for the 9-proton. In (3) the first dissociation



step must involve the 9-proton, *i.e.* the ionisation sequence is 9-H, 7-H. The value of $pK_2 - pK_1$ for (3) is about 8, *i.e.* the first dissociation of this compound is confined to position 9.

[1] D. Lichtenberg, F. Bergmann, and Z. Neiman, *Israel J. Chem.*, 1972, **10**, 805.

[2] D. Lichtenberg and F. Bergmann, *J.C.S. Perkin I*, 1973, 789.

The low pK values of (3) and (9) are similar to those of other purine derivatives bearing both 3- and 9-substituents (H or Me), in which steric strain can be relieved by anion formation.[3,4] For example in all uric acids with a free N(9)H system, the latter undergoes dissociation first, the pK values being in the range 5·5—6·0.[5]

Methylation of Anions of Purine-6,8-diones.—(a) *Monoanions.* Electrophilic alkylation of the monoanions should parallel conversion of the anions into the neutral molecules. However, ionisation, as a reversible process, is thermodynamically controlled, whereas N-alkylation is essentially irreversible and thus subject to kinetic control. Furthermore, because of the bulk of the alkylating reagent, methylation is much more sensitive to steric interference than is binding of a proton. In the series of 6-methylthiopurin-8-ones, these criteria have proved their value for interpretation or prediction of the direction of methylation.[6]

The anions of (3) and (9) do not react with aqueous dimethyl sulphate, even at reflux temperature (Table 4) (no indication of O-methylation was found). Apparently approach of the alkylating agent to position 9, bearing the negative charge, is hindered by the 3-methyl group.

On the other hand, the anion of (10) is methylated without difficulty at N-7 to yield (15), but because of the instability of either (10) or (15) at the pH used, considerable amounts of decomposition products are also found.

The anions of the dimethyl derivatives (7), (8), and (11) are all converted into the same end-product, (14), the yield increasing in the order given for a fixed reaction time of 3 h. This suggests that the rate of alkylation of anions decreases in the order N-1 > N-7 > N-9.

At pH 9—10, the 7-methyl derivative (4) is present predominantly as monoanion, with a small percentage of dianion (Table 2). Therefore attack of a methylating agent should involve mainly position 9 [formation of (11)] and to a lesser degree N-1, leading to compound (7). However the anion of (11) is again rapidly attacked to yield (14) (see above). Indeed, paper chromatography at the beginning of the reaction revealed the presence of (11), whereas after 3 h only a mixture of (7) and (14) remained, in which the latter predominated. These results are in accord with the ionisation sequence already proposed.

The monoanion of the 9-methyl derivative (5) is a mixture of tautomers and thus can be attacked at N-1 and N-7. We should expect formation of a mixture of (8) and (11), with the former predominating, as suggested before. Both these primary products can be converted into (14), albeit at different rates. Small amounts of (11) were indeed obtained; however the 1,9-dimethyl derivative (8) comprised the bulk of the product (Table 4). These results show again that position 7, which is sterically hindered, can be methylated.

The monoanions of (1) and (2) are attacked exclusively at position 9. Chromatographic analysis established the reaction paths as (1) ⟶ (5) ⟶ (8) and (2) ⟶ (8) ⟶ (14), respectively. No signs of reaction at N-7 [formation of (4) or (7)] were found, in contrast to the observations with the monoanion of (5). This suggests that in (1) and (2) dissociation of the 9-proton is mainly responsible for monoanion formation.

(b) *Dianions of compounds* (1)—(5). In the dianion of (3), position 7 is the first to accept a proton. In agree-

TABLE 4

Methylation of the anions of purine-6,8-diones in aqueous solution [a]

		Monoanions				Dianions			
				Products formed				Products formed	
No.	Posns. of Me groups	pH	Yield [b] (%)	Posns. of Me groups	No.	pH	Yield [b] (%)	Posns. of Me groups	No.
(1)		9·5—10·5	50	9 (+1,9) [c]	(5), [(8)]	>12	40	1 + 3 + 7 + 9 +1,9 [d] (+1,7,9)	(2), (3), (4), (5), (8) [(14)]
(2)	1	10—13	40	1,9 + 1,7,9 [e]	(8), (14)	14	60	1,7 + 1,9 [f] (+1,7,9)	(7), (8), [(14)]
(3)	3	8—12		(no reaction)		>14	70	3,7	(9)
(4)	7	9—10	60	1,7 + 1,7,9 [g] (+7,9)	(7), (14), [(11)]	14	70	1,7 + 7,9 +3,7 [h]	(7), (11), (9)
(5)	9	9—10	60	1,9 (+7,9 + 1,7,9)	(8), [(11), (14)]	>14	80	1,9 + 7,9 +1,7,9 [i]	(8), (11), (14)
(6)	1,3			(decomposition) [j]					
(7)	1,7	10—11	20	1,7,9	(14)				
(8)	1,9	10—11	60	1,7,9	(14)				
(9)	3,7	9—10		(no reaction)					
(10)	3,9	10—11	40	3,7,9 [k]	(15)				
(11)	7,9	13—14	80	1,7,9	(14)				

[a] All reactions with Me_2SO_4 in water at reflux temperature for 3 h. For 1 g of monoanion, 0·6 ml of Me_2SO_4 was used; for 1 g of dianion, 0·5 ml Me_2SO_4. Samples for paper chromatography were taken after 1, 10, and 30 min and after 1 and 3 h. They were immediately neutralised and their n.m.r. spectra measured, to investigate the presence of OMe groups. [b] Overall yield. [c] Compounds in square brackets were formed only in trace amounts. [d] Ratio of products 8 : 1 : 1 : 2 : 2. [e] Ratio 5 : 1. [f] Ratio 4 : 1. [g] Ratio 1 : 2; (11) was present only in the early stages of the reaction. [h] Ratio 10 : 2 : 3. [i] Ratio 3 : 1 : 1. [j] Methylation of compound (6) could not be determined because the compound decomposed above pH 8 (no reaction below pH 8). [k] Owing to the thermal instability of (10) and (15), considerable amounts of degradation products were formed.

[3] D. Lichtenberg, F. Bergmann, and Z. Neiman, *J. Chem. Soc.* (*C*), 1971, 1676.

[4] D. Lichtenberg, F. Bergmann, and Z. Neiman, *J.C.S. Perkin II*, 1972, 1676.

[5] F. Bergmann and S. Dikstein, *J. Amer. Chem. Soc.*, 1955, **77**, 691.

[6] F. Bergmann, M. Rahat, and D. Lichtenberg, *J.C.S. Perkin I*, 1973, 1225.

ment with this, alkylation of the dianion yields exclusively (9) (Table 4). The reactivity of the dianion is remarkable in view of the resistance to alkylation of the monoanion and supports the explanation advanced above for the latter phenomenon.

The dianion of (2) gives a 4 : 1 mixture of (7) and (8), with traces of (14). This result supports the assumption that the main ionisation sequence of (2) is 9-H, 7-H.

The dianion of (5) gives a mixture of the same products as the monoanion, but the proportions of (11) and (14) are greater (Table 4). These results, together with the observations on methylation of the monoanion of (5), suggest that the main dissociation sequence is 1-H, 7-H.

With the dianion of (4), position 1 is attacked in preference to N-9, in accord with the fact that the dianion binds the first proton at N-1. However, in addition, methylation occurs at position 3. This requires that position 9 is unsubstituted and that a negative charge is present in the pyrimidine ring. These conditions can be fulfilled only in the dianions of two derivatives, *viz.* (1) and (4).

The dianion of (1) yields a mixture of alkylation products, of which six components have been identified. The appearance of (4) indicates that the 7-position also participates in the ionisation process. Dissociation of the 1-proton permits spreading of the negative charge to N-3 and leads to the formation of (3).

Cation Formation.—In formation of the cations of compounds of class (b), the 2-H n.m.r. signal is shifted downfield by 0·70—0·80 p.p.m. (see Table 3) and $\lambda_{max.}$ shows a small hypsochromic displacement (0—4 nm). Thus these derivatives may undergo protonation predominantly at N-1 to form fixed cations, similar to (IIIC) and (IVB).

In compounds (1), (2), (4), and (7), the corresponding shift in δ_{2-H} is near 0·7 p.p.m., but $\lambda_{max.}$ shows a bathochromic shift of 11—15 nm. This leads to a close similarity of the absorption maxima of these four cations with those of classes (b) and (c), suggesting that here protonation N-3 may make an important contribution.

In the 9-methyl derivatives (5), (8), (11), and (14), which all bear a proton or a methyl group at position 1, protonation at N-3 would create fixed cations with steric strain. This process either is incomplete or does not occur at all, as manifested by the relatively small downfield shift of the 2-H signal (0·09—0·55 p.p.m.). It appears possible that in these four derivatives protonation takes place preferentially on one of the carbonyl groups. Protonation at one of the oxygen atoms must occur also in the formation of dications by compounds (6), (12), and (13). Table 6 shows that in all members of the present series, the net charges at the oxygen atoms are considerably higher than at N-3.

The pK values for cation formation (Table 2) are clearly divided into two groups. In class (b), the values are in the range +1 to +1·5. In class (a), we find values below −1·5. Thus N-1 in class (b) shows greater basicity than N-3 in class (a). This is also manifested in the superdelocalisabilities and net charges for these two positions (Table 6).

TABLE 5

Methylation of neutral molecules of purine-6,8-diones [a]

No.	Posns. of Me groups	Reaction [b]		Yield (%)	Products	
		Time (h)	Temp.		Posn. of Me groups	No.
(1)		6	Reflux	75	1 + 1,3 + 1,9 [c]	(2), (6), (8)
(2)	1	2·5	Reflux	60	1,3	(6)
(3)	3	96	Room temp.	60	1,3	(6)
(4)	7	2	Reflux	40	1,7 [d]	(7)
(5)	9	24	Reflux		(no reaction)	
(6)	1,3	2	Reflux		1 + 1,9 [e]	(2), (8)
(7)	1,7	120	50 °C	40	1,3,7	(12)
(8)	1,9	96	Reflux		(no reaction)	
(9)	3,7	4	95 °C	60	1,7 + 1,3,7 [f]	(7), (12)
(10)	3,9	48	Room temp.	40	1,3,9	(13)
(11)	7,9	96	Reflux		(no reaction)	

[a] All reactions with methyl iodide in dimethylformamide. [b] For 1 g of substrate, 35 ml of solvent were used, but for compound (3), 100 ml and for compound (4), 250 ml were needed. [c] Ratio of products 8 : 3 : 2. [d] The primary product, identified on paper chromatograms, is (12), which decomposes thermally to (7). [e] These products are also formed in the absence of methyl iodide and result from thermal degradation. [f] Ratio 1 : 1; at 95° the primary product (12) suffers slow decomposition to (7).

Methylation of Neutral Molecules.—Electrophilic alkylation of neutral molecules is analogous to their protonation, *i.e.* only unsubstituted nitrogen atoms or the oxygen atoms of the carbonyl groups can be attacked. The conditions for the methylation reactions described in this paragraph varied widely (Table 5). In some cases elevated temperatures were needed to initiate the reaction, with the result that the primary products underwent secondary changes, as will be shown.

In accord with the sites of protonation in (2) and (3), the primary methylation product of either derivative is (6). However the latter is thermolabile and, when heated alone, decomposes to a mixture of (2) and (8) (Table 5). This indicates that part of (6) undergoes 3-dealkylation and part suffers a shift of the 3-methyl group to position 9. This process resembles the thermal degradation of the 1,3-dibenzylhypoxanthine cation, which yields a variety of products by benzyl migration.[7]

[7] J. H. Montgomery, K. Hewson, S. J. Clayton, and H. J. Thomas, *J. Org. Chem.*, 1966, **31**, 2202.

Compound (3) is attacked by methyl iodide even at room temperature, at which (6) is stable. In compound (2), attack of the methylating agent at N-3 is made difficult by the presence of the N(9)H system. Therefore this reaction (in dimethylformamide) requires reflux temperature, which causes thermal degradation of (6) resulting in changes in composition of the mixture with time.

The greater reactivity of (3), as compared to (2), is again reflected by the data in Table 6.

TABLE 6

Superdelocalisabilities for electrophilic attack and net charges of nitrogen atoms in the pyrimidine ring and of carbonyl oxygen atoms in purine-6,8-diones

No.	Posns. of Me groups	Superdelocalisabilities for electrophilic attack at N-1	N-3	O-6	O-8	Net charge N-1	N-3	O-6	O-8
Class (a)									
(1)		1·085	1·154	1·015	0·782	+0·214	−0·261	−0·442	−0·424
(2)	1	1·149	1·179	1·039	0·782	+0·256	−0·264	−0·445	−0·424
(4)	7	1·089	1·157	1·022	0·806	+0·214	−0·260	−0·442	−0·425
(5)	9	1·085	1·155	1·023	0·800	+0·214	−0·261	−0·443	−0·425
(7)	1,7	1·154	1·183	1·046	0·807	+0·256	−0·263	−0·444	−0·425
(8)	1,9	1·149	1·180	1·046	0·800	+0·256	−0·264	−0·445	−0·425
(11)	7,9	1·089	1·158	1·030	0·826	+0·214	−0·260	−0·442	−0·426
(14)	1,7,9	1·154	1·184	1·054	0·836	+0·256	−0·263	−0·445	−0·426
Class (b)									
(3)	3	1·192	1·169	1·053	0·782	−0·308	+0·286	−0·462	−0·425
(9)	3,7	1·195	1·174	1·066	0·807	−0·309	+0·286	−0·461	−0·426
(10)	3,9	1·192	1·169	1·060	0·801	−0·309	+0·286	−0·462	−0·425
(15)	3,7,9	1·195	1·175	1·068	0·826	−0·309	+0·286	−0·462	−0·427

The above results with compound (2) may also explain the observations with (1) as substrate. At reflux temperature, the first product from (1) is the hydroiodide of (3), N-3 being the only position available for attack. Thermal dissociation converts this salt into the free base of (3), which reacts further to give (6). Compound (6) then decomposes to a mixture of (2) and (8) (see Table 5).

The 1,7-dimethyl derivative (7) reacts at moderate temperatures to yield (12). Alkylation of the 7-methyl derivative (4), on the other hand, requires reflux temperature. The first product, identified on chromatograms, is 1,3,7-trimethylpurine-6,8-dione (12), which at the high temperature used undergoes progressive demethylation to give (7). The same sequence is observed during the reaction of the 3,7-dimethyl derivative (9), but here the conditions required are less drastic. It thus appears probable that (4) is first attacked slowly at position 3, a process paralleling the reaction path of (1). At the high temperature required for methylation of (4), the intermediate hydroiodide of (9) dissociates and the free base is rapidly converted into (12), which then decomposes slowly. We thus propose the following sequence for methylation of (4): (4) ⟶ (9) ⟶ (12) ⟶ (7).

The 9-methyl derivatives (5), (8), and (11) resist alkylation even at reflux temperatures (about 150°), a reaction for which only position 3 would be available. The negative results correspond to the observation that protonation of these three derivatives at N-3 meets with difficulties, as shown *inter alia* by the small deshielding of the 2-H in the cations. On the other hand, the 3,9-dimethyl derivative (10), which undergoes protonation at N-1, is alkylated at the same position at room temperature to yield (13).

We have inferred (above) that in (5), (8), and (11), the carbonyl groups are the main participants in protonation. However we have not encountered any methoxy-derivatives in the alkylation experiments. Presumably any methoxy-group would be cleaved readily by the hydroiodic acid formed simultaneously.

Chemical Shifts of N-*Methyl Substituents*.—The 1- and 3-methyl signals resemble those of the corresponding hypoxanthines.[1] However the 8-oxo-group causes an upfield shift of about 0·4 p.p.m. for the 7- and 9-methyl signals. Increasing *N*-methylation does not influence much the position of each individual signal, with the exception of the 3,9-dimethyl combinations.[3,4,6] Thus in compounds (10) and (15), the 3-methyl group is deshielded by 0·22 and 0·17 p.p.m., respectively, and the 9-methyl substituent by 0·35 and 0·33 p.p.m., respectively, relative to the positions of the corresponding signals for compounds (3) and (5) (see Table 3). In the cations of (10) and (15), deshielding of the 9-methyl group is reduced to about one third of the value for the neutral molecules.

Evidence for the Structures of New Purines; Synthetic Procedures.—Compound (9) was obtained by alkaline hydrolysis of 3,7-dimethyl-6-methylsulphonylpurin-8-one[8] (Table 7). The n.m.r. spectrum of (9) (Table 3) supports the conclusion that both *N*-methyl substituents have retained their original position. In addition, the location of the methyl groups in (9) follows from the fact that the same compound is obtained by methylation of the dianion of either (3) or (4). Preparation of (4) and (11) by a route analogous to that for (9) is preferable to the methods described in the literature.[9,10]

The structure of (6) follows from its formation by

[8] F. Bergmann, M. Rahat, and I. Tamir, *J.C.S. Perkin I*, 1974, 450.

[9] E. Fischer, *Ber.*, 1899, **32**, 1849.

[10] E. Fischer, *Ber.*, 1884, **17**, 335.

alkylation of either (2) or (3). Similarly, the 1,7-dimethyl derivative (7) results from alkylation of either the dianion of (2) or the neutral form of (4). Compound (12) was obtained from both (7) and (9) (see Table 5).

The 1,9- (8) and 3,9- (10) dimethyl derivatives, as well as the 3,7,9-trimethyl homologue (15) were obtained by oxidation of the corresponding 6-mercaptopurin-8-ones.[8] Compound (8) proved identical with that prepared by Fischer [11] by a different method.

1,3,9-Trimethylpurine-6,8-dione (13) was formed by methylation of the neutral molecule of (10). The product obtained differed in every respect from (15) and exhibited properties characteristic of members of class (c) in general (see Tables 1—3).

EXPERIMENTAL

M.p.s were determined with a Fisher–Johns apparatus. Microanalyses were performed by F. Strauss, Oxford, and M. Goldstein, Jerusalem. U.v. spectra were measured on a Hitachi–Perkin-Elmer 124 spectrophotometer and n.m.r. spectra on a JEOL MH-100 instrument (internal standard sodium 3-trimethylsilyl[2,2,3,3-$^2H_4$]propionate). All n.m.r. measurements were carried out in D_2O at 70°; pH was adjusted by the use of $NaHCO_3$, Na_2CO_3, NaOD, $CF_3{\cdot}CO_2H$, and D_2SO_4.

p*K* Values were derived from plots of $\lambda_{max.}$ as function of pH.

For paper chromatography (descending) on Whatman no. 1 paper, the following solvents were used (A) n-butanol–acetic acid–water (12 : 3 : 5 v/v), (B) propan-2-ol–dimethylformamide–ammonia (*d* 0·88) (13 : 5 : 2 v/v), (C) ethanol–dimethylformamide–water (3 : 1 : 1 v/v). Theophylline (R_F 0·68 in all solvents) served as standard for evaluation of R_F values. Spots were located by their fluorescence under a Mineralight u.v. lamp (λ *ca.* 254 nm).

Purines.—The following compounds were prepared by known methods: purine-6,8-dione (1), [12] its 3- (3) [13] and 9-methyl (5) derivatives,[14] and the 1,7,9-trimethyl homologue (14).[15]

General Procedures for New Purines.—(a) *Alkaline hydrolysis of 6-methylsulphonylpurin-8-ones.* 7-Methyl- (4), 3,7-dimethyl- (9) and 7,9-dimethyl-purine-6,8-dione (11) were prepared by refluxing a suspension of the corresponding 6-methylsulphonylpurin-8-one [9] (1·5 g) in 2N-NaOH (6 ml) for the period indicated in Table 7. The hydrolysis products were precipitated by acidification to pH 4; yields *ca.* 90%.

(b) *Oxidation of 6-mercaptopurin-8-ones.* Compounds (8), (10), and (15) were prepared as indicated in Table 8.

Isolation of Purines from Methylation Reactions (*Table* 9). —*1-Methylpurine-6,8-dione* (2). From the mixture resulting from alkylation of the neutral molecule of (1) (see Table 5), the solvent was removed *in vacuo*. The residue was refluxed for 10 min with 5% KOH, in the presence of charcoal, to destroy (6). The solution was filtered and neutralised to precipitate a mixture of (2) and (8). Compound (8), together with a small proportion of (2), was removed by repeated extraction with boiling ethanol (75 ml g^{-1}). The insoluble portion was recrystallised from water. The product was identical with authentic material.[16]

[11] E. Fischer, *Ber.*, 1899, **32**, 258.
[12] R. K. Robins, *J. Amer. Chem. Soc.*, 1958, **80**, 6671.
[13] F. Bergmann, G. Levin, A. Kalmus, and H. Kwietny-Govrin, *J. Org. Chem.*, 1961, **26**, 1504.

1,3-Dimethylpurine-6,8-dione betaine (6). A solution of 3-methylpurine-6,8-dione (3) (1 g) in dimethylformamide (20 ml) was treated at room temperature with methyl iodide (5 ml) for 4 days. The iodide salt of (6) was precipitated by addition of 1 : 1 ether–acetone (200 ml). The solid was

[14] A. Albert and D. J. Brown, *J. Chem. Soc.*, 1954, 2060.
[15] E. Fischer, *Ber.*, 1899, **32**, 1852.
[16] D. J. Brown and J. S. Harper, *J. Chem. Soc.*, 1961, 1298.

TABLE 7

Alkaline hydrolysis of 6-methylsulphonylpurin-8-ones

Posns. of Me groups	Reaction [a] time	Product	Yield [b] (%)	Crystal form
	5 h	*c*	0	Needles
3	5 h	(3)	90	Plates
7	15 min	(4)	90	Rectangular plates
3,7	10 min	(9) [d]	90	Prisms
7,9	5 min	(11)	95	Needles

[a] In all cases, 2N-NaOH was used; except for the 3-methyl derivative, where 5% Na_2CO_3 was employed. [b] All products were crystallised from water and decomposed above 300°. [c] No reaction. [d] Found: C, 46·4; H, 4·6; N, 31·1. $C_7H_8N_4O_2$ requires C, 46·7; H, 4·4; N, 31·1%.

TABLE 8

Oxidation of 6-mercaptopurin-8-ones to purine-6,8-diones [a]

Posns. of Me groups	Reaction time	Product [b]	Yield (%)	Solvent	Crystal form
1,9	15 min [c]	(8)	50	H_2O	Needles
3,9	1 h [d]	(10) *	40	H_2O	Prisms
3,7,9	1 h [e]	(15) †	40	$Me_2N{\cdot}CHO$	Prisms

[a] An alkaline solution of the 6-mercaptopurin-8-one was treated with 30% H_2O_2 (4 ml g^{-1}) at room temp. [b] All compounds showed decomposition above 300°. [c] Oxidation in 2N-NaOH. After 1 h, solid $NaHSO_3$ was added until the pH was 7. The precipitated (8) [11] was filtered off and recrystallised. [d] Reaction in N-NaOH. After 1 h, an excess of methanol was added and the mixture left at room temp. overnight. Inorganic salts were filtered off, the filtrate was brought to dryness *in vacuo*, and the residue was recrystallised. [e] Reaction in saturated $NaHCO_3$; work-up as for the 3,9-dimethyl derivative.

* Found: C, 44·7; H, 5·1; N, 30·1. $C_7H_8N_4O_2,0{\cdot}5H_2O$ requires C, 44·4; H, 4·8; N, 29·6%. † Found: C, 49·3; H, 5·4, N, 28·7. $C_8H_{10}N_4O_2$ requires C, 49·5; H, 5·2; N, 28·9%.

TABLE 9

Purines formed by alkylation of purine-6,8-diones with methyl iodide in dimethylformamide

No.	Posns. of Me groups	Product	Yield (%)	M.p. or decomp. (°C)	Solvent	Crystal form
(1)		(2) [a]	35	>300	H_2O	Rectangular plates
(3)	3	(6) * [d]	60	>300	[b]	Rods
(4)	7	(7) †	40	>400	H_2O	Rods
(7)	1,7	(12)	40 [c]	217	PrOH	Prisms
(10)	3,9	(13) ‡	40 [c]	247	EtOH	Prisms

[a] See ref. 16. [b] For purification of (6) see Experimental section. [c] Isolated as picrate; the amount of pure (12) available was insufficient for analysis, but the structure was established beyond doubt (see Table 3). [d] Purified by precipitation by acetone from minimum volume of 87% aqueous methanol.

* Found: C, 46·4; H, 4·3; N, 30·8. $C_7H_8N_4O_2$ requires C, 46·7; H, 4·4; N, 31·1%. † Found: C, 46·4; H, 4·6; N, 30·8%. Required figures as for (6). ‡ Found: C, 39·5; H, 3·0; N, 22·8. $C_{14}H_{13}N_7O_9$ requires C, 39·7; H, 3·1; N, 23·2%.

suspended in ethanol (20 ml) and stirred with sodium acetate (300 mg) at room temperature for 30 min. The betaine of (6) was filtered off and purified as described in Table 9. Refluxing a solution of (6) in dimethylformamide for 2 h yielded a mixture of (2) and (8) in a ratio of *ca.* 3 : 1.

1,7-*Dimethylpurine*-6,8-*dione* (7). Methylation of (4) as neutral molecule (Table 5) was prolonged until the primary product (12) had disappeared almost completely (2 h). The solvent was removed *in vacuo* and the residue treated with boiling N-NaOH and charcoal. The solution was filtered and neutralised with glacial acetic acid.

1,3,7-*Trimethylpurine*-6,8-*dione* (12) *picrate*. From the mixture obtained from (7) and methyl iodide in dimethylformamide (see Table 5), the product (12) was precipitated by addition of ether–acetone, as described for compound (6). Because of the instability of (12), it was isolated as the picrate.

1,3,9-*Trimethylpurine*-6,8-*dione* (13) *picrate*. The compound was precipitated from the solution in dimethylformamide resulting from methylation of the neutral molecule of (10), as described for (6). Pure (13) was isolated as the picrate.

Molecular Orbital Calculations.—Molecular orbitals obtained by the HMO method were utilised for calculation of superdelocalisabilities for electrophilic attack and of net charges.[17,18] A FORTRAN computer program, written by Dr. A. Meyer (Department of Organic Chemistry, Hebrew University, Jerusalem) was used. Parameters were taken from ref. 19. Computations were performed on a CDC 6400 computer.

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[18] K. Fukui, T. Yonezawa, and C. Nagata, *Bull. Chem. Soc. Japan*, 1954, **27**, 423.

[19] B. Pullman and A. Pullman, 'Quantum Biochemistry,' Interscience, London, 1963, p. 108.