Studies on the Chemical Reactivity and the Physical Properties of Allopurinol (Pyrazolo[3,4-*d*]pyrimidin-4-one) and Related Compounds

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Pyrazolo[3,4-*d*]pyrimidines resemble purines by avoiding disubstitution at the *peri*-positions 1 and 7, but they also resist 1,2-disubstitution in the pyrazole ring. INDO calculations show that 1,2-disubstituted derivatives of allopurinol are the least stable.

The anion of allopurinol is present as a mixture of tautomeric forms and yields upon methylation 1,5-, 2,5-, and 2,7-dimethyl derivatives. Alkylation of 4-methylthiopyrazolo[3,4-*d*]pyrimidine, either as the neutral molecule or as the anion, offers a convenient approach to the synthesis of many mono- and di-methyl derivatives, which serve as precursors for methylated allopurinols.

ALTHOUGH allopurinol (pyrazolo[3,4-*d*]pyrimidin-4-one) (1) has been widely studied for its biochemical and clinical effects,[1-4] its chemical reactivity has remained largely unexplored. We describe here the alkylation of (1) and its *N*-methyl derivatives, and from these experiments we obtained information on tautomerisation and ionisation. We have also studied the alkylation of 4-methylthiopyrazolo[3,4-*d*]pyrimidines. Our observations will be compared with the behaviour of the corresponding purines.

RESULTS AND DISCUSSION

Tautomerism in the Allopurinol Series.—The $\lambda_{max.}$ of (1) is identical with the values for the 5-methyl (4) and the 1,5-dimethyl (6) derivatives (Table 1). Thus it might be supposed that in aqueous solution (1) is present in its 1,5-di-NH form. However, introduction of an NMe group generally shifts $\lambda_{max.}$ by a few nm to longer wavelengths, as in the pairs (1)/(6) and (3)/(7). Therefore (1) should absorb at a shorter wavelength than *e.g.* (2). In fact its absorption maximum is found between those of (2) and (3), indicating that (1) is a mixture of the 1,5- and 2,5-di-NH tautomers. By similar reasoning, 5-methylallopurinol (4) is present in aqueous solution as a mixture of 1- and 2-NH tautomers. The ^{13}C n.m.r. spectrum of allopurinol supports the presence of both tautomers in solution.[5] The *X*-ray interferogram of (1) shows a hydrogen bridge between the 1-NH group and 7-N of another molecule, while 2-N is bound to the hydrogen of a 5-NH group, suggesting that in the crystal allopurinol is present as the 1,5-di-NH tautomer.[6]

The *p*-quinoid structure of the pyrimidine moiety in compounds (5) and (9) causes a bathochromic shift of $\lambda_{max.}$ of 20—25 nm, relative to compounds (1) and (3). The close values of $\lambda_{max.}$ for 7-methyl- (5) and 2,7-dimethyl-allopurinol (9) indicate that the former is present in aqueous solution as the 2-NH tautomer (Scheme 1). The presence of the 1-NH form would cause an appreciable bathochromic shift of the u.v. maximum of (5), as in the 1,7-dimethyl isomer (8). It is thus evident that (5) avoids the simultaneous presence of substituents at positions 1 and 7, because of steric interference. This phenomenon is analogous to the interference between 3- and 9-substituents in the purine series.[7,8] MO calculations (see later) indicate that the 2-NH form of (5) is more stable than the 1-NH tautomer.

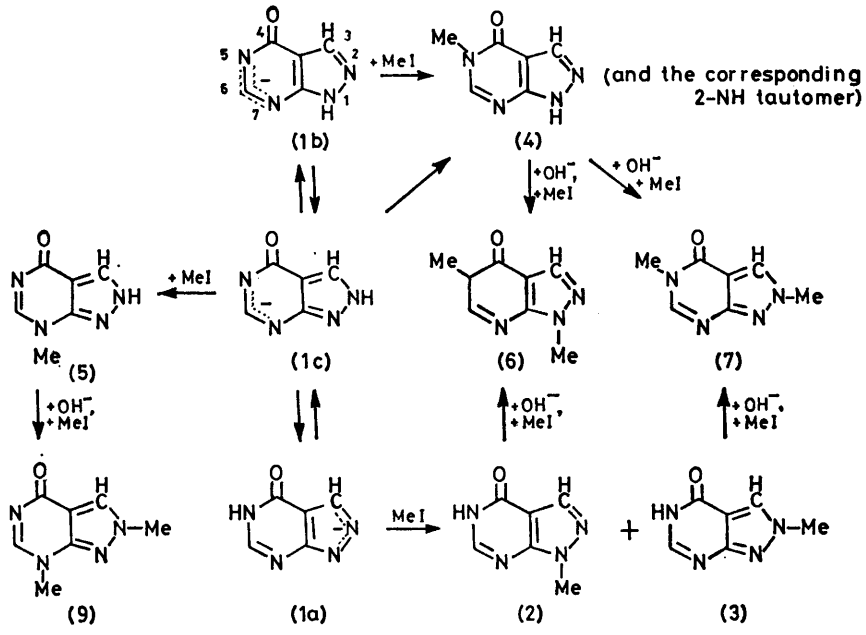
Anion Formation in Allopurinols.—Dissociation of the 5-NH group in the pyrimidine moiety of (2) and (3) leads to a marked bathochromic shift of $\lambda_{max.}$ of 23 ± 2 nm (Table 1). In contrast, ionisation of an NH-group in the pyrazole moiety of (4) and (5) causes a very small bathochromic or a marked hypsochromic displacement of $\lambda_{max.}$. Allopurinol itself occupies an intermediate position, *i.e.* monoanion formation is accompanied by a

TABLE 1

U.v. absorption spectra and dissociation constants of allopurinols

Compound	Substituents in allopurinol	$\lambda_{max.}$/nm [a]					pF for	
		n	a	Δ(a − n)	c	Δ(c − n)	Anion	Cation
(1)		252	261 [b]	+9	255	+3	10.5 [b]	+0.4
(2)	1-Methyl	249	270	+21	260	+11	9.4	< −1.8
(3)	2-Methyl	255	280	+25	257	+2	10.5	−0.5
(4)	5-Methyl	252	253	+1	257	+5	9.5	−0.5
(5)	7-Methyl	277	261	−16	254	−23	8.5	+3.5
(6)	1,5-Dimethyl	252			264	+12		−1.5
(7)	2,5-Dimethyl	260			262	+2		−1.5
(8)	1,7-Dimethyl	292			268	−24		+7.0
(9)	2,7-Dimethyl	280			260	−20		+3.2
(10)	5,7-Dimethyl iodide	261 [c]			266	+5		−0.2
(11)	2,5,7-Trimethyl iodide				272			
(12)	1-Ethoxycarbonyl	268	279	+11	263	−5	7.0	−0.75
(13)	1-Ethoxycarbonyl-5-methyl	273			269	−4		−0.5

[a] Letters a, n, and c refer respectively to the neutral molecule, the anion, and the cation. [b] The dianion of allopurinol shows $\lambda_{max.}$ 278 and 257 nm; pK *ca.* 13. [c] This value characterises the zwitterion of (10).



SCHEME 1

moderate bathochromic shift of 9 nm, indicating the presence of the tautomeric forms (1a)—(1c) (Scheme 1). Di-anion formation shifts the absorption maximum of (1) by a further 17 nm, *i.e.* somewhat less than the displacement of $\lambda_{max.}$ characteristic for anion formation in the pyrimidine ring of (2) and (3). It is concluded that (1a) makes the larger contribution to the tautomeric mixture of the monoanions of (1), *i.e.* the main ionisation sequence of (1) is 1(2) ⟶ 5. This conclusion is supported by the similar chemical shifts of the anions of (1) and (4) (Table 2).

Cation Formation in Allopurinols.—The allopurinols are divided into two classes (Table 1). All 7-methyl derivatives have pK values above +3, while all others show pK values between +0.4 and −1.8. The latter range is characteristic for protonation in the pyrazole moiety, with some exceptions, to be discussed below. Attachment of a proton to the pyrazole ring causes only small downfield shifts of the 3-H signals [Table 2, compounds (1), (2), and (6)].

The fixed cation of 5,7-dimethylallopurinol (10) shows $\delta_{6\text{-H}}$ 9.80. The large downfield shifts of the 6-H signals in the cations of 7-methyl- (5) and 2,7-dimethyl-allopurinol (9) (1.0 and 0.76 p.p.m., respectively) indicate attachment of the proton mainly to position 5, thus forming an amidinium-like structure resembling (10).

TABLE 2

N.m.r. spectra of allopurinols (δ)

Compound	Substituents	3-H			6-H			N-Me [a]			Others		
		n [b]	a [b]	c [b]	n	a	c	n	a	c	n	a	c
(1)		8.02	7.84	8.22	8.16	7.84	8.27						
(2)	1-Methyl [c]	8.13	7.98	8.33	8.20	8.11	8.43	3.96	3.93	4.07			
(3)	2-Methyl [c]	8.04	8.05	8.65	8.56	8.29	9.09	4.09	3.99	4.18			
(4)	5-Methyl [c]	8.13	7.81	8.66	8.30	7.84	9.10	3.43	3.37	3.62			
(5)	7-Methyl [c]	8.37	8.22	8.75	8.28	8.14	9.28	3.80	3.70	4.06			
(6)	1,5-Dimethyl	8.05		8.09	8.37		8.39	(1) 3.92		3.95			
								(5) 3.48		3.45			
(7)	2,5-Dimethyl	8.25		8.77	8.55		9.27	(2) 4.04		4.13			
								(5) 3.44		3.64			
(8)	1,7-Dimethyl	8.24		8.39	8.79		9.05	(1) 4.18		4.26			
								(7) 3.98		4.10			
(9)	2,7-Dimethyl	8.25		8.79	8.41		9.17	(2) 4.01		4.13			
								(7) 3.65		3.96			
(10)	5,7-Dimethyl [d]			9.06			9.80	(5)		3.55			
								(7)		3.92			
(11)	2,5,7-Trimethyl [d]			8.99			9.74	(2)		4.11			
								(5)		3.57			
								(7)		3.91			
(12)	1-Ethoxycarbonyl	8.32	8.23	8.33	8.27	8.21	8.37				(CH_2)	4.49	4.47	4.49
											(Me)	1.39	1.36	1.39
(13)	1-Ethoxycarbonyl-5-methyl	8.34		8.56	8.56		8.78	(5) 3.48		3.79	(CH_2)	4.49		4.73
											(Me)	1.38		1.61

[a] Figures in parentheses indicate position of the methyl substituent. [b] n = Neutral form; a = anion; c = cation. [c] Assignment of 3-H and 6-H signals in these compounds is based on the nuclear Overhauser effect. [d] Measured as iodide salt.

TABLE 3

Physical properties of pyrazolo[3,4-*d*]pyrimidine-4-thiones

Compound	Substituents	$\lambda_{max.}$ (nm) n [b]	a [b]	c [b]	pK for Anion	Cation	δ 3-H n [c]	a	c	6-H n	a	c	N-Me [a] n	a	c
(14)		321	317 [c]	332	8.3 [c]	−1.1	8.14 [c]	8.15	8.26	8.26	8.17	8.32			
(15)	1-Methyl	322	320	327	7.5	< −3	8.20	8.16	8.35	8.20	8.07	8.24	3.94	3.94	4.00
(16)	2-Methyl	332	334	337	7.5	+0.5	8.66	8.60	8.67	8.05	8.05	8.12	4.06	4.04	4.08
(17)	7-Methyl	352	339	334	7.8	+2.4	8.30	8.29	8.97	8.50	8.50	9.34	3.74	3.73	3.98
(18)	1,5-Dimethyl	320		331		−2.5	8.13		8.35	8.66		8.72	(1) 3.91		4.13
													(5) 3.80		3.72
(19)	2,7-Dimethyl [d]			357					8.90			9.31	(2)		4.10
													(7)		3.88

[a] Figures in parentheses indicate position of the methyl substituent. [b] n = Neutral form, a = anion, c = cation. [c] The dianion of (14) is characterised as follows: $\lambda_{max.}$ 308 nm; p*K* 12.3; δ_{3-H} 8.12; δ_{6-H} 8.14. [d] This compound was available only as the hydriodide; the free base is unstable.

TABLE 4

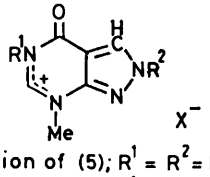
Physical properties of 4-methylthiopyrazolo[3,4-*d*]pyrimidines and related compounds

Compound	Substituents	$\lambda_{max.}$/nm n [b]	c [b]	pK Anion	Cation	δ 3-H n	c	6-H n	c	N-Me [a] n	c	S-Me n	c	Others	n	c
4-Methylthio-derivatives																
(20)		291 [c]	302	9.6	+1.2	8.32 [c]	8.40	8.76	8.98			2.73	2.81			
(21)	1-Methyl	288	300		+0.1	8.28	8.50	8.75	8.83	4.01	4.07	2.72	2.90			
(22)	2-Methyl	305	308		+1.7	8.76	9.27	8.73	9.03	4.25	4.32	2.77	2.97			
(23)	7-Methyl	308	328		+5.0	8.97	9.40	8.80	9.36	4.15	4.19	2.83	2.92			
(24)	2,7-Dimethyl iodide		326				9.35		9.45	(2)	4.31		2.92			
										(7)	4.14					
(25)	1-Ethoxycarbonyl	294	306		−0.5	8.95	9.26	8.60	8.99			2.73	3.21	(CH_2)	4.52	4.82
														(Me)	1.40	1.61
4-Methoxy-derivatives																
(26)	7-Methyl	265	257		−3.0	8.36		8.77		4.11				(OMe)	4.21 [d]	

[a] Numbers in parentheses indicate position of the methyl substituent. [b] n = Neutral molecule, c = cation. [c] The anion of (20) shows $\lambda_{max.}$ 287 nm; δ_{3-H} 8.20; δ_{6-H} 8.32; and δ_{S-Me} 2.72. [d] Assignment of the two methyl signals in (26) was made by the following method: 4-methylthiopyrazolo[3,4-*d*]pyrimidine (20) reacted with CD_3I to give the 7-CD_3 derivative (23a). The latter reacted with sodium methoxide to give (26), deuteriated only in the 7-methyl substituent.

This is analogous to similar observations in the purine series.[9,10]

For 5-methyl- (4) and 2,5-dimethyl-allopurinol (7), Δδ (n—c) * is 0.53 and 0.52 p.p.m., respectively, for the



Cation of (5); $R^1 = R^2 = H$
Cation of (9); $R^1 = H$; $R^2 = Me$
Fixed cation of (10); $R^1 = Me$; $R^2 = H$
Fixed cation of (11); $R^1 = R^2 = Me$

3-H signals, and 0.80 and 0.72 p.p.m. for the 6-H bands (Table 2). Therefore these compounds may form tautomeric cations (Scheme 2). Such a possibility should also be considered for the 2-methyl derivative (3), for which we find Δδ (n—c) 0.61 p.p.m. for 3-H and 0.53 p.p.m. for 6-H.

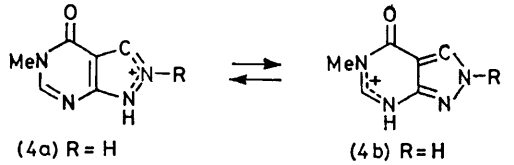
These data reflect the relative instability of 1,2-disubstituted cations, as is also indicated by their low p*K* values.

* This symbol indicates the difference in δ values between the neutral form n and the cation c.

Tautomerism and Ionisation in Pyrazolo[3,4-d]*pyrimidine-4-thiones and the Corresponding 4-Methylthio-derivatives.*—The absorption maxima of (14) and its 1-methyl (15) and 1,5-dimethyl derivatives (18) are close to each other and quite different from the $\lambda_{max.}$ of the 2-methyl derivative (16) (Table 3). Therefore in aqueous solution, (14) is present mainly as the 1,5-di-NH tautomer.

Monoanion formation in (14) involves the 5-NH group, as indicated by similar u.v. and n.m.r. spectra of the anions of (14) and (15). Dissociation of the 5-NH group in the 4-thioxo-derivatives (14)—(16) causes only small changes of $\lambda_{max.}$ (Table 3), in contrast to the large bathochromic shifts for anion formation in compounds (2) and (3). This is due to resonance with the 4-thiocarbonyl group, in analogy to the marked hypsochromic shift upon *S*-methylation (see Table 4).

Protonation of the 4-thioxo-derivatives leads to similar spectral changes to those observed for the cor-



(4a) R = H
(7a) R = Me

(4b) R = H
(7b) R = Me

SCHEME 2

responding allopurinols, and can be interpreted in the same way. Again the large downfield shift of the 6-H signal in the 7-methyl derivative (17) ($\Delta\delta$ 0.84 p.p.m.) indicates formation of an amidinium-like cation by protonation at N-5. On the other hand, such a large shift is missing for (23), the 4-methylthio-analogue of compound (17), which protonates in the pyrazole ring.

The $\lambda_{max.}$ of 4-methylthiopyrazolo[3,4-*d*]pyrimidine (20) is intermediate between the absorption maxima of its 1- (21) and 2-methyl (22) derivatives (Table 4), *i.e.* in an aqueous solution of (20) both 1- and 2-NH tautomers are present.

Alkylation as a Model for Electrophilic Attack on Allopurinols.—(a) *Methylation of anions.* Cheng and Robins reported the formation of 1,5-dimethylallopurinol (6), when (1) was treated with methyl iodide in the presence of potassium hydroxide.[11] The same product was also obtained by methylation of the anion of 1-methylallopurinol (2). The authors concluded that the pathway of alkylation is (1)→(2)→(6). In our hands, methylation of (1) with an excess of MeI gave three products, *i.e.* 1,5- (6) (*ca.* 60%), 2,5- (7) (*ca.* 30%), and 2,7-dimethylallopurinol (9) (*ca.* 10%). We confirm that methylation or (2) yields exclusively (6). We have further observed that (3) gives exclusively (7), while (4) produces a mixture of (6) and (7). Thus the latter two derivatives could result from alkylation of either (4) or from its isomers (2) and (3), formed in the first step of the reaction chain of (1). We have not succeeded in identifying any of the monomethyl intermediates in the alkylation of allopurinol, apparently because their rate of methylation exceeds the rate of their formation. Therefore in the tentative Scheme 1, we have included both alternative pathways for formation of (6) and (7).

The 2,7-dimethyl derivative (9) results only from alkylation of 7-methylallopurinol (5). It thus appears that the tautomeric anion (1c) is transformed into the 7-methyl derivative (5), which then serves as a precursor of (9). Scheme 1 is based on the hypothesis that the anion of (1) is a mixture of the tautomers (1a), (1b), and (1c).

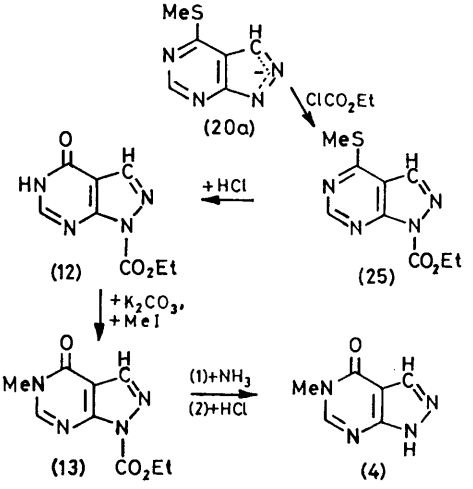
(b) *Methylation of neutral forms of allopurinols.* Compound (1) proved resistant to alkylation, in contrast to the facile conversion of hypoxanthine into the 7,9-dimethylhypoxanthinium ion.[12] However, attack at either position 1 or 2 in the pyrazole ring would involve formation of 1,2-disubstituted derivatives, which are energetically unfavourable (see Table 6).

Compound (5) is attacked by methyl iodide exclusively at position 5 to yield the fixed cation of 5,7-dimethylallopurinol (10). This reaction parallels conversion of 3-methylhypoxanthine into the 1,3-dimethylhypoxanthinium ion.[13]

The neutral forms of 2,5- (7) and 2,7-dimethylallopurinol (9) undergo further alkylation to give the same 2,5,7-trimethyl derivative (11). The latter was also obtained from (1) directly (see Experimental section). In every case, (11) crystallised first as the black tri-iodide (11a), which was converted by sodium sulphite into the colourless mono-iodide (11). The similarity of the u.v. and n.m.r. spectra of (11) and the 5,7-dimethylallopurinol cation (10) should be noted.

A surprising result was obtained by heating a solution of 1-methylallopurinol (2) in methyl toluene-*p*-sulphonate to 150 °C. The drastic conditions overcame the steric hindrance to substitution at N-7, leading to 1,7-dimethylallopurinol (8). It should be recalled that all attempts to synthesise the analogous 3,9-dimethylhypoxanthine have so far failed.*

Synthesis of Monoalkyl Derivatives of Allopurinol.—As mentioned before, we have not succeeded in isolating any of the monomethylated allopurinols during alkylation of the anion of (1). However, 4-methylthiopyrazolo[3,4-*d*]pyrimidine (20) lends itself to monoalkylation in various ways.



SCHEME 3

The neutral form of (20) was transformed into the 7-methyl derivative (23) by reaction with MeI. Acid hydrolysis of (23) yielded (5). The reaction (20)→(23) parallels the conversion of 6-methylthiopurine to its 3-methyl derivative.[14] Compound (23) was obtained as its hydriodide. The free base of (23) reacted further with MeI to give the 2,7-dimethyl derivative (24), which crystallised from the reaction mixture as the brown tri-iodide. Sodium sulphite reduced the latter to the mono-iodide (24), acid hydrolysis of which yielded (9).

The anion (20a) produced with MeI a mixture of the 1- (21) and 2-methyl (22) derivatives, which served as convenient sources of compounds (2) and (3) by alkaline hydrolysis [see Table 5 (*c*)].

The synthesis of 5-methylallopurinol (4) proved more difficult. The anion (20a) gave with ethyl chloroformate exclusively the 1-ethoxycarbonyl derivative (25) (Scheme 3). The absence of the 2-ethoxycarbonyl isomer is surprising, in view of the course of methylation of (20a) described above. Cautious hydrolysis of (25) with 6N-HCl at 37 °C produced 1-ethoxycarbonylallopurinol (12) in low yield. The anion of (12) was then alkylated at

* Recently, the synthesis of 3,9-dimethylhypoxanthine has been achieved (T. Itaya and K. Ogana, *Heterocycles*, 1977, **6**, 965).

TABLE 5

Substitution reactions of pyrazolo[3,4-*d*]pyrimidines

(*a*) Thiation of allopurinols [a]

Compound used	Product formed	Yield (%)	M.p. or decomp. (°C)	Crystal form and colour	Fluorescence [b]
Allopurinol (1)	(14)	100	>360	Yellowish needles	Violet
1-Methylallopurinol (2)	(15)	89	>300	Colourless needles	Brilliant violet
1,5-Dimethylallopurinol (6)	(18)	100	238—239	Fine colourless needles	Orange

(*b*) Thiohydrolysis of substituted 4-methylthiopyrazolo[3,4-*d*]pyrimidines

Compound	Substituents	Reaction conditions [c]	Product formed	M.p. or decomp. (°C)	Solvent for crystallisation	Crystal form and colour	Fluorescence [b]
(22)	2-Methyl	Reflux for 60 min	(16)	>300	Water	Colourless needles	Greenish-yellow
(23)	7-Methyl	Stand 5 min at room temperature	(17)	>300	Ethanol	Yellow plates	Bright yellow
(24)	2,7-Dimethyl	Reflux for 10 min	(19) [d]	242—246	Acetic acid	Yellowish cubes	Orange

(*c*) Hydrolysis of substituted 4-methylthiopyrazolo[3,4-d]pyrimidines

		Reaction							
Compound	Substituent	Medium	Temp. (°C)	Time/h	Product formed	Yield (%)	M.p. or decomp. (°C)	Solvent for crystallisation	Crystal form [e]
(21)	1-Methyl	1N-NaOH in 50% MeOH	50	2	(2)	100	>300	Water	Needles
(22)	2-Methyl	1N-NaOH in 50% MeOH	50	3.5	(3)	100	>300	Water	Amorphous
(23)	7-Methyl	6N-HCl	50	1	(5)	100	>300	Water	Bushels of needles
(25)	1-Ethoxycarbonyl	6N-HCl	37	18	(12)	12	228—230	Propan-2-ol	Needles

(*d*) Analyses of new compounds

		Analysis (%)							
		Calculated				Found			
Compound	Formula	C	H	N	Others	C	H	N	Others
(4)	$C_6H_6N_4O$	48.00	4.00	37.33		48.30	4.02	37.33	
(5)	$C_6H_6N_4O$	48.00	4.00	37.33		48.18	3.91	37.18	
(7)	$C_7H_8N_4O$	51.22	4.88	34.15		51.48	4.99	34.40	
(8)	$C_{14}H_{16}N_4O_4S$	50.00	4.76	16.66	S, 9.52	49.74	5.06	16.48	9.88
(10)	$C_7H_9IN_4O$				I, 43.49				43.12
(11)	$C_8H_{11}IN_4O$	31.37	3.59	18.30	I, 41.50	31.49	3.64	18.33	41.75
(11a)	$C_8H_{11}I_3N_4O$	17.14	1.96	10.00	I, 68.04	17.39	1.94	9.56	68.00
(12)	$C_8H_8N_4O_3$	46.15	3.84	26.92		45.63	3.55	27.66	
(13)	$C_9H_{10}N_4O_3$	48.65	4.50	25.23		48.45	4.54	25.24	
(17)	$C_6H_6N_4S$	43.37	3.61	33.79	S, 19.27	43.15	3.43	33.35	18.97
(19)	$C_7H_8N_4S$	46.66	4.44	31.11	S, 17.77	46.81	4.57	31.14	18.20
(22)	$C_7H_8N_4S$	46.66	4.44	31.11	S, 17.77	46.40	4.44	31.07	17.34
(23)	$C_7H_9IN_4S$	27.27	2.92	18.18		27.10	2.95	18.46	
(24)	$C_8H_{11}IN_4S$	29.81	3.42	17.39	S, 9.94	30.16	3.47	17.70	10.07
					I, 39.44				39.35
(24a)	$C_8H_{11}I_3N_4S$	16.66	1.90	9.72		16.49	1.66	10.00	
(25)	$C_9H_{10}N_4O_2S$	45.37	4.20	23.53	S, 13.44	45.24	4.27	23.78	13.32
(26)	$C_7H_{12}N_4O_3$	42.00	6.00	28.00		41.60	5.98	28.15	

[a] All thiations at 140 °C, time 30 min (see Experimental section); all products were recrystallised from water. [b] Under a Mineralight u.v. lamp, emitting light of λ *ca.* 255 nm. [c] Compounds (22) and (23) (as iodides) were dissolved in 1N-KOH. Hydroxgen sulphide was bubbled through the solution during the time indicated. The pH was then brought to 6 to precipitate the 4-thioxo-derivative formed. Yield was quantitative. For thiohydrolysis of (24), an aqueous solution was refluxed, while H_2S was bubbled through. [d] Compound (19) underwent slow spontaneous decomposition. Because of the very small amount available, (19) was not obtained in analytically pure form. [e] All the products were colourless.

position 5 to 1-ethoxycarbonyl-5-methylallopurinol (13). Hydrolysis of the latter with cold ammonia, followed by treatment with hydrochloric acid, led to compound (4) (Scheme 3).

Some Nucleophilic Substitutions of Pyrazolo[3,4-d]*-pyrimidines.*—(a) *Thiation of 4-oxo-derivatives.* Table 5 (*a*) summarises the conditions for conversion of three allopurinols into the corresponding 4-thioxo-derivatives. The short reaction times and the high yields are noteworthy in view of the tedious thiation of hypoxanthines.[15]

(b) *Thiohydrolysis of 4-methylthiopyrazolo*[3,4-d]*pyrimidines.* Both the 2- (22) and the 7-methyl (23) derivatives reacted readily with K_2S. The former required 1 h reflux to give (16), but when hydrogen sulphide was bubbled through a solution of (23) in KOH at room temperature, (17) was formed within 5 min. The 2,7-dimethyl derivative (24) was unstable in alkali, but underwent rapid thiohydrolysis to (19), when hydrogen sulphide was bubbled through its refluxing aqueous solution [Table 5 (*b*)].

The high reactivity of (23) and (24) finds a close

parallel in the ready thiohydrolysis of 3-methyl-6-methylthiopurine and of 3,7-dimethyl-6-methylthiopurinium iodide.[16] However, the reaction of the 2-methyl derivative (22) is unusual, in view of the refractoriness of the analogous 7-methyl-6-methylthiopurine.

(c) *Alkaline hydrolysis of 4-methylthiopyrazolo*[3-4-d]*pyrimidines.* The 1- (21) and 2-methyl (22) derivatives were cleaved by reflux with 1N-NaOH in 50% methanol for 2—3 h to yield compounds (2) and (3), respectively. On the other hand, all 7-methyl derivatives of (20) decomposed in warm alkali; they could however be hydrolysed with concentrated hydrochloric acid.

(d) *Exchange of a 4-methylthio-group by methoxide ion.* Only the 7-methyl derivatives of (20) reacted smoothly with methoxide. Thus (23) was converted into the 4-methoxy-derivative (26).

Assignment of N.M.R. Signals to the Aromatic Protons of Pyrazolo[3,4-d]*pyrimidines.*—The signals of the two aromatic protons are usually distinguishable by their different shape: the 6-H band has a smaller height and a greater width than the 3-H signal. The proton at position 6 is located between two nitrogens, and the quadrupoles of the latter are responsible for its shorter relaxation time. The assignment, based on line shape, was always in excellent agreement with measurements of the nuclear Overhauser effect (NOE), which are indicated in Table 2.

Steric Interference between 1- and 7-Substituents in Pyrazolo[3,4-d]*pyrimidines.*—Comparison of the two methyl signals in (8) with the corresponding ones in (2) and (5) reveals the following downfield shifts in (8): 1-Me, neutral form $\Delta\delta$ 0.22; cation, 0.19 p.p.m.; 7-Me, 0.18 and 0.04 p.p.m., respectively (Table 2). This indicates that protonation relieves the strain between the two *peri*-methyl groups, but still causes a considerable downfield shift of the 1-Me signal. A similar phenomenon has been observed for 3,9-dimethylpurine-6,8-dione, when passing from the neutral form to the cation.[17]

Theoretical Calculations.—Energies and dipole moments were calculated by the INDO method.[18] In Table 6, the possible tautomers of each compound are arranged in order of decreasing stability. The following observations can be made. (*a*) The most stable form of allopurinol is the 1,5-di-NH tautomer and the least stable one the 1,2-di-NH tautomer. Likewise in compounds (2) and (3) and all the dimethyl derivatives, the 1,2-disubstituted form is always the least stable, and 1,2-dimethylallopurinol is still unknown. Furthermore the spectral data for 2-NH and 2-NMe derivatives indicate that whenever protonation occurs at position 1, the 7-NH tautomer is also formed (Scheme 2). Alkylation of 2,5-dimethylallopurinol (7) takes place at N-7 and not at N-1, to yield compound (11).

(*b*) The 2-NH form of 5-methylallopurinol (4) is more stable than the 1-NH tautomer. However the anion of (4) is attacked by methylating agents to about the same degree at N-1 and N-2 (see Scheme 1).

(*c*) For 7-methylallopurinol (5), the 2-NH tautomer is considerably more stable than the 1-NH form, in accordance with the spectral and chemical properties of this compound.

(*d*) The calculated dipole moments roughly follow the order of energies, the least stable form having the highest dipole moment.

TABLE 6

Energies and dipole moments of allopurinols, calculated by the INDO method. Tautomers in each group are arranged according to descending values of total energy. The most stable form is in italics

Compound	Tautomer	Electronic energy (atomic units) [a]	Bond energy (atomic units)	Total energy (kcal mol^{-1})	Dipole moment (Debye units)
(1), Allopurinol	1,5-di-NH	−357.588	−7.695	*−64 881.521*	4.34
	2,5-di-NH	−356.345	−7.542	−64 785.852	1.35
	2,7-di-NH	−357.467	−7.541	−64 784.856	5.58
	1,7-di-NH	−359.675	−7.505	−64 762.257	8.99
	1,2-di-NH	−357.705	−7.479	−64 745.845	9.91
(2), 1-Methylallopurinol	5-NH	−416.620	−8.886	*−70 295.575*	4.32
	7-NH	−418.247	−8.816	−70 251.851	9.03
	2-NH	−416.425	−8.788	−70 233.830	10.06
(3), 2-Methylallopurinol	5-NH	−411.831	−8.853	*−70 274.563*	1.41
	7-NH	−413.151	−8.850	−70 273.116	5.80
	1-NH	−414.993	−8.789	−70 234.602	10.18
(4), 5-Methylallopurinol	2-NH	−415.776	−8.849	*−70 272.164*	1.41
	1-NH	−415.754	−8.837	−70 264.519	3.67
(5), 7-Methylallopurinol	2-NH	−416.657	−8.860	*−70 278.944*	5.55
	1-NH	−420.485	−8.818	−70 252.771	9.05
(7), 2,5-Dimethylallopurinol		−473.650	−10.159	*−75 760.797*	1.46
(6), 1,5-Dimethylallopurinol		−475.806	−10.147	−75 753.320	3.71
(9), 2,7-Dimethylallopurinol		−475.879	−10.159	*−75 760.661*	5.84
(8), 1,7-Dimethylallopurinol		−484.833	−10.128	−75 741.189	9.06
1,2-Dimethylallopurinol [b]		−478.758	−10.097	−75 722.291	10.40

[a] 1 Atomic unit = 628.4 kcal mol^{-1}. [b] This compound is still unknown.

EXPERIMENTAL

M.p.s were determined on a Fisher–Johns apparatus. 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 °C, unless stated otherwise. The pH was adjusted by the use of $NaHCO_3$, Na_2CO_3, NaOD, CF_3COOH, and D_2SO_4. pK Values were derived from plots of $\lambda_{max.}$ as a function of pH. For paper chromatography by the descending method on Whatman paper no. 1, 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); and (C) ethanol–dimethylformamide–water (3 : 1 : 1, v/v). Theophylline (R_F 0.68 in all solvents) served as a standard for evaluation of R_F values. Spots were located by their fluorescence under a Mineralight u.v. lamp ($\lambda_{max.}$ *ca.* 254 nm).

Known Compounds.—Allopurinol (1) and pyrazolo[3,4-*d*]pyrimidine-4-thione (14) were gifts of Dr. G. B. Elion, Wellcome Research Laboratories, Triangle Park, North Carolina. 4-Methylthiopyrazolo[3,4-*d*]pyrimidine (20) was prepared according to the literature.[19] In addition, compounds (2), (3), (6), (15), (16), (18), (21), and (22) have been described by Robins and his co-workers [11,19] or in the patent literature,[20] but they were synthesised by different procedures, starting usually from the pyrazole moiety.

General Procedure for Thiation of Allopurinols.—A suspension of an allopurinol (2.5 g) and phosphorus pentasulphide (10 g) in β-picoline (100 ml) was stirred and refluxed for 30 min. The solvent was removed *in vacuo* and the residue treated with boiling water (75 ml) for 15 min. The mixture was then kept in ice for 30 min; the solid portion was filtered off and recrystallised from water, with addition of charcoal (see Table 5).

Methylation of the Anion of Allopurinol (1).—Solutions of (1) (5 g) in 7% KOH (60 ml) and of MeI (20 g) in methanol (50 ml) were mixed and warmed to 50 °C for 6 h. A further 5 g of MeI was added, and warming was continued for another 4 h. The pH was adjusted to 7 and the solution brought to dryness *in vacuo*. The residue was treated several times with boiling benzene to extract the 1,5-dimethyl derivative (6), which crystallised from n-butyl acetate as needles, m.p. 191—195 °C,[11] yield 1.7 g (28%). The residue, insoluble in benzene, was recrystallised from n-butanol to give the 2,5-dimethyl isomer (7) as needles, m.p. 295—298 °C, yield 0.8 g (13%). Upon evaporation of the butanol filtrate, the residue (0.3 g) showed the n.m.r. spectrum of 2,7-dimethylallopurinol (9).

In another experiment, the reaction mixture, after 10 h at 50 °C, was replenished with 5 g of MeI and set aside at room temperature for three weeks. The dark brown solution was brought to dryness and the residue was recrystallised from n-butanol, yielding black prismatic rods of the tri-iodide (11a), m.p. 136—138 °C, yield 8.2 g (40%).

When sodium sulphite was added to a methanolic solution of (11a), the latter became colourless. The inorganic salts were removed by filtration, the filtrate brought to dryness, and the residue crystallised from n-butyl alcohol as needles, m.p. 226—229 °C.

1,7-*Dimethylallopurinol Toluene-p-sulphonate* (8).—A mixture of 1-methylallopurinol (2) (2 g) and methyl toluene-*p*-sulphonate (30 ml) was heated to 150 °C for 15 min, and the homogenous solution so obtained was kept at 110—120 °C for 2 h. Acetone (250 ml) was then added to precipitate the reaction product, which crystallised from acetonitrile as rods, m.p. 257—260 °C, yield 2.1 g (45%).

2,5-*Dimethylallopurinol* (7).—To a solution of 2-methylallopurinol (3) (0.5 g) in 0.1N-NaOH (5 ml) was added MeI (1 ml) and the mixture was stirred at 37 °C for 2 h. The mixture was brought to dryness and the residue of 2,5-dimethylallopurinol purified as described above.

Methylation of the Anion of 5-Methylallopurinol (4).—A mixture of (4) (1 g), dissolved in 0.1N-NaOH (5 ml), and methyl iodide (1 ml) was stirred at room temperature for 24 h. The residue which remained after evaporation of the solvent was chromatographed on paper, to separate 1,5- (6) and 2,5-dimethylallopurinol (7), which were then identified by their spectral properties.

Methylation of the Neutral Form of 7-Methylallopurinol (5).—A solution of (5) (0.9 g) in DMF (30 ml) and MeI (15 ml) was set aside at room temperature for 2 h. Addition of ether (500 ml) precipitated 5,7-dimethylallopurinol iodide (10), which crystallised from n-butyl alcohol as needles, decomposing at 252—254 °C, yield 0.8 g (46%).

Methylation of 4-Methylthiopyrazolo[3,4-d]*pyrimidines.*—(a) *Methylation of the anion of 4-(methylthio)pyrazolo*[3,4-d]*pyrimidine* (20a). To a stirred solution of (20) (3 g) in methanol (150 ml) and MeI (1.5 ml), kept at 50 °C, was added dropwise 1N-NaOH (20 ml) during 45 min, and stirring was continued for 4 h. The solution was then brought to dryness *in vacuo* and the residue treated with dilute ammonia (20 ml). The insoluble portion was filtered off, and recrystallised from isopropanol to give 1-methyl-4-methylthiopyrazolo[3,4-*d*]pyrimidine (21) as rods, m.p. 131—133 °C,[11] yield 1.8 g (55%).

The ammoniacal filtrate was neutralised with glacial acetic acid, and the precipitate recrystallised from n-butyl acetate. Upon rapid crystallisation, 2-methyl-4-methylthiopyrazolo[3,4-*d*]pyrimidine (22) was obtained as fine, long needles, while slow crystallisation yielded prismatic blocs, m.p. 171—173 °C,[21] yield 0.8 g (24%).

(b) *Methylation of the neutral form of* (20). A solution of (20) (1.4 g) in DMF (28 ml) and MeI (10 ml) was kept at 50 °C for 3 h. Upon cooling to −20 °C, 7-methyl-4-methylthiopyrazolo[3,4-*d*]pyrimidine hydriodide (23a) crystallised, and was recrystallised from acetic acid as long yellow needles, m.p. 226—228 °C, yield quantitative.

A suspension of the above salt in water had a pH of *ca.* 4. The pH was adjusted to 7 by cautious addition of ammonia. The free base (23) was crystallised from water as yellow needles, decomposing at 146—148 °C. The free base (23) proved to be unstable; it released methanethiol upon storage and blackened.

Methylation of 7-Methyl-4-methylthiopyrazolo[3,4-d]*pyrimidine.*—A solution of (23a) (0.15 g) and sodium acetate (40 mg) in DMF (10 ml) and MeI (1 ml) was set aside at room temperature for 12 h. Upon addition of water, the brown tri-iodide (24a) precipitated, and was crystallised from n-butyl alcohol as pointed needles, m.p. 154—155 °C; yield *ca.* 90%.

A methanolic solution of (24a) was decolourised with sodium sulphite. The mixture was filtered and the filtrate brought to dryness. From isopropanol, compound (24) crystallised in branched, yellow needles, m.p. 200 °C.

1-*Ethoxycarbonyl-4-methylthiopyrazolo*[3,4-d]*pyrimidine* (25).—To a solution of (20) (1 g) in 1N-NaOH (7 ml), stirred at 0 °C, was added dropwise ethyl chloroformate (1.8 ml). During 30 min., a white precipitate formed. The pH was adjusted to 8 and the solid filtered off and crystallised from methanol as rectangular plates, m.p. 112—114 °C, yield 1.6 g (75%).

4-*Methoxy-7-methylpyrazolo*[3,4-*pyrimidine* (26).—To a

solution of (23a) (0.3 g) in methanol (20 ml), kept at room temperature, were added with stirring 2 equiv. of sodium methoxide in absolute methanol. After 30 min, the solution was neutralised with glacial acetic acid and brought to dryness *in vacuo*. The residue was again dissolved in methanol and the product precipitated by addition of ether. Compound (26) crystallised from benzene as rods, m.p. 230—233 °C.

Calculations.—For calculations by the INDO method, program CNINDO-174, No. 281 QPCE 136-COORDINATE was used on a CDC-6400 computer of the Hebrew University. Bond lengths and bond angles were derived from molecular models, using the formula of Dewar *et al.*[21] Bond orders were obtained from Hückel program calculations.

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