Purine Studies. Part I. Stability to Acid and Alkali. Solubility. Ionization. Comparison with Pteridines.

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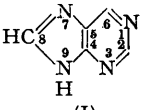
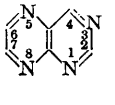
Syntheses of several new monosubstituted purines are described. In general, purines were found to be more stable than the corresponding pteridines: the electronic distribution responsible for this is discussed. Unexpectedly, 2-monosubstituted purines underwent a reversal of the Traube synthesis, giving 4 : 5-diaminopyrimidines.

The cumulative effect of hydroxyl groups is to make purines progressively less soluble in water, but (contrary to E. Fischer's prediction) amino-groups *in excess of one* increase the solubility. The ionization constants of 34 purines are measured and discussed.

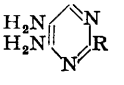
THIS study of the simpler purines follows the lines of a study of the simpler pteridines (Albert, Brown, and Cheeseman, *J.*, 1951, 474; 1952, 1620, 4219; Albert and Brown, *J.*, 1953, 74). The desirability of comparing purine (I) and pteridine (II) arises from the



(I)
(The hydrogen atom shown on $N_{(9)}$ is shared with $N_{(7)}$)



(II)



(III*a*; R = OH)
(III*b*; R = SMe)

similarity of their structure, and from the essential catalytic role played by pteridines in the natural synthesis of purines (Woolley and Pringle, *J. Amer. Chem. Soc.*, 1950, **72**, 634). Moreover, pteridines can be formed from purines by ring enlargement and may be formed thus in Nature (Albert, *Biochem. J.*, in the press).

Stability.—The results given in Table 1 were obtained by refluxing the purines with acid or alkali, as was done with the pteridines (Albert, Brown, and Cheeseman, *J.*, 1952, 4219). It is evident that purine, unlike pteridine, is highly stable: as with pteridine, hydroxyl groups stabilize it against attack by acid. But whereas pteridine is also stabilized by hydroxyl groups against attack by alkali, the reverse effect is found in the purine series when two or more hydroxyl groups are present, so that uric acid is distinctly unstable to alkali. The nature of this reaction is known to be oxidative (Brandenberger, *Helv. Chim. Acta*, 1954, **37**, 641).

The exceptional instability of 2-hydroxypurine to acids was further investigated. After only 5 minutes' boiling with N-sulphuric acid, appreciable quantities of 4-amino-5-formamido-2-hydroxypyrimidine and 4 : 5-diamino-2-hydroxypyrimidine (III*a*) were found. After two hours, no 2-hydroxypurine remained, and (III*a*) was isolated as the sparingly soluble quartasulphate in 25% yield (much of it had broken down further).

TABLE 1. *Decomposition (%) of purines.*

(A) From ammonia evolved, assumed to be mole per mole, (B) from spectrophotometric estimation of unchanged material (both 1 hr. at 100°).

	N-H_2SO_4		10N-NaOH	
Purine derivative	A	B	A	B
Unsubstituted	12	8 [b]	0	1
2-Hydroxy	12	92	2	2
6-Hydroxy (hypoxanthine)	1	2	1	1
8-Hydroxy	0	0	0	0
2 : 6-Dihydroxy (xanthine)	3	2	9·5	8
2 : 8-Dihydroxy	5	5	6	4
6 : 8-Dihydroxy	5	4	7	6
2 : 6 : 8-Trihydroxy (uric acid)	3	4	21	18
2-Mercapto	8	88	[c]	[c]
2-Amino [a]	10	4	1	1
$N_{(9)}$-Methyl	1	3	5	98

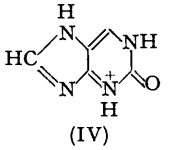
[a] Critical conditions for non-interference by 2 : 4 : 5-triaminopyrimidine were found at 267 mμ (pH 0). The yield of triaminopyrimidine in the acid hydrolysis was shown to be 5% by paper chromatography (solutions of known strength as controls). [b] Cf. pteridine (74%). [c] Not attempted.

These reactions were also followed chromatographically on paper. By avoiding the usual excess of acid in its preparation (Johns, *J. Biol. Chem.*, 1912, **11**, 69; Tafel and Ach, *Ber.*, 1901, **34**, 1170), the yield of 2-hydroxypurine was improved.

Dilute acid readily attacked 2-mercaptopurine also (see Table 1), giving 4 : 5-diamino-2-mercaptopyrimidine. However, 2-aminopurine was not greatly affected under these conditions, although 2 : 4 : 5-triaminopyrimidine was found chromatographically in the product. No evidence of hydrolysis to (III*a*) could be found in either case. 2-Methylthiopurine was not attacked by acid.

Introduction of electron-releasing substituents into 2-hydroxypurine stabilizes it towards acids. For example, 2 : 6-dihydroxypurine (xanthine) resists 10N-hydrochloric acid at 100° (Fischer, *Ber.*, 1898, **31**, 2562), and uric acid withstands boiling 7N-hydrochloric acid or concentrated sulphuric acid at 100°. Other examples of this stabilization will be found in Table 1; also we found 2 : 6 : 8-triaminopurine was stable to N-sulphuric acid at 100°.

The instability to acids of 2-hydroxypurine must involve a cation, *e.g.*, (IV) or even (V), capable of stabilizing the pyrimidine ring at the expense of the glyoxaline ring.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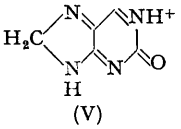
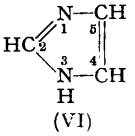
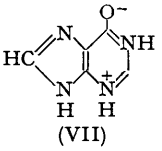

(V)


(VI)


(VII)

Another example of a special instability caused by a 2-substituent is provided by the reaction of 2-methylthiopurine with aliphatic amines. 2-Methylthiopurine, when heated with mono- or di-methylamine at 155° for 24 hours, broke down to 4 : 5-diamino-2-methylthiopyrimidine (III*b*), whereas 6- and 8-methylthiopurine gave only the expected alkylaminopurines [for proof of the constitution of (III*b*) see p. 2067]. Neither methylamine nor 10N-sodium hydroxide affected 2-methylthiopurine at 100° (30 min.).

The instability to alkali of $N_{(9)}$-methylpurine (Table 1) is in line with Fischer's observation that hydroxypurines become highly sensitive to boiling N-potassium hydroxide when they are so substituted that they can no longer produce anions. For example, caffeine (1 : 3 : 7-trimethylxanthine) was completely destroyed in ¼ hour, whereas xanthine was barely

affected in 20 hours (*Ber.*, 1898, **31**, 3266). 5-Amino-4-methylaminopyrimidine was isolated almost quantitatively after action of N-sodium hydroxide on 9-methylpurine (1 hour at 100°), and compared with authentic material (Brown, *J. Appl. Chem.*, 1954, **4**, 72).

The reason for the increased stability of purine over pteridine is to be found in the different distribution of electrons in the two systems. Glyoxaline (VI) has a benzene-like

TABLE 2. *Physical properties of purines.*

No.	Purine derivative	Solubility in H_2O, 20° (±2°) 1 in	Solubility in H_2O, 100° 1 in	pK_a (in H_2O) and concn. (M) at which determined (20°)		R_F	Source
1	Unsubstituted	2	<1 [i]	—		0·80	A
	anion	—	—	8·93 (±0·02)	0·01	—	—
	cation	—	—	2·39 (±0·04) [j]	0·1	—	—
2	6-Methyl	5	—	—		0·85	B
	anion	—	—	9·02 (±0·02)	0·01	—	—
	cation	—	—	2·6	0·1	—	—
3	8-Methyl	18	6	—		0·85	A
	anion	—	—	9·37 (±0·05)	0·01	—	—
	cation	—	—	2·85 (±0·06)	0·1	—	—
4	$N_{(3)}$-Methyl	<2 [b]	—	—		0·85	A
	cation	—	—	2·36 (±0·03)	0·1	—	—
5	8-Phenyl	5,900	600	—		0·90	A
	anion	—	—	8·09 (±0·06)	0·001	—	—
	cation	—	—	2·68 (±0·07)	0·0017 [k]	—	—
6	6-Chloro	180	—	—		[r]	C
	anion	—	—	7·82 (±0·04)	0·01	—	—
	cation	—	—	<2	0·03	—	—
7	2-Hydroxy (+1H_2O)	380	22	—		0·40	A
	anion (mono)	—	—	8·43 (±0·03)	0·02	—	—
	anion (di)	—	—	11·90 (±0·07)	0·02	—	—
	cation	—	—	1·69 (±0·04)	0·02	—	—
8	6-Hydroxy (hypoxanthine)	1,400 [c]	70 [c]	—		0·55	F
	anion (mono)	—	—	8·94 (±0·02)	0·005	—	—
	anion (di)	—	—	12·10 (±0·03)	0·02	—	—
	cation	—	—	1·98 (±0·02)	0·025 [k]	—	—
9	8-Hydroxy	240	12	—		0·70	A
	anion (mono)	—	—	8·24 ±(0·03)	0·005	—	—
	anion (di)	—	—	>12	—	—	—
	cation	—	—	2·58 (±0·06)	0·005	—	—
10	2 : 6-Dihydroxy (xanthine)	2,000 [de]	400 [e]	—		[s]	D
	anion (mono)	—	—	7·44 (±0·04) [l]	0·001	—	—
	anion (di)	—	—	11·12 (±0·04)	0·001	—	—
11	2 : 8-Dihydroxy	26,000 [f]	2500	—		0·30	E
	anion [d]	—	—	7·45 (±0·10) [f]	—	—	—
12	6 : 8-Dihydroxy	6,000	350	—		0·40	A
	anion (mono)	—	—	7·65 (±0·07)	0·002 [k]	—	—
	anion (di)	—	—	9·87 (±0·07)	0·002 [k]	—	—
13	2 : 6 : 8-Trihydroxy (uric acid)	39,500 [g]	1262 [g]	5·4 [m]	—	0·30	—
14	2-Methoxy	200	—	—		0·90	A
	anion	—	—	9·2	0·01	—	—
	cation	—	—	2·44 (±0·08)	0·001	—	—
15	6-Methoxy (+0·5H_2O)	225	—	—		0·85	B
	anion	—	—	9·16 (±0·05)	0·005	—	—
	cation	—	—	2·21 (±0·02)	0·02	—	—
16	2-Amino	120	3·5	—		0·55	A
	anion	—	—	9·93 (±0·03)	0·05	—	—
	cation (mono)	—	—	3·80 (±0·01)	0·05	—	—
	cation (di)	—	—	−0·28 [f]	—	—	—
17	6-Amino (adenine)	1,100 [h]	40 [h]	—		0·70	D
	anion	—	—	9·8 [n]	—	—	—
	cation (mono)	—	—	4·22 (±0·02)	0·005	—	—
	cation (di)	—	—	<1	0·05 [k]	—	—
18	8-Amino	2,000	600	—		0·50	A
	anion	—	—	9·36 (±0·08)	0·003	—	—
	cation	—	—	4·68 (±0·02)	0·01 [k]	—	—
19	2 : 6-Diamino	420	17	—		0·55	D
	anion	—	—	10·77 (±0·05)	0·02	—	—
	cation (mono)	—	—	5·09 (±0·05)	0·02	—	—
	cation (di)	—	—	<1	—	—	—

TABLE 2. (*Continued.*)

No.	Purine derivative	Solubility in H_2O, 20° (±2°) 1 in	Solubility in H_2O, 100° 1 in	pK_a (in H_2O) and concn. (M) at which determined (20°)		R_F	Source
20	2 : 6 : 8-Triamino	200	20	—		0·40	B, H
	anion	—	—	10·79 (±0·04)	0·033	—	—
	cation (mono)	—	—	6·23 (±0·07)	0·033	—	—
	cation (di)	—	—	2·41 (±0·05)	0·033	—	—
21	6-Methylamino	850	50	—		0·80	A, F
	anion	—	—	9·99 (±0·05)	0·007	—	—
	cation (mono)	—	—	4·18 (±0·02)	0·007	—	—
	cation (di)	—	—	<1	—	—	—
22	8-Methylamino	450	60	—		0·60	A
	anion	—	—	9·56 (±0·04)	0·01	—	—
	cation	—	—	4·78 (±0·04)	0·01	—	—
23	2-Dimethylamino	3,000	—	—		0·90	A, B
	anion	—	—	10·22 (±0·10)	0·002	—	—
	cation	—	—	4·02 (±0·03)	0·002	—	—
24	6-Dimethylamino	120	15	—		0·85	A
	anion	—	—	10·5 [p]	0·02	—	—
	cation (mono)	—	—	3·87 (±0·04)	0·02	—	—
	cation (di)	—	—	<1	—	—	—
25	8-Dimethylamino	150	3	—		0·65	A
	anion	—	—	9·73 (±0·03)	0·02	—	—
	cation (mono)	—	—	4·80 (±0·03)	0·03	—	—
	cation (di)	—	—	<1	—	—	—
26	2-Amino-6-hydroxy (guanine)	200,000	—	—		[s]	D
	anion (mono)	—	—	9·2 [n]	—	—	—
	anion (di)	—	—	12·3 [n]	—	—	—
	cation	—	—	3·3 [n]	—	—	—
27	6-Amino-2-hydroxy (*iso*guanine)	16,000	4000	—		0·40	A
	anion	—	—	8·99 (±0·2)	—	—	—
	cation	—	—	4·51 (±0·2) [f]	—	—	—
28	2-Mercapto	2,500	300	—		0·35	A
	anion (mono)	—	—	7·15 (±0·05)	0·0025	—	—
	anion (di)	—	—	10·4 [p]	0·0025	—	—
	cation	—	—	0·5 (±0·3) [f]	—	—	—
29	6-Mercapto	4,000	180	—		0·60	F
	anion (mono)	—	—	7·77 (±0·02)	0·0015	—	—
	anion (di)	—	—	10·84 (±0·04)	0·0015	—	—
	cation	—	—	<2·5 [q]	—	—	—
30	8-Mercapto	2.200	60	—		0·80	A
	anion (mono)	—	—	6·64 (±0·02)	0·0025	—	—
	anion (di)	—	—	11·16 (±0·09)	0·0025	—	—
	cation	—	—	<2·5 [q]	—	—	—
31	2-Methylthio	2,200	150	—		0·90	A
	anion	—	—	8·91 (±0·03)	0·0025	—	—
	cation	—	—	1·91 (±0·07)	0·0025	—	—
32	6-Methylthio	1,300	20	—		0·90	F
	anion	—	—	8·74 (±0·01)	0·005	—	—
	cation	—	—	0 (±0·2) [f]	—	—	—
33	8-Methylthio	650	65	—		0·90	A
	anion	—	—	7·67 (±0·01)	0·01	—	—
	cation	—	—	2·95 (±0·05)	0·01	—	—
34	2-Amino-8-phenyl	—	—	—		—	G
	anion	—	—	9·20 (±0·02)	0·001	—	—
	cation	—	—	3·98 (±0·07)	0·001	—	—

[a] No evidence of other species could be found spectrometrically. [b] 1 g. is dissolved by 1·7 ml. Fischer, *Ber.*, 1897, **30**, 2227. [d] At 10°. [e] Strecker, *Annalen*, 1861, **118**, 151 (*q.v.* for discussion of earlier figures). [f] Determined spectrometrically. [g] His and Paul, *Z. physiol. Chem.*, 1901, **31**, 29; Rossi, *Biochem. Z.*, 1913, **54**, 299. [h] Kossel, *Z. physiol. Chem.*, 1886, **10**, 254; Tafel and Ach, *Ber.*, 1901, **34**, 1175. [i] 1 g. is dissolved by 0·9 ml. [j] Equiv. to 2·30 at 0·001M. [k] Back-titrated. [l] Cf. 7·7 (Ogston, *J.*, 1935, 1376; hydrogen electrode). [m] Bernouilli and Loebenstein, *Helv. Chim. Acta*, 1940, **23**, 245. [n] Taylor, *J.*, 1948, 765. [p] Decomposed by alkali. [q] Spectrophotometry revealed no inflections down to pH 0. [r] Travels with front. [s] Is not visible in this solvent, but 3% ammonium chloride was suitable.

Sources: A, This paper. B, Kindly presented by Dr. Gertrude Elion (unpublished work). C, Kindly presented by Dr. Aaron Bendich (unpublished work). D, Commercial preparation, purified until a single spot was obtained (paper chromatography), and the potentiometric titration curve became regular. E. Johns, *Amer. Chem. J.*, 1911, **45**, 84. F, Elion, Burgi, and Hitchings, *J. Amer. Chem. Soc.*, 1952, **74**, 411. G, Falco, Elion, Burgi, and Hitchings, *ibid.*, p. 4897. H, Cavalieri and Bendich, *ibid.*, 1950, **72**, 2587.

inertness. It is stable to boiling 60% hydriodic acid, to chromic–sulphuric acid, and to distillation over lime (Ackermann, *Z. physiol. Chem.*, 1910, **65**, 508). It forms 4-nitroglyoxaline with nitric acid at 100° (Fargher and Pyman, *J.*, 1919, **115**, 219). This aromatic behaviour is derived from a sextet of unlocalized π-electrons, two being supplied from each double bond and the remaining two from the NH group. Delocalization is so effective as to leave that nitrogen atom with a formal positive charge. In acid and alkaline solution, glyoxaline is stablilized by further resonance, the anion and the cation being symmetrical structures. Benziminazole, in which a glyoxaline and a benzene nucleus are combined, is also stable, *e.g.*, it is unchanged by 10N-hydrochloric acid at 270° (O. Fischer, *Ber.*, 1889, **22**, 644). In pyrimidine both nitrogen atoms are doubly bound (of the type –N=) and hence have a formal negative charge. Each of these atoms competes for the six π-electrons, but the C : N= ratio of 4 : 2 does not endanger stability under mild conditions (however, pyrimidine is more sensitive to alkali than pyridine; Lythgoe and Rayner, *J.*, 1951, 2323).

Pteridine (II), which consists of two 6-membered rings, has 10 ring-atoms contributing 10 π-electrons which tend to accumulate on the four negatively charged nitrogen atoms (note the highly unfavourable C : N= ratio of 6 : 4). The outstanding instability of pteridine to acid and alkali (and its stabilization by electron-releasing substituents) has been traced to this electronic distribution (Albert, Brown, and Cheeseman, *J.*, 1952, 4219). In purine, on the contrary, the excess of π-electrons provided by the glyoxaline ring (5 ring-atoms : 6 π-electrons) plus the presence of a positively charged nitrogen atom in this ring, create a molecule in which the π-electron density is nowhere very low. That there is sharing of π-electrons between the glyoxaline and the pyrimidine ring follows from the ionization constants (see below). It is relevant that, whereas glyoxaline couples with diazotized aniline (in the 2-position; Fargher and Pyman, *loc. cit.*), we find that purine does not couple. However, it does couple if an electron-releasing substituent is introduced into the 2- or the 6-position: Fischer (*Z. physiol. Chem.*, 1909, **60**, 69) showed that this coupling took place in the 8-position. Pteridines, on the other hand, do not couple even when three electron-releasing groups are present (Albert, Brown, and Cheeseman, *J.*, 1952, 1620). Possible evidence for a slight localization of π-electrons in the glyoxaline ring is furnished by the ease with which the hitherto unknown 8-aminopurine diazotizes and couples (red) with β-naphthol, like the similarly constituted 2-aminoglyoxaline (Fargher and Pyman, *loc. cit.*). 2-Amino-, 6-amino-, and 2 : 6-diamino-purine do not couple.

Solubility in Water.—The solubility of a number of purines has been determined and is shown in Table 2. The following remarks apply especially to solubility at 20°. Purine (I), unlike pteridine (II), has a hydrogen available for hydrogen-bond formation, and presumably exists in the solid phase as a chain of molecules, hydrogen-bonded between the 7- and the 9-positions of neighbours. This would account for the high m. p. (213°) compared with that of pteridine (140°). Evidently this type of hydrogen-bond does not compete favourably with hydrogen bonding to water molecules, because purine is very soluble in water, even more so than pteridine which cannot form hydrogen bonds. Understandably, the solubility is lowered by lipoid-solubilizing groups (*e.g.*, Me, Cl, MeS, Ph; Nos. 2, 3, 6, 31, 32, 33, and 35), the larger groups having the greater effect. On the other hand, a 9-methyl group (No. 4) slightly increases the solubility by preventing hydrogen-bonding (m. p. 161° and, unlike purine, lipoid-soluble).

The monohydroxypurines (Nos. 7—9) resemble the hydroxypteridines in being one hundred (or more) times less soluble in water than the parent substance. This decrease is attributed to unusually strong crystal-lattice forces originating in hydrogen bonding between the hydroxy-groups and the ring-nitrogen atoms (Albert, Brown, and Cheeseman, *J.*, 1952, 4219). This increased strength, it is now suggested, comes from the reinforcement afforded by strong dipoles, of the type (VII). In harmony with this concept, methoxypurines are more soluble than the corresponding hydroxypurines (see Nos. 14 and 15; 8-methoxypurine is still unknown).

The dihydroxypurines (Nos. 10—12) are less soluble than the monohydroxypurines; trihydroxypurine (No. 13) is the least soluble of all. Various figures for xanthine (No. 10) are available in the literature, and we have used Strecker's because he has given the subject

critical attention. 2 : 8-Dihydroxypurine resembles xanthine in being apparently amorphous and tending to give supersaturated solutions, but the other substances in Table 2 reached equilibrium rapidly and deposited well-defined crystals.

The monoaminopurines (Nos. 16—18) are all less soluble than purine. The order of insolubility ($2 < 6 < 8$) for the monoaminopurines also holds if the 7- or the 9-hydrogen atom is replaced by methyl (Fischer, *Ber.*, 1899, **32**, 435). Fischer (*ibid.*, p. 498) suggested that the polyamino-purines would be less soluble still, in analogy with the hydroxypurines. This is not so, as Nos. 19 and 20 show (cf. 13 and 20, for instance). It is evident that, in this series at least, amino-groups in excess of one can be more attracted to water than to other molecules of the purine. As would be expected from our hypothesis of hydrogen bonding, the monomethylaminopurines are more soluble than the aminopurines, and the dimethylaminopurines more soluble still (Nos. 21—25). However, 2-dimethylaminopurine forms an exception: it is surprisingly insoluble although normal in other respects (*e.g.*, in the relation of the spectra, and of the pK's, to those of the parent compound).

As with pteridines, the aminohydroxypurines (Nos. 26 and 27) are far less soluble than the corresponding dihydroxy- or diamino-purines. This is attribtued to the dipole's being much more powerful in such substances. Similarly, 6-amino-2 : 8-dihydroxypurine is soluble only 1 in 500,000 at 23° (Bendich, *J. Biol. Chem.*, 1950, **183**, 267).

The mercaptopurines (Nos. 28—30) are less soluble than the corresponding hydroxypurines (Nos. 7—9). Because neither hydrogen bonding nor dipole moments should be as great as in the hydroxy-analogues, the principal consideration must be the lower hydrophilic properties of sulphur in comparison with oxygen. Hence it is not surprising that *S*-methylation (Nos. 31—33) improves solubility less effectively than does *O*-methylation.

Ionization.—A number of ionization constants have been determined (see Table 2). The ionization of purine, not previously investigated, yields an anion of pK_a 8·9 (*i.e.*, a little stronger than phenol) and a cation of pK_a 2·4 (*i.e.*, considerably weaker than aniline or pyridine). The values for glyoxaline (VI) [13 and 7·0 respectively (Kirby and Neuberger, *Biochem. J.*, 1938, **32**, 1146; Wieland and Schneider, *Annalen*, 1953, **580**, 159)] are changed, on the addition of a benzene ring (giving benziminazole), to 12·3 and 5·5 respectively, *i.e.*, the acid strength has become stronger and the base strength weaker (Albert, Goldacre, and Phillips, *J.*, 1948, 2240). The base strength of pyrimidine is very weak (1·3; Albert, Goldacre, and Phillips, *loc. cit.*) and, as it is a more electron-attracting nucleus than benzene, the above values for purine may seem a natural consequence of combining the glyoxaline and the pyrimidine nucleus. It cannot be disputed that the anion is formed by the loss of a proton from the glyoxaline ring, but it does not follow that the cation is formed by the capture of a proton in the glyoxaline ring. The cationic pK_a (2·4) is just as compatible with a pyrimidine nucleus enriched by the electrons which the glyoxaline nucleus is surrendering. This poses a problem which diffraction crystallography could solve.

Methyl substituents show their usual (but feeble) acid-weakening and base-strengthening properties (Nos. 2 and 3). 9-Methylpurine has no acidic properties because methyl has replaced the ionizable hydrogen. The 8-phenylpurines (Nos. 5 and 34) show the expected acid-strengthening. Chlorine (in No. 6) proves to be acid-strengthening and base-weakening, as expected.

The introduction of a hydroxyl group into purine (Nos. 7—9) permits the formation of two anions. It is not easy to determine, in each case, whether the imino- or the hydroxy-group is the stronger. For example, the anionic pK's of purine, 6-hydroxypurine, and 6-hydroxy-9-methylpurine are the same (8·9). Fortunately the hydroxyl group in 6-hydroxypurine is so placed with regard to $N_{(7)}$ as to form chelate complexes: it was found that a steady stability constant can be obtained from the titration curves (in the presence of various metallic cations) only if it is assumed that the group which chelates has the lower anionic pK_a (Albert, *Biochem. J.*, 1953, **54**, 646). This implies that the value 8·9 belongs to the hydroxyl group of 6-hydroxypurine. This form of reasoning does not help with the isomers, which do not form chelate complexes.

Attention is drawn to the cationic constant of 6-hydroxypurine, hitherto overlooked, apparently because it does not influence the spectrum [pK's of 10·3 and 0·6 obtained for

this substance by a solubility method (Filitti, *J. Chim. phys.*, 1935, **32**, 1) are evidently incorrect]. Further hydroxyl groups progressively increase acidic strength (Nos. 10—13). They also weaken basic strength: solubility difficulties prevent exact measurement, but Wood's figure of pK_a 0·8 for xanthine may be a guide (*J.*, 1906, **89**, 1840).

As expected, an amino-substituent (Nos. 16—26) is acid-weakening. The rather large consequent increase in basic strength indicates that the principal basic centre has shifted to a guanidino-group which includes the amino-substituent. It is relevant that *X*-ray crystallography of adenine (No. 17) hydrochloride has revealed that the proton is on $N_{(1)}$ (Cochran, *Acta Cryst.*, 1951, **4**, 81). As would be expected, further substitution by amino-groups increases the base-strengthening effect, and triaminopurine (No. 20) has pK_a 6·2, the most basic of known purines. The mono- and di-methylation of the amino-purines progressively decreases acid strength without greatly changing base strength (Nos. 21—25).

As would be expected, the mercaptopurines (Nos. 28—30) are more acidic than the hydroxy-analogues (Nos. 7—9). The effect of a methylthio-group in a benzene nucleus is slightly to weaken bases and either to strengthen acids or not affect them (Bordwell and Cooper, *J. Amer. Chem. Soc.*, 1952, **74**, 1058). These effects are observed also in Nos. 31—33, but the base-strengthening in No. 33 was unexpected.

In comparisons of the ionization of purine and pteridine, the most important differences are that the latter is a stronger base (pK_a 4·1) and has no imino-group with which to form an anion. No equivalent has been found in the purine series to (*a*) the hysteresis encountered in titrating 6-hydroxypteridine (Albert, Brown, and Cheeseman, *J.*, 1952, 1620), (*b*) the curious base-weakening properties of methylpteridines (to be reported later), or (*c*) the mutual weakening of amino- and hydroxy-groups in aminohydroxypteridines (Albert, Brown, and Cheeseman, *J.*, 1952, 4219).

Syntheses.—Purine is usually prepared from uric acid, in 2% yield, by Fischer's method (*Ber.*, 1898, **31**, 2550). The recent accessibility of 4 : 5-diaminopyrimidine (Brown, *J. Appl. Chem.*, 1952, **2**, 239) prompted us to re-examine Isay's method (*Ber.*, 1906, **39**, 250), in which this amine is heated with formic acid to 210° and the product distilled. The usual poor yields were found to be caused by destruction during the distillation: the purine was present in the reaction mixture as a formate from which it was liberated by calcium carbonate refluxing in alcohol (85% yield). Attempts to methylate purine were unsuccessful, including (*a*) use of methyl sulphate at pH 8·5, (*b*) heating with methyl iodide alone, or in aqueous sodium hydroxide, (*c*) use of methyl toluene-*p*-sulphonate at 150°, (*d*) heating with formic acid and formaldehyde, and (*e*) heating the mercurichloride with methyl iodide. It also resisted hydroxymethylation with formaldehyde.

9-Methylpurine was obtained in excellent yield by refluxing 5-amino-4-methylaminopyrimidine (Brown, *J. Appl. Chem.*, 1954, **4**, 72) with formic acid. In the usual method a trivial yield is obtained, by a lengthy series of reactions, from uric acid (Fischer, *loc. cit.*).

2-Dimethylaminopurine was prepared by formylating 4 : 5-diamino-2-dimethylaminopyrimidine in the 5-position and heating the product at 260°. A specimen prepared from these intermediates under other conditions (Miss G. Elion, unpublished work) was used to confirm the unexpectedly low solubility (Table 2). A similar attempt to prepare 2-methoxypurine gave only a 10% yield because diformylation largely supervened: the second product was not basic and was thus probably 4 : 5-diformamido-2-methoxypyrimidine. 4-Amino-2-methoxy-5-nitropyrimidine resisted formylation even by acetic formic anhydride. Isay's synthesis of 2-aminopurine (*loc. cit.*, *Ber.*) has been improved.

2-Mercaptopurine was obtained by refluxing 4 : 5-diamino-2-mercaptopyrimidine with formic acid. When this work was complete, a synthesis of this substance under more vigorous conditions, and in lower yield, was described by Robins, Dille, Willits, and Christensen (*J. Amer. Chem. Soc.*, 1953, **75**, 263). With chloroacetic acid, it gave 2-carboxymethylthiopurine, but this could not be hydrolysed to 2-hydroxypurine even on 3 hours' refluxing with 48% hydrobromic acid. 2-Mercaptopurine was converted into 2-methylthiopurine with methyl iodide. The position of the methyl group was confirmed by direct synthesis: 4-amino-2-chloro-5-nitropyrimidine with sodium methyl sulphide gave 4-amino-2-methylthio-5-nitropyrimidine; this was hydrogenated to the 4 : 5-diamine (III*b*), which was condensed with formic acid to give the 5-formamido-derivative.

This was cyclized to 2-methylthiopurine at 185°. 4 : 5-Diamino-2-methylthiopyrimidine was also prepared by the methylation of 4 : 5-diamino-2-mercaptopyrimidine.

Some improved syntheses of 6-substituted purines are given in the Experimental section.

Among several new 8-substituted purines, 8-phenylpurine was prepared by benzoylating 4 : 5-diaminopyrimidine, separating the di- and the mono-benzoyl derivative, and heating the latter at 230°. 8-Methylthiopurine was made by methylating 8-mercaptopurine. It evolved methanethiol copiously when boiled with N-hydrochloric acid, thus confirming the attachment of the methyl group to S. When heated with ammonia, methylamine, and dimethylamine it gave respectively 8-amino-, 8-methylamino-, and 8-dimethylamino-purine. 6 : 8-Dihydroxypurine was synthesized in a new and more convenient way from urea and 4 : 5-diamino-6-hydroxypyrimidine. Improvements have been affected in the preparation of 8-methyl-, 8-hydroxy-, and 8-mercapto-purine. The last-named was converted into 8-carboxymethylthiopurine which gave only a poor yield of 8-aminopurine when heated with ammonia. A trace of 8-aminopurine was obtained by the action of cyanogen bromide on 4 : 5-diaminopyrimidine, a reaction that is more successful in the benziminazole series (Pierron, *Ann. Chim.*, 1908, **15**, 193).

Experimental

Yields of substances that lack a m. p. refer to the stage where they appeared homogeneous in paper chromatography in 3% aqueous ammonium chloride or butanol–5N-acetic acid. Microanalyses were by Mr. P. R. W. Baker, Beckenham. R_F values (as in Table 2) were obtained in butanol–5N-acetic acid (2 : 1) by the descending method, picric acid (R_F 0·80) being used as a control.

Solubilities.—Those recorded in Table 2 were determined as described by Albert, Brown, and Cheeseman, *J.*, 1952, 4219.

Ionization Constants.—Except where otherwise noted in Table 2, these were determined potentiometrically, as described by Albert, Brown, and Cheeseman (*J.*, 1951, 474). The pK's of pyrimidines required for the spectrometric analyses reported in Table 1 were taken from Part II.

Decomposition by Alkali.—Each purine (0·001 mole) was heated under reflux with 10N-sodium hydroxide (10 ml.) at 110° (bath) for 1 hr., then steam-distilled in a Kjeldahl apparatus for 20 min., at constant volume. The evolved ammonia was trapped in 0·1N-hydrochloric acid (25 ml.), and determined by back-titration. The contents of the distillation-flask were then diluted (after neutralization, where necessary) with an appropriate buffer solution to 10^{-4}M. The densities, at wave-lengths known to be significant for the starting material, in its various ionic species, were then compared in an ultra-violet spectrophotometer, of which the solvent cell contained an aqueous solution of the same amount of the same salts brought to the same pH.

Each spectrometrically determined decomposition figure in Table 1 was calculated from that peak showing the greatest loss in density (compared with the corresponding peak in the corresponding species of untreated substance). This examination of a number of peaks, and species, was designed to minimize error due to the possibility of the decomposition products' having some peaks at wave-lengths near to those of the starting material.

Decomposition by Acid.—The purines (0·001 mole) were heated as above, but with N-sulphuric acid (10 ml.). The resulting solution (or suspension) was diluted to 50 ml., of which 25 ml. were transferred to a Kjeldahl apparatus. 10N-Sodium hydroxide (3 ml.) was added and the contents were steam-distilled (at constant volume) for 20 min., the evolved ammonia being determined as above. Corrections were made, where necessary, for any ammonia evolved through alkaline decomposition during this distillation.

Purine.—4 : 5-Diaminopyrimidine (4·4 g., 0·04 mole; Brown, *J. Appl. Chem.*, 1952, **2**, 239) and formic acid (20 ml.) were heated at 100° under carbon dioxide for 15 min., then at 210° during 45 min. and at 210° for 30 min. The product was rapidly cooled, powdered finely, and heated to constant weight at 110—120°. The dark powder was refluxed for 3 hr. in alcohol (100 ml.) with calcium carbonate (2 g.). The suspension was filtered and the residue was dried, finely ground, and refluxed for 30 min. with alcohol (40 ml.). The combined filtrates, taken to dryness gave 4·1 g. of purine (85%), m. p. 212—213°. Sublimation at 175°/0·01 mm. gave faintly yellow crystals (93% recovery), m. p. 212—213°; recrystallization, by dissolution in boiling

alcohol (1 g. in 13 ml.) and concentration to one-third volume, removed the yellow colour (90% recovery), m. p. 212—213° (cf. 211—212° given by Fischer, *Ber.*, 1898, **31**, 2550).

9-Methylpurine.—5-Amino-4-methylaminopyrimidine (2 g.) was refluxed with formic acid (98%; 4 ml.) for 2 hr. The acid was removed at 100°/20 mm. Alcohol (4 ml.) was added to the oily residue and the mixture evaporated. Sublimation at 140—150°/20 mm. gave white crystals (1·7 g.) of 9-methylpurine. After recrystallisation from *iso*butyl methyl ketone (7 parts), it had m. p. 159—161° [Fischer gives 162—163° (corr.)] (Found: N, 41·5. Calc. for $C_6H_6N_4$: 41·8%). The compound discolours slowly in sunlight.

2-Hydroxypurine.—A solution of 2-aminopurine (2·7 g.; 0·02 mole) in acetic acid (10 ml.) and water (10 ml.) was super-cooled to 20°. Sodium nitrite (1·4 g.) in water (6 ml.) was added dropwise during 5 min. at < 30°. Next day, the gelatinous precipitate was filtered off, recrystallized from water (20 parts) and dried at 110°, giving 2-hydroxypurine as creamy-white crystals (65%), homogeneous on paper chromatography with aqueous ammonium chloride (3%) (Found: C, 39·0; H, 3·9; N, 36·4. Calc. for $C_5H_4ON_4,1H_2O$: C, 39·0; H, 3·9; N, 36·35%). When boiled with 2 equivs. of N-sulphuric acid for 2 hr. and adjusted to pH 4·5, 2-hydroxypurine gave a precipitate of 4 : 5-diamino-2-hydroxypyrimidine quartasulphate, which was recrystallised from 100 parts of boiling water (25% yield) (Found: C, 32·0; H, 4·2; N, 37·3. Calc. for $C_4H_6ON_4,\frac{1}{2}H_2SO_4$: C, 31·9; H, 4·35; N, 37·2%).

2-Aminopurine.—2 : 4 : 5-Triaminopyrimidine (7·5 g.; Albert, Brown, and Cheeseman, *J.*, 1951, 474), formylmorpholine (15 ml.; Médard, *Bull. Soc. chim.*, 1936, **3**, 1343), and formic acid (3 ml., 1·2 equivs.) were heated under reflux at 200° for 1 hr. Acetone (50 ml.) precipitated the 2-aminopurine which was dissolved in boiling water (25 ml.). The solution was passed through a wide filter, cooled to 50°, and diluted with 3N-nitric acid (12 ml.). After refrigeration, the nitrate was filtered off, suspended in boiling water (15 ml.), and brought to pH 6—8 with N-sodium citrate (1 ml.) and 6N-sodium hydroxide. The solution was boiled with carbon, filtered, and refrigerated, giving buff-coloured crystals of 2-aminopurine (50%). This was recrystallised from 4 parts of boiling water (carbon) until colourless, and became anhydrous when dried at 110°.

2-Dimethylaminopurine.—Crude 4 : 5-diamino-2-dimethylaminopyrimidine (3 g.; Albert *et al.*, *loc. cit.*) was refluxed with 98% formic acid (10 ml.) for 20 min., evaporated at 100° *in vacuo*, treated with water (40 ml.), warmed to 60°, and brought by ammonia to pH 7. The black precipitate was collected after refrigeration and crystallized (carbon) from water (140 ml.) (yield, 1·2 g.). Recrystallization from water (90 parts; carbon) gave white needles of *4-amino-2-dimethylamino-5-formamidopyrimidine*, m. p. 235° (Found: C, 46·15; H, 6·1; N, 38·9. $C_7H_{11}ON_5$ requires C, 46·4; H, 6·1; N, 38·65%). This (0·9 g.) was plunged into a bath at 255—260° until evolution of water was complete (about 3 min.). The residue (0·72 g.) was recrystallized twice from water (45 parts; carbon) to give white 2-dimethylaminopurine, m. p. 222—223° (Found: C, 51·55, H, 5·6; N, 43·0. Calc. for $C_7H_9N_5$: C, 51·5; H, 5·6; N, 42·9%).

2-Methoxypurine.—Crude, dried 4 : 5-diamino-2-methoxypyrimidine (1 g.; Albert *et al.*, *J.*, 1952, 4219) and acetic formic anhydride (5 ml.) were heated at 50° for 1 hr. The solution was evaporated *in vacuo*, giving a solid (A). Alcohol (5 ml.) was added and the mixture evaporated. The residue was sublimed at 160°/0·1 mm. for 2 hr. The sublimate was recrystallized *at once* from alcohol (6 ml.), to give white nodules of *2-methoxypurine*, m. p. 205—206° (10%) (Found: C, 48·0; H, 4·15; N, 37·1. $C_6H_6ON_4$ requires C, 48·0; H, 4·05; N, 37·3%). It is soluble in 25 parts of boiling alcohol. The preparation could not be scaled up without loss. Formic acid could not be substituted for the anhydride. When the solid A (above) was stirred with hot water (5 ml.), a solid (0·5 g.) separated. This was recrystallised from alcohol (75 parts), giving white needles of 4 : *5-diformamido-2-methoxypyrimidine*, m. p. 180—181° (decomp.) (Found: C, 42·9; H, 4·1; N, 28·5. $C_7H_8O_3N_4$ requires C, 42·85; H, 4·1; N, 28·55%).

2-Mercaptopurine.—Formic acid (98%; 100 ml.) and 4 : 5-diamino-2-mercaptopyrimidine (20 g.) were refluxed vigorously for 3 hr. The suspension was kept at 0° for 12 hr. and the solid filtered off. A second crop was obtained by concentration. It was dissolved in 0·25N-sodium hydroxide (700 ml.), and reprecipitated with acetic acid (pH 4) (yield, 16 g., 73%). It was twice recrystallized from water (130 parts) to give yellow needles of 2-mercaptopurine which, dried at 110°, retained 0·25 mol. of water, which was lost at 150° [Found: C, 38·3; H, 3·05; N, 35·7; S, 20·3. Calc. for $(C_5H_4N_4S)_4,H_2O$: C, 38·3; H, 2·9; N, 35·75; S, 20·45%]. It decomposed slowly about 250°.

2-Carboxymethylthiopurine.—2-Mercaptopurine quartahydrate (2·4 g.) and chloroacetic acid (1·5 g.) in water (100 ml.) were refluxed for 2½ hr. After refrigeration the solid (2·8 g.) was filtered off and washed with water (5 ml.). Recrystallization from water (65 parts; carbon)

gave 80% recovery of white plates of 2-*carboxymethylthiopurine* (anhyd. after being dried at 130°), decomp. *ca.* 200° (Found: N, 26·7. $C_7H_6O_2N_4S$ requires N, 26·7%).

2-*Methylthiopurine*.—Methyl iodide (2·6 ml.) was added to a solution of 2-mercaptopurine hydrate (6·5 g.) in 5% aqueous sodium hydroxide (40 ml.), with shaking, during 20 min. After 2 hr. the whole was brought to *ca.* pH 5. It was refrigerated and the solid was filtered off and recrystallized from boiling water (700 ml.), to give 2-*methylthiopurine* as white needles (4·35 g., 64%), m. p. 250—255° (decomp.) (Found: C, 43·4; H, 3·55; N, 33·7; S, 18·9. $C_6H_6N_4S$ requires C, 43·35; H, 3·65; N, 33·7; S, 19·3%). It was readily soluble in hot alcohol (see below for an alternative synthesis).

4-*Amino*-2-*methylthio*-5-*nitropyrimidine*.—Methanethiol [freshly generated from *S*-methylthiourea sulphate (25 g.) and 5N-sodium hydroxide (40 ml.)] was passed into ethanolic N-sodium hydroxide (75 ml.). The solution was added during 5 min. to 4-amino-2-chloro-5-nitropyrimidine (8·75 g.) in hot ethanol (875 ml.). After 10 min., carbon dioxide was passed through the solution until it reached room temperature. The mixture was chilled and filtered. The solid was ground under water and refiltered. The yellow residue (8·0 g.; m. p. 180°) was recrystallized twice from alcohol (50 parts), giving colourless plates (5·3 g.) of 4-*amino*-2-*methylthio*-5-*nitropyrimidine*, m. p. 181—183°, which become yellow in light (Found: C, 32·5; H, 3·3; N, 29·6. $C_5H_6O_2N_4S$ requires C, 32·25; H, 3·25; N, 30·1%).

4 : 5-*Diamino*-2-*methylthiopyrimidine*.—(a) *From* 4-*amino*-2-*methylthio*-5-*nitropyrimidine*. The nitro-compound (4 g.), suspended in ethanol (600 ml.), was hydrogenated (16 hr.) over 10% palladised strontium carbonate (1 g.). The filtrate was evaporated to dryness and the residue recrystallised from water (50 ml.) to give 2·4 g. of pink product (m. p. 154°). Purification as in (b) gave identical material (solubility, m. p., mixed m. p., paper chromatogram).

(b) *From* 2-*methylthiopurine*. 2-Methylthiopurine (1·5 g.) was heated with 20% aqueous dimethylamine (5 ml.) at 155—160° for 24 hr. Evaporation gave a brown residue which, crystallised (carbon) from water (8 ml.), gave a buff powder (0·6 g.; m. p. 150—155°). It was recrystallised from water (6 ml.) and then from ethyl acetate (15 ml.), giving faintly pink laths of 4 : 5-*diamino*-2-*methylthiopyrimidine* (0·3 g.), m. p. 157—159° (Found: C, 38·75; H, 5·05; N, 36·1; S, 20·45. $C_5H_8N_4S$ requires C, 38·45; H, 5·15; N, 35·9; S, 20·5%).

(c) *By methylation of* 4 : 5-*diamino*-2-*mercaptopyrimidine*. 4 : 5-Diamino-2-mercaptopyrimidine (1·4 g.) in N-potassium hydroxide (11 ml.) was shaken with methyl iodide (0·7 ml.) for 5 min., kept for 20 min. at room temperature, and refrigerated (yield 1·5 g.). Recrystallization from ethyl acetate (50 ml.) gave 1·05 g. of 4 : 5-diamino-2-methylthiopyrimidine, m. p. 157—159°.

4-*Amino*-5-*formamido*-2-*methylthiopyrimidine*.—4 : 5-Diamino-2-methylthiopyrimidine (0·8 g.) was refluxed with 98% formic acid (6 ml.) for 4 hr. The formic acid was removed in a vacuum and the residue dissolved in boiling water (30 ml.). The pH was adjusted to 5, and the solution refrigerated. The product (0·6 g.) was recrystallized from water, giving 4-*amino*-5-*formamido*-2-*methylthiopyrimidine*, m. p. 180—190° (resolidifies; see below) (Found: N, 30·75; S, 17·15. $C_6H_8ON_4S$ requires N, 30·4; S, 17·4%).

2-*Methylthiopurine* (see alternative method, above).—The above formamido-compound (0·15 g.) was heated for 5 min. after it melted (bath at 185°). The cooled residue was recrystallized from water (16 ml.), giving white needles (0·1 g.) of 2-methylthiopurine, m. p. 249—255°.

6-*Dimethylaminopurine*.—Only the hydrochloride has been described (Elion, Burgi, and Hitchings, *J. Amer. Chem. Soc.*, 1952, **74**, 411). The free base was prepared by heating 6-methylthiopurine with dimethylamine (as described for the 8-isomer, below) and also as follows. 6-Chloropurine was prepared by the action of phosphoryl chloride on 6-hydroxypurine according to an unpublished method kindly sent us by Dr. A. Bendich, and was recrystallized from water. 6-Chloropurine (0·5 g.) was heated at 100° for 1 hr. with 15% methanolic dimethylamine (11 ml.). The product was chilled and the crystals (0·32 g.) were recrystallized from alcohol (10 ml.) giving 0·22 g. of white 6-*dimethylaminopurine*, m. p. 257° (Found: C, 51·7; H, 5·5; N, 42·9. $C_7H_9N_5$ requires C, 51·5; H, 5·6; N, 42·9%). It was identical with the product made from 6-methylthiopurine (mixed m. p.; chromatogram). A further crop was obtained from the mother-liquors.

6-Methylaminopurine, prepared by the method of Elion *et al.* (*loc. cit.*), who give m. p. 312—314°, had m. p. 306°, but gave correct analyses (Found: C, 48·3; H, 4·55. Calc. for $C_6H_7N_5$: C, 48·3; H, 4·7%).

6-*Amino*-2-*hydroxypurine* (iso*Guanine*).—The sulphate (Bendich, Tinker, and Brown, *J. Amer. Chem. Soc.*, 1948, **70**, 3113) was recrystallised from N-sulphuric acid [Found: N, 33·35; S, 7·7. Calc. for $(C_5H_5ON_5)_2,H_2SO_4,H_2O$: N, 33·45; S, 7·7%]. The base was precipitated by acetic acid from a solution of this salt (2·75 g.) in 0·25N-sodium hydroxide (110 ml.), washed well

with water, and recrystallized from water (4000 parts) as colourless needles (Found: N, 46·2. Calc. for $C_5H_5ON_5$: N, 46·35%).

8-*Phenylpurine*.—Benzoyl chloride (4·2 g., 1 equiv.) was added dropwise to 4 : 5-diaminopyrimidine (3·3 g.) in boiling anhydrous pyridine (35 ml.). Sodium hydrogen carbonate (2·52 g.) and water (15 ml.) were added to the cooled product and the whole was taken to dryness at 100°. The residue was boiled with water (350 ml.) and filtered from 4 : 5-*dibenzamidopyrimidine* (10% of pale, non-basic crystals from 13 parts of alcohol, m. p. 179°; very soluble in chloroform) (Found: C, 68·0; H, 4·4; N, 17·65. $C_{18}H_{14}O_2N_4$ requires C, 67·9, H, 4·4; N, 17·6%). The filtrate (350 ml.), concentrated to 20 ml., deposited 45% of 4-*amino*-5-*benzamidopyrimidine*, from 90 parts of water or 45 of alcohol, forming colourless crystals, m. p. 225° (Found: C, 61·6; H, 4·4; N, 26·0. $C_{11}H_{10}ON_4$ requires C, 61·7; H, 4·7; N, 26·2%). 4-Amino-5-benzamidopyrimidine (1 g.) was heated at 230° for 20 min. The residue, twice recrystallized from 33 parts of alcohol, gave 60% of colourless 8-*phenylpurine*, m. p. 261° (Found: C, 67·4; H, 4·0; N, 28·5. $C_{11}H_8N_4$ requires C, 67·3; H, 4·1; N, 28·6%).

8-*Methylpurine*.—The following method, based on Isay's process (*Ber.*, 1906, **39**, 250) gives an improved yield melting 7° higher. Acetic anhydride (25 ml.) and 4 : 5-diaminopyrimidine (5 g.) were heated to 210° during 20 min. in an open vessel under carbon dioxide, and kept at 210° (bath-temp.) for 20 min. longer. The crude product was extracted with alcohol (200 ml.), and the solution boiled with carbon (3 g.), and filtered. Concentration to 50 ml. and refrigeration gave yellow crystals which sublimed at 220°/0·01 mm. The yellow sublimate was dissolved in alcohol (500 ml.) and allowed to percolate through a column of activated alumina (2 × 8 cm.). The column was washed with a further 50 ml. The percolate and washings were evaporated to dryness (2·5 g.). The residue, recrystallized from *iso*butyl methyl ketone (550 ml.; carbon), gave white 8-methylpurine, m. p. 271—273° (Found: C, 53·65; H, 4·6. Calc. for $C_6H_6N_4$: C, 53·75; H, 4·5%).

8-*Hydroxypurine* (cf. Isay, *loc. cit.*).—Urea (7·5 g.) and 4 : 5-diaminopyrimidine (5 g.) were heated at 165° for 45 min. The crude product (4·4 g.), recrystallized twice from water (25 parts), gave white 8-hydroxypurine (2·3 g.), m. p. 305—307° (decomp.) (Found: C, 44·55; H, 3·0. Calc. for $C_5H_4ON_4$: C, 44·15; H, 2·95%).

2 : 8-*Dihydroxypurine*.—When prepared according to Johns (*Amer. Chem. J.*, 1911, **45**, 84) this was inhomogeneous (paper chromatography) and was purified by dissolution in boiling 0·4N-ammonia (100 parts; charcoal) and precipitation with acetic acid. Dried at 150° *in vacuo* it was unmelted at 350° (Found: C, 39·75; H, 2·8; N, 36·3. Calc. for $C_5H_4O_2N_4$: C, 39·5; H, 2·65; N, 36·85%).

6 : 8-*Dihydroxypurine*.—Urea (6 g.) and 4 : 5-diamino-6-hydroxypyrimidine (7·5 g.) were heated at 170° for 20 min. The cooled residue, crystallized from water (1400 ml.; insoluble residue rejected), gave 6 : 8-dihydroxypurine (3·8 g., 42%), white needles, unmelted at 350°. After recrystallization from water (330 parts) and drying at 110° it retained $0·67H_2O$, but at 160° it became anhydrous (Found: C, 39·25; H, 2·7%).

8-*Mercaptopurine*.—Isay's method (*loc. cit.*) was advantageously modified as follows. Thiourea (17 g.) and 4 : 5-diaminopyrimidine (12 g.) were heated at 195—200° for 30 min. The black residue was removed from the flask by soaking it in water (30 ml.) for 12 hr. and then extracted by boiling water (800 ml.) at pH 4—5. Treatment with carbon (10 g.) and refrigeration gave 6·9 g. of crude pink material. Three recrystallizations from water (80 parts; carbon) gave long, faintly yellow needles of 8-mercaptopurine, decomp. 300—310° (Found: S, 21·0. Calc. for $C_5H_4N_4S$: S, 21·05%).

8-*Methylthiopurine*.—8-Mercaptopurine (6·3 g.) was methylated similarly to the 2-isomer (see above). Enough water (*ca.* 200 ml.) was added to dissolve the solid at 100°. Upon refrigeration, 4·6 g. (69%) of material resulted. Recrystallization (carbon) from water (60 parts) produced white needles of 8-*methylthiopurine*, m. p. 257—259° (Found: C, 43·35; H, 3·65; S, 19·05. $C_6H_6N_4S$ requires C, 43·35; H, 3·65; S, 19·3%).

8-*Carboxymethylthiopurine*.—Crude 8-mercaptopurine (1·6 g.) and chloroacetic acid (1·2 g.) in water (50 ml.) were refluxed for 2 hr. The hot solution was brought to pH 2 and, after chilling, the solid (1·5 g.) was filtered off and dissolved in 0·1N-sodium hydroxide (100 ml.). Treatment at 60—70° with carbon, filtration, and precipitation (pH 2) with N-sulphuric acid (this cycle was repeated) gave 8-*carboxymethylthiopurine* as a white powder, decomp. slowly above 220° (Found: N, 26·75; S, 15·2. $C_7H_6O_2N_4S$ requires N, 26·7; S, 15·25%). It crystallizes (slowly) from dimethylformamide.

8-*Aminopurine*.—8-Methylthiopurine (2·5 g.) was heated with ammonia (*d* 0·89; 6 ml.), water (6 ml.), and traces of copper acetate and copper bronze at 155—160° for 24 hr. The

mixture was taken to dryness and the residue recrystallised from water (*ca.* 800 ml.), giving 1·15 g. of product. Two further recrystallizations from water (600 parts; carbon) gave small white needles of 8-*aminopurine*, m. p. <360° (Found: C, 44·4; H, 3·7; N, 51·6. $C_5H_5N_5$ requires C, 44·45; H, 3·7; N, 51·8%).

8-*Methylaminopurine*.—8-Methylthiopurine (1·3 g.) was heated with aqueous methylamine (25%; 6 ml.) at 150° for 24 hr. The yellow residue after evaporation was twice recrystallized from water (60 parts), to give white 8-*methylaminopurine* (0·5 g.), m. p. 332—334° (decomp.) (Found: C, 48·6; H, 4·6; N, 47·1. $C_6H_7N_5$ requires C, 48·3; H, 4·7; N, 47·0%).

8-*Dimethylaminopurine*.—8-Methylthiopurine (1·45 g.) and 20% aqueous dimethylamine (5 ml.) were heated at 140° for 24 hr. After evaporation to dryness, the residue was recrystallized from water (6 ml.) (yield 0·95 g.). Another recrystallization gave white needles of 8-*dimethylaminopurine*, m. p. 292° (decomp.) (Found: C, 51·5; H, 5·6; N, 42·9. $C_7H_9N_5$ requires C, 51·5; H, 5·6; N, 42·9%).

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