827. *Potential Anti-purines. Part II.* The Synthesis of 6- and 9-Substituted Purines and 8-Azapurines.*

By C. L. Leese and G. M. Timmis.

The synthesis is described of a number of purines and their 8-aza-derivatives substituted in the 9- or the 6- and 9-positions, all related to adenine and its riboside.

In the biosynthesis of nucleic acid and certain related co-factors, *e.g.*, purine riboside triphosphates, two routes have been well established in a number of organisms, namely, that starting with glycine amide ribotide and that which utilises a simple purine and converts it in one step into its 9-(ribose phosphate) derivative (ribotide). A third route,[1] involving purine ribosides, *e.g.*, (I), is distinguished from these in being more difficult to discover, and although it therefore appears to be of only minor importance in normal growth it might be important in malignant growth. On this hypothesis we have made analogues of adenine with cyclic substituents at the 9-position (II; R = H) which to some extent simulate sterically the riboside adenosine; these might interfere therefore with the third pathway. In addition, substitution in the amino-group of adenine leads to compounds which have remarkable inhibitory effects on the division of cells in tobacco wound callus tissue in the case of 6-furfurylaminopurine, and on the regeneration of tentacles in hydra in the case of 6-ω-phenylalkylaminopurines.[2] In the hope that an additive or, better, a synergic effect might be obtained we have combined suitable substitution at the 6- and the 9-position of adenine (cf. II). Since replacement of the 8-methine group in purines by nitrogen has yielded compounds with interesting growth-inhibitory properties, *e.g.*, 8-azaguanine, we have also made derivatives of 8-aza-adenine.

The preparation of the intermediate 4 : 5-diamino-6-substituted secondary aminopyrimidines (III; R = phenethyl, *cyclo*hexyl, or furfuryl) is described in the preceding paper. Treatment of these with nitrous acid resulted in ring closure between the 5-amino-group and the secondary amino-group with the formation of 9-substituted 8-aza-adenines (IV; R = NH_2, R′ = phenethyl, *cyclo*hexyl, or furfuryl). In no case was there any evidence for the formation of the isomeric 6-substituted amino-8-azapurines which might have been formed by the alternative mode of ring closure. The products, isolated essentially pure in high yield, were insoluble in alkali, indicating the absence of an acidic triazole-hydrogen atom, a fact which was confirmed by the ultraviolet absorption spectra.

9-Furfuryl-8-aza-adenine has pK_a 2·6 at 22°; the acidic pK_a (which is 5·8 for 8-aza-adenine) is absent, showing that the hydrogen in the triazole ring must be replaced by the furfuryl group. Moreover it was possible to convert one of these 8-aza-adenine derivatives (IV; R = phenethyl) into the 8-azahypoxanthine (IV; R = OH, R′ = phenethyl) by hot nitrous acid. Similar treatment of 9-*cyclo*hexyl- and 9-furfuryl-8-aza-adenine caused extensive decomposition.

The 9-substituted adenine derivatives (II, R = H, R′ = phenethyl, *cyclo*hexyl, or furfuryl) were prepared by formylation and cyclisation of the sulphates of the triaminopyrimidines (III) in formamide at 180°, mixtures with the isomeric 6-(substituted amino)-purines (II; R′ = H) being formed. The 6-(substituted amino)-purines were extracted with N-sodium hydroxide. The identity of these products, formed in minor yield, was finally established by unambiguous synthesis by treatment of 6-methylthiopurine with the appropriate amine.

* Part I, *J.*, 1958, 804.

[1] Aaronson and Rodgriguez, *J. Bacteriol.*, 1957, **74**, 807; Goldthwaite, *J. Clin. Invest.*, 1957, **36**, 1572; Gordon, Intrieri, and Brown, *J. Biol. Chem.*, 1957, **229**, 641; Brockman, Sparks, and Simpson, *Biochim. Biophys. Acta*, 1957, **26**, 671.

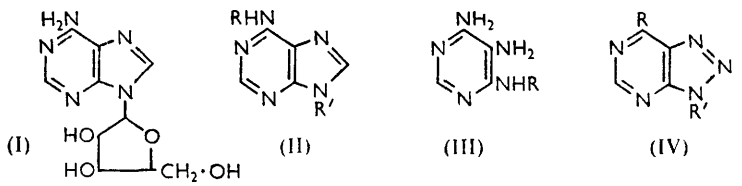
[2] Skinner and Shive, *J. Amer. Chem. Soc.*, 1955, **77**, 6692; Ham, Eakin, Skinner, and Shive, *ibid.*, 1956, **78**, 2648; Skinner, Shive, Ham, Fitzgerald, and Eakin, *ibid.*, p. 5097; Skinner, Gardner, and Shive, *ibid.*, 1957, **79**, 2843.

The structures of the pure 9-substituted adenines were confirmed by the light-absorption data (Table). The spectra are similar to that of adenosine and since the curves remain unaltered between pH 7 and pH 12 the absence of an acidic centre in the glyoxaline ring is confirmed. In contrast the 6-substituted adenines have spectra resembling that of adenine. The pK_a^2 values arise from the ionisation of the glyoxaline-hydrogen atom and the pK_a^1 values represent protonation of a basic centre in the pyrimidine ring.

Compound	pH	$\lambda_{max.}$	$10^{-3}\varepsilon$	$\lambda_{min.}$	$10^{-3}\varepsilon$	pK_a^1	pK_a^2
Adenine	2	263	13·1	229	2·5	2·2	9·8
	12	269	12·3	237	3·35		
6-Phenethylaminopurine	2	273·5	16·2	234	2·82	4·2	10·1
	7	269	17·46	232	2·36		
	12	275	17·7	240	3·57		
6-*cyclo*Hexylaminopurine	2	272	17·1	233	2·5	4·2	10·2
	7	269·5	18·2	230·5	2·14		
	12	275	18·2	241	3·36		
9-*cyclo*Hexyladenine	2	261	14·6	233	3·1	4·19	—
	7—12	262	14·7	229	2·5		
9-*cyclo*Hexyl-6-*cyclo*hexylamino-purine	2	267	17·9	236	2·8	4·4	—
	7—12	271	17·1	234	1·9		
Adenosine	2	257	14·6	230	3·5	3·45	*
	12	260	14·9	227	2·25		
9-Furfuryl-8-aza-adenine	0·9N-HCl	262	12·5	235	5·9	—	—
	5·8—12	279	11·9	236	3·1	†	

* Adenosine exhibits a further dissociation $pK_a^2 = 12{\cdot}5$ due to ionisation of the sugar moiety.
† See text.

For the preparation of the 9-alkyl-6-alkylaminopurines and their 8-aza-derivatives, 4 : 6-bisalkylamino-5-aminopyrimidines were prepared from 4 : 6-dichloro-5-nitropyrimidine. Cyclisation with formamide and nitrous acid then yielded the purines and 8-aza-



purines respectively in high yield, there being no opportunity for the formation of isomers. The syntheses of 6-*cyclo*hexylaminopurine [3] and 6-amino-9-*cyclo*hexylpurine,[4] from 6-chloropurine and *cyclo*hexylamine and from 6-chloro-9-*cyclo*hexylpurine and ammonia respectively, have recently been described but these methods require the use of pressure vessels and appear to be less convenient than ours.

Experimental

Analyses are by Mr. P. R. W. Baker, Beckenham.

9-Substituted Adenine and 8-Aza-adenine Derivatives.—*9-Phenethyl-8-aza-adenine.* 4 : 5-Diamino-6-phenethylaminopyrimidine (0·5 g.), dissolved in water (10 ml.) containing 2N-hydrochloric acid (2·2 ml.) was treated at 0° dropwise with sodium nitrite (0·19 g.) in water (3 ml.). The *azapurine* (0·45 g., 86%) was precipitated immediately and recrystallised from ethanol yielding white plates, m. p. 218—219° (Found: C, 60·4; H, 4·9; N, 34·8. $C_{12}H_{12}N_6$ requires C, 60·0; H, 5·0; N, 34·9%).

9-Phenethyl-8-azahypoxanthine. 9-Phenethyl-8-aza-adenine (1 g.) in 1·25N-sulphuric acid (60 ml.) at 70° was treated during 10 min. with sodium nitrite (2 g.) in water (10 ml.). The solution was boiled for 5 min., then cooled in ice. A white solid (0·4 g.) separated which from methanol yielded white needles of *9-phenethyl-8-azahypoxanthine*, m. p. 262—263° (Found: C, 60·2; H, 4·7; N, 29·0. $C_{12}H_{11}ON_5$ requires C, 59·7; H, 4·6; N, 29·0%). Neutralisation

[3] Sutherland and Christensen, *J. Amer. Chem. Soc.*, 1957, **79**, 2251.
[4] Montgomery and Temple, *ibid.* 1958, **80**, 409.

of the original filtrate with ammonia yielded unchanged 9-phenethyl-8-aza-adenine (0·5 g.), m. p. 217—218°. Thus there was an 80% conversion of the adenine into the hypoxanthine.

9-*Furfuryl*-8-*aza-adenine*. 4 : 5-Diamino-6-furfurylaminopyrimidine (0·5 g.) in ice-cold water (20 ml.) and 2N-hydrochloric acid (2·5 ml.) was treated with sodium nitrite (0·19 g.) in water (15 ml.) during 10 min. After a further 10 min., 9-*furfuryl*-8-*aza-adenine* (0·5 g., 95%) was removed by filtration and obtained as white plates, m. p. 227—228°, from ethanol (Found: C, 50·0; H, 3·6; N, 38·8. $C_9H_8ON_6$ requires C, 50·0; H, 3·7; N, 38·8%).

9-cyclo*Hexyl*-8-*aza-adenine*. 4 : 5-Diamino-6-*cyclo*hexylaminopyrimidine (1·5 g.) in water (100 ml.) and 2N-hydrochloric acid (7·3 ml.) with sodium nitrite (0·55 g.) in water (10·0 ml.) as before gave the *aza-adenine* (1·5 g., 95%), plates, m. p. 264—265° (from ethanol) (Found: C, 55·3; H, 6·2; N, 38·4. $C_{10}H_{14}N_6$ requires C, 55·0; H, 6·5; N, 38·5%). Attempted conversion into the hypoxanthine resulted in 80% recovery of the starting material. Prolonged treatment with hot nitrous acid caused decomposition.

9-*Phenethyladenine*. 4 : 5-Diamino-6-phenethylaminopyrimidine sulphate (1·7 g.) and formamide (5 ml.) were heated at 180—190° for 20 min., cooled, and diluted with water (50 ml.). The solid (1·4 g.), m. p. 150—163°, which was isolated was suspended in 2N-sodium hydroxide (15 ml.), warmed for 1 min. on a steam-bath, cooled, and filtered. The residue (1·05 g., 84%), on recrystallisation from aqueous ethanol, yielded 9-*phenethyladenine*, m. p. 179—180° (Found: C, 65·2; H, 5·5; N, 29·3. $C_{13}H_{13}N_5$ requires C, 65·2; H, 5·5; N, 29·2%). Neutralisation of the alkaline extract with acetic acid yielded 6-phenethylaminopurine (0·2 g., 11%), m. p. 245—246°, identical with a sample prepared by heating 6-methylthiopurine with phenethylamine (Skinner *et al.*, 1956, ref. 2).

9-*Furfuryladenine*. 4 : 5-Diamino-6-furfurylaminopyrimidine sulphate (5 g.) in formamide (12 ml.) gave similarly 9-*furfuryladenine* (1·3 g., 37%), m. p. 190—191°, needles from ethanol (Found: C, 55·9; H, 4·3; N, 32·3. $C_{10}H_9ON_5$ requires C, 55·8; H, 4·2; N, 32·5%). The alkaline extract on neutralisation with acetic acid deposited 6-furfurylaminopurine (kinetin) (0·35 g., 10%), m. p. 269—270°, identical with a specimen prepared by heating 6-methylthiopurine with furfurylamine.

9-cyclo*Hexyladenine*. 4 : 5-Diamino-6-*cyclo*hexylaminopyrimidine sulphate (2·35 g.) in formamide (6 ml.) yielded 9-*cyclo*hexyladenine,[4] m. p. 197—198°, plates from benzene (Found: C, 61·3; H, 6·8; N, 32·5. Calc. for $C_{11}H_{15}N_5$: C, 60·8; H, 6·9; N, 32·2%). This afforded a *picrate* (from saturated ethanolic picric acid), m. p. 295° (decomp.) (Found: N, 25·0. $C_{17}H_{18}O_7N_8$ requires N, 25·1%). Acidification of the alkaline extract with acetic acid yielded a trace of crystals, m. p. 205—206°, identical with 6-*cyclo*hexylaminopurine (see below).

6-cyclo*Hexylaminopurine*. 6-Methylthiopurine (0·8 g.), *cyclo*hexylamine (1 g.), and water (2 ml.) were heated at 150° for 24 hr. in a sealed tube, then on a steam-bath to expel methanethiol, and treated with water (10 ml.) and adjusted to pH 5 with acetic acid. Ice-cooling yielded a white solid (0·6 g.) which gave 6-*cyclo*hexylaminopurine[3] (0·4 g., 38%), m. p. 206—207°, as needles from methanol (Found: C, 61·2; H, 7·0; N, 32·2. Calc. for $C_{11}H_{15}N_5$: C, 60·8; H, 6·9; N, 32·2%). The *picrate*, from ethanolic picric acid, was obtained as yellow needles (from water), m. p. 242—243° (Found: N, 23·8. $C_{17}H_{18}O_7N_8,H_2O$ requires N, 24·2%).

5-*Nitro*-4 : 6-*di*(*phenethylamino*)*pyrimidine*. 4 : 6-Dichloro-5-nitropyrimidine[5] (5 g.) in ether (200 ml.) was slowly treated with phenethylamine (16 g.) in ether (20 ml.). After 18 hr. the ether was removed *in vacuo* and the solid residue extracted with water (100 ml.). The *product* (8·15 g., 87%) was isolated and obtained from light petroleum (b. p. 60—80°) as pale yellow needles, m. p. 133—134° (Found: C, 66·1; H, 5·9; N, 18·9. $C_{20}H_{21}O_2N_5$ requires C, 66·1; H, 5·8; N, 19·2%).

4 : 6-*Di*(*furfurylamino*)-5-*nitropyrimidine*. This *compound* (7·74 g., 95%) was obtained by the general method from 4 : 6-dichloro-5-nitropyrimidine (5 g.) and furfurylamine (13 g.). Recrystallisation from benzene–light petroleum (b. p. 80—100°) gave white needles, m. p. 132—133° (Found: C, 53·4; H, 4·3; N, 21·9. $C_{14}H_{13}O_4N_5$ requires C, 53·3; H, 4·1; N, 22·2%).

4 : 6-*Di*(cyclo*hexylamino*)-5-*nitropyrimidine*. This *compound* (6·47 g., 78%) was similarly obtained from 4 : 6-dichloro-5-nitropyrimidine (5 g.) and *cyclo*hexylamine (13 g.). It formed pale yellow needles, m. p. 135—136°, from light petroleum (b. p. 80—100°) (Found: C, 60·2; H, 7·9; N, 21·8. $C_{16}H_{25}O_2N_5$ requires C, 60·1; H, 7·9; N, 21·9%).

5-*Amino*-4 : 6-*di*(*phenethylamino*)*pyrimidine*. 5-Nitrodi(phenethylamino)pyrimidine (8·17 g.), suspended in ethanol (300 ml.), was reduced with hydrogen and Raney nickel and the filtrate

[5] Boon, Jones, and Ramage, *J.*, 1951, 96.

evaporated to dryness *in vacuo* to yield the *product* (7·5 g., 95%), m. p. 126—127°. It was obtained from benzene–light petroleum (b. p. 60—80°) as white plates, m. p. 131—131·5° (Found: C, 71·9; H, 6·8; N, 21·2. $C_{20}H_{23}N_5$ requires C, 72·0; H, 6·9; N, 21·0%).

5-*Amino*-4 : 6-*di*(*furfurylamino*)*pyrimidine*. 4 : 6-Difurfurylamino-5-nitropyrimidine (6·13 g.) in ethanol (200 ml.) similarly gave this *product* (4·90 g., 88%), plates, m. p. 117° [from light petroleum (b. p. 60—80°)] (Found: C, 59·1; H, 5·3; N, 24·8. $C_{14}H_{15}O_2N_5$ requires C, 58·9; H, 5·3; N, 24·5%).

5-*Amino*-4 : 6-*di*(cyclo*hexylamino*)*pyrimidine*. This *compound* (4·15 g., 81%) was similarly obtained by the catalytic hydrogenation of 4 : 6-di(*cyclo*hexylamino)-5-nitropyrimidine (5·64 g.) in ethanol (200 ml.). It was purified by dissolution in dilute acetic acid and precipitation with dilute ammonia, yielding white prisms, m. p. 299—300° (Found: C, 66·3; H, 9·1; N, 24·4. $C_{16}H_{27}N_5$ requires C, 66·4; H, 9·4; N, 24·2%).

9-*Substituted* 6-(*Substituted amino*)-*purines and*-8-*aza-purines*.—9-*Phenethyl*-6-*phenethylamino*-8-*azapurine*. 5-Amino-4 : 6-di(phenethylamino)pyrimidine (1 g.) in glacial acetic acid (20 ml.) and water (10 ml.) was cooled in ice and treated dropwise with sodium nitrite (0·25 g.) in water (1 ml.). The precipitated *azapurine* (0·77 g., 74%) yielded white needles, m. p. 201°, from ethanol (Found: C, 69·6; H, 5·5; N, 24·6. $C_{20}H_{20}N_6$ requires C, 69·7; H, 5·8; N, 24·4%).

9-*Furfuryl*-6-*furfurylamino*-8-*azapurine*. This *compound* (0·93 g., 89%) was obtained when 5-amino-4 : 6-di(furfurylamino)pyrimidine (1 g.) in glacial acetic acid (5 ml.) and water (25 ml.) was treated with sodium nitrite (0·25 g.) in water (2 ml.) as in the previous experiment. The pure compound, m. p. 125—126°, obtained from ethanol exhibited dimorphism (Found: C, 56·4; H, 4·0; N, 28·7. $C_{14}H_{12}O_2N_6$ requires C, 56·7; H, 4·0; N, 28·3%).

9-cyclo*Hexyl*-6-cyclo*hexylamino*-8-*azapurine*. This *azapurine* (0·95 g., 91%) was similarly obtained from 5-amino-4 : 6-di(*cyclo*hexylamino)pyrimidine (1 g.) in glacial acetic acid (15 ml.) and water (15 ml.) by sodium nitrite (0·25 g.) in water (2 ml.). Recrystallisation from aqueous ethanol yielded prisms, m. p. 166—167° (Found: C, 63·8; H, 7·9; N, 28·1. $C_{16}H_{24}N_6$ requires C, 63·9; H, 8·0; N, 27·9%).

9-*Phenylethyl*-6-*phenylethylaminopurine hydrochloride*. 5-Amino-4 : 6-di(phenethylamino)-pyrimidine (0·5 g.) in formamide (2 ml.) containing 1 drop of concentrated sulphuric acid was heated at 180—190° for 10 min. The cooled mixture was diluted with ethanol (5 ml.) and poured into 18% w/v hydrochloric acid (30 ml.). After ice-cooling the crystalline *purine hydrochloride* was filtered off and recrystallised from 2N-hydrochloric acid as plates (0·35 g., 60%), m. p. 230—231° (Found: C, 66·7; H, 5·8; N, 18·7; Cl, 8·9. $C_{21}H_{21}N_5$,HCl requires C, 66·3; H, 5·8; N, 18·4; Cl, 9·3%).

9-*Furfuryl*-6-*furfurylaminopurine*. 5-Amino-4 : 6-di(furfurylamino)pyrimidine (1 g.) was heated at 180—190° for 15 min. in formamide (3 ml.) containing formic acid (0·5 ml.). Dilution with water (30 ml.) and ice-cooling yielded the *purine* (0·97 g., 91%), needles, m. p. 128·5—129° from benzene–light petroleum (b. p. 60—80°) (Found: C, 61·0; H, 4·3; N, 23·9. $C_{15}H_{13}O_2N_5$ requires C, 61·0; H, 4·4; N, 23·7%).

9-cyclo*Hexyl*-6-cyclo*hexylaminopurine hydrochloride*. 5-Amino-4 : 6-di(*cyclo*hexylamino)-pyrimidine (0·5 g.) was heated with formamide (3 ml.) and formic acid (0·2 ml.) at 180—190° for 25 min. The cooled mixture was dissolved in ethanol (3 ml.) and poured into water (40 ml.) containing aqueous ammonia (5 ml.; *d* 0·88). Ice-cooling caused slow solidification of the oily product which then was dissolved in ethanol (10 ml.) and saturated with dry hydrogen chloride, to yield a white crystalline hydrochloride (0·45 g.), m. p. 226—229°. This was purified twice by dissolution in ethanol and saturation of the solution with dry hydrogen chloride, to yield needles of the pure *salt* (0·40 g., 68%), m. p. 233—235° (Found: C, 59·9; H, 7·8; N, 20·6; Cl, 11·0. $C_{17}H_{25}N_5$,HCl requires C, 60·7; H, 7·8; N, 20·8; Cl, 10·5%).

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Chester-Beatty Research Institute,
Institute of Cancer Research: Royal Cancer Hospital,
Fulham Road, London, S.W.3. [*Received, May 27th*, 1958.]