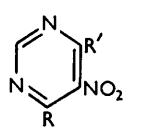
560. *The Synthesis of Kinetin and Some Related Heterocyclic Compounds.*

By R. Hull.

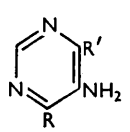
Ring closure of 4 : 5-diamino-6-furfurylaminopyrimidine with ethyl orthoformate and acetic anhydride has yielded the unexpected 6-furfurylaminopurine (kinetin). The isomeric 6-amino-9-furfurylpurine and kinetin are formed by ring closure of the same pyrimidine with formamide. Other purines and triazolopyrimidines are also described.

Furfuryl- and furyl-purines and -pyrimidines are of interest as analogues of ribosides and deoxyribosides in which the furanose structure is replaced by furan. Such compounds could conceivably act as antiviral agents by antagonising the formation of nucleic acids which form an integral part of virus structures, or might have interesting effects on cell metabolism.

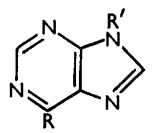
During this investigation an attempt was made to synthesise 9-furfuryladenine (V), an analogue of adenosine. This involved the preparation of 4 : 5-diamino-6-furfurylaminopyrimidine (IV), followed by conversion of this into the purine. Theoretically ring closure



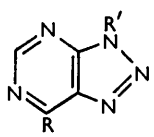
(I) — (III) (VIII)



(IV) (IX)



(V) — (VII) (X)



(XI) (XII)

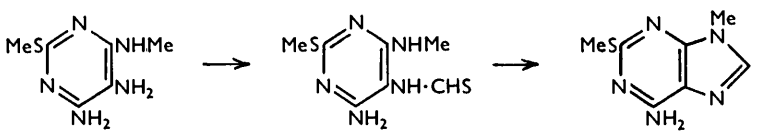
	R	R′
(I)	Cl	Cl
(II)	NH_2	Cl
(III)	NH_2	$NH{\cdot}C_5H_5O$
(IV)	NH_2	$NH{\cdot}C_5H_5O$

	R	R′
(V)	NH_2	C_5H_5O
(VI)	$NH{\cdot}C_5H_5O$	H
(VII)	$NAc{\cdot}C_5H_5O$	H
(VIII)	Cl	$NH{\cdot}C_5H_5O$

	R	R′
(IX)	Cl	$NH{\cdot}C_5H_5O$
(X)	—	C_5H_5O
(XI)	NH_2	C_5H_5O
(XII)	OH	C_5H_5O

C_5H_5O = furfuryl.

could occur in two ways, giving a purine substituted either by furfurylamino at position 6 (VI) or by furfuryl at position 9 (V). The ring closure of 5 : 6-diaminopyrimidines containing a substituted amino-group in position 4 has been investigated by Todd and his coworkers in experiments on the synthesis of nucleosides. They found,[1] for example, that ring closure of the methylaminopyrimidine (XIII), *via* the thioformamido-compound (XIV), gave only one product, the 9-substituted purine (XV). Later they found that a similar ring closure took place with a 4-tri-*O*-acetylribosylaminopyrimidine.[2] However, when xylose replaced the ribose portion, mixtures of the 9- and the 6-substituted purine were obtained.[3]



(XIII) (XIV) (XV)

A newer method for the synthesis of purines was suggested by the observation by Goldman, Marsico, and Gazzola[4] that 4 : 5-diaminopyrimidines on treatment with ethyl

[1] Baddiley, Lythgoe, McNeil, and Todd, *J.*, 1943, 383.
[2] Baddiley, Kenner, Lythgoe, and Todd, *J.*, 1944, 657.
[3] Baddiley, Lythgoe, and Todd, *J.*, 1944, 318; Kenner, Lythgoe, and Todd, *ibid.*, p. 652; Howard, Lythgoe, and Todd, *J.*, 1945, 556.
[4] Goldman, Marsico, and Gazzola, *J. Org. Chem.*, 1956, **21**, 599.

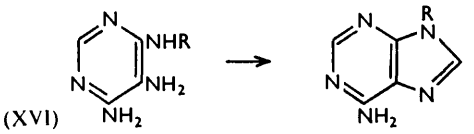
orthoformate and acetic anhydride yielded purines in good yield. In the presence of these reagents, 4 : 5-diamino-6-furfurylaminopyrimidine (IV) gave a substance, $C_{10}H_9ON_5$, which was readily soluble in aqueous sodium hydroxide, was reprecipitated from alkaline solution with acetic acid, and formed a silver salt. These properties were inconsistent with the expected 6-amino-9-furfurylpurine (V), but were in agreement with the isomeric 6-furfurylaminopurine (VI).

Great interest has centred on this compound (VI). It was originally isolated from a nucleic acid preparation,[5] during a search for a cell-division factor. The compound was shown to be 6-furfurylaminopurine,[6] and it was subsequently synthesised from 6-chloro-[7] or 6-methylthio-purine [5] and furfurylamine. It has been named " kinetin."

Comparison of the infrared absorption spectra of the compound, $C_{10}H_9ON_5$, and kinetin, as prepared by an unambiguous method, showed that they were identical. If the mild alkaline treatment was omitted after reaction of the pyrimidine (IV) with ethyl orthoformate and acetic anhydride then acetylkinetin (VII) resulted. This compound is new, since it is stated [6] that kinetin cannot be acetylated. Presumably in the ring closure of the pyrimidine (IV), acetylation of the furfurylamino-group takes place before ring closure to the purine.

Recent work on 6-(substituted amino)purines [8] suggests a widespread importance of these substances in processes of cell division, at least within the plant kingdom.

Daly and Christensen [9] have investigated the possibility of using pyrimidine intermediates for the preparation of analogues of kinetin. However, they found that cyclisation of the substituted aminopyrimidines (XVI; R = Ph, CH_2Ph, or Me) using formamide or sodium dithioformate gave only the 9-substituted purines (XVII).



(XVI) (XVII)

The behaviour of formamide on the furfurylaminopyrimidine (IV) was then investigated. A mixture of kinetin (VI) and the isomeric 9-furfurylpurine (V) resulted, easily separable by the solubility of the former in alkali. Proof of the structure (V) was obtained by preparing the compound from 6-chloro-9-furfurylpurine (X; R = Cl) and alcoholic ammonia under pressure.

Since triazolopyrimidines are of interest as antagonists of folic acid and purines in chemotherapy,[10] the diaminopyrimidine (IV) was treated with nitrous acid. Only one of the two possible isomers was obtained and this was regarded as the expected aminotriazolopyrimidine (XI). In conformity with this it was deaminated by nitrous acid at 100°, yielding the hydroxy-compound (XII).

The several pyrimidine intermediates required in the above investigation were obtained from 4 : 6-dichloro-5-nitropyrimidine (I). The furfurylaminopyrimidine (VIII) was obtained as an oil by addition of furfurylamine and triethylamine as their acetates to the dichloropyrimidine (I). Reduction with zinc dust then gave the amino-compound (IX); the same compound was also obtained from 5-amino-4 : 6-dichloropyrimidine [11] and furfurylamine. The diaminopyrimidine (IV) was obtained by a standard procedure involving stepwise nucleophilic attack on (I), to give the nitro-compound (III), followed by

[5] Miller, Skoog, Von Saltza, and Strong, *J. Amer. Chem. Soc.*, 1955, **77**, 1392.
[6] Miller, Skoog, Okumura, Von Saltza, and Strong, *ibid.*, p. 2662; 1956, **78**, 1375.
[7] Bullock, Hand, and Stokstad, *ibid.*, p. 3693.
[8] Skinner and Shive, *ibid.*, 1955, **77**, 6692; Ham, Eakin, Skinner, and Shive, *ibid.*, 1956, **78**, 2648; Skinner, Shive, Ham, Fitzgerald, and Eakin, *ibid.*, p. 5097; Sutherland and Christensen, *ibid.*, 1957, **79**, 2251; Skinner, Gardner, and Shive, *ibid.*, p. 2843.
[9] Daly and Christensen, *J. Org. Chem.*, 1956, **21**, 177.
[10] Timmis, *J. Pharm. Pharmacol.*, 1957, **9**, 84.
[11] Brown, *J. Appl. Chem.*, 1954, **4**, 72.

catalytic reduction. Early attempts to synthesise the chloropurine (X; R = Cl) were unsuccessful, owing possibly to the high reactivity of the halogen atom. In one experiment, reaction of the pyrimidine (IX) with ethyl orthoformate and acetic anhydride followed by mild treatment with alcoholic alkali to destroy the excess of reagent gave 6-ethoxy-9-furfurylpurine (X; R = OEt). Omission of the alcoholic alkaline treatment gave the halogenated purine in low yield.

Experimental

4-Amino-6-furfurylamino-5-nitropyrimidine.—4-Amino-6-chloro-5-nitropyrimidine [12] (3·5 g.) in dioxan (50 ml.) was added slowly during ½ hr. with stirring to furfurylamine (3·7 ml.) in dioxan (10 ml.). After a further 1½ hr. water was added and the *product* (4·6 g.) collected. It crystallised from ethanol in needles, m. p. 181—182° (Found: C, 45·8; H, 4·0; N, 30·0. $C_9H_9O_3N_5$ requires C, 46·0; H, 3·8; N, 29·8%).

4 : 5-Diamino-6-furfurylaminopyrimidine.—4-Amino-6-furfurylamino-5-nitropyrimidine (4·6 g.) in methanol (100 ml.) was hydrogenated over Raney nickel at room temperature and pressure. After filtration from the catalyst, the solution was evaporated. The residue (3·0 g.) crystallised from water (carbon) and gave the *aminopyrimidine* as prisms, m. p. 120—121° (Found: C. 53·0; H, 5·4; N, 34·7. $C_9H_{11}ON_5$ requires C, 52·7; H, 5·4; N, 34·1%).

6-Furfurylaminopurine (Kinetin).—4 : 5-Diamino-6-furfurylaminopyrimidine (7 g.) in ethyl orthoformate (33 ml.) and acetic anhydride (33 ml.) was heated under reflux during 1½ hr. Excess of reagent was removed under diminished pressure. 2·5N-Sodium hydroxide (70 ml.) and alcohol (35 ml.) were added to the residue, and the solution was kept at 40° during 20 min. Excess of alcohol was removed under diminished pressure and the solution was neutralised with acetic acid. The precipitated *product* (7 g.) crystallised from alcohol in leaf-shaped needles, m. p. 266° (sealed tube) (Found: C, 56·2; H, 4·4; N, 32·1. Calc. for $C_{10}H_9ON_5$: C, 55·8; H, 4·2; N, 32·6%). It was undepressed in melting point on admixture with kinetin. A solution in 0·05N-sulphuric acid gave a white precipitate with silver nitrate. The infrared absorption spectrum of the compound (Nujol smear) was identical with that of authentic kinetin.

6-(Acetylfurfurylamino)purine.—4 : 5-Diamino-6-furfurylaminopyrimidine (1·05 g.) in ethyl orthoformate (5 ml.) and acetic anhydride (5 ml.) was heated under reflux during 1½ hr. Excess of reagent was removed under reduced pressure and the residue (0·5 g.) was washed with water. Recrystallisation from alcohol gave *6-(acetylfurfurylamino)purine* as colourless needles, m. p. 151° (Found: C, 56·1; H, 4·3; N, 27·2. $C_{12}H_{11}O_2N_5$ requires C, 56·1; H, 4·3; N, 27·2%).

4-Chloro-6-furfurylamino-5-nitropyrimidine.—Furfurylamine (5·35 g.) and triethylamine (5·55 g.) were brought to pH 8 with 9N-acetic acid with cooling. This solution was added slowly to a stirred solution of 4 : 6-dichloro-5-nitropyrimidine (9·7 g.) in dioxan (45 ml.) at 10—15°. After 2 hr. the mixture was added to ice-water (400 ml.), and the oil was separated and combined with the ether extract of the supernatant phase. The extracts were washed with water and dried (Na_2SO_4), and the ether was removed. Distillation of the residual oil (11·9 g.) gave a small quantity of unchanged 4 : 6-dichloro-5-nitropyrimidine followed by the *product* as a pale brown oil (b. p. 165—170°/15 mm.) (Found: C, 42·3; H, 2·7; N, 22·0. $C_9H_7O_3N_4Cl$ requires C, 42·4; H, 2·75; N, 22·0%).

5-Amino-4-chloro-6-furfurylaminopyrimidine.—(*a*) 5-Amino-4 : 6-dichloropyrimidine [11] (1·7 g.) in hot dioxan (12 ml.) was added to furfurylamine (1·26 g.) and triethylamine (1·3 g.) in dioxan (3 ml.), and the whole was heated under reflux during 7 hr. The solution was set aside for 2 days, then the triethylamine hydrochloride was collected and washed with dioxan. The filtrate and washings were evaporated to dryness. The residue (2 g.), after being washed with water, crystallised from aqueous alcohol (carbon) and gave the *furfurylaminopyrimidine* as colourless needles, m. p. 137—138° (Found: C, 48·2; H, 4·0; N, 24·9. $C_9H_9ON_4Cl$ requires C, 48·1; H, 4·0; N, 24·95%).

(*b*) 4-Chloro-6-furfurylamino-5-nitropyrimidine (0·55 g.) was added to a rapidly stirred suspension of zinc dust (2·1 g.) in water (20 ml.) at 90—95°. After 15 minutes' stirring the mixture was filtered, and the filtrates were adjusted to pH 10 with aqueous ammonia and extracted with chloroform (3 × 50 ml.). The combined extracts were dried (Na_2SO_4) and the solvent was removed. Recrystallisation of the residue from aqueous alcohol (carbon) gave 5-amino-4-chloro-6-furfurylaminopyrimidine as needles, m. p. 136°, identical with the above.

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

6-*Chloro*-9-*furfurylpurine*.—5-Amino-4-chloro-6-furfurylaminopyrimidine (2·0 g.) in acetic anhydride (8 ml.) and ethyl orthoformate (8 ml.) was heated under reflux during 3 hr. Excess of reagent was carefully removed under diminished pressure. Trituration with ice-cold aqueous alcohol and storage gave the *purine* (0·25 g.; m. p. 96—97°) which crystallised from light petroleum (b. p. 80—100°) as prisms, m. p. 97—99° (Found: C, 51·4; H, 2·9; N, 24·0. $C_{10}H_7ON_4Cl$ requires C, 51·2; H, 3·0; N, 23·9%).

Condensation of Formamide with 4 : 5-*Diamino*-6-*furfurylaminopyrimidine*.—4 : 5-Diamino-6-furfurylaminopyrimidine (5·0 g.) and formamide (27 ml.) were heated under gentle reflux for 15 min. Ice-water was added to the cooled mixture, and the solid (3·8 g.; m. p. 176—180°) was extracted with 2N-sodium hydroxide (20 ml.). 6-Furfurylaminopurine (kinetin) [1·0 g.; m. p. 263—264° (sealed tube)] separated when this filtrate was neutralised with acetic acid. The alkali-insoluble material (1·65 g.; m. p. 188—189°) crystallised from water (carbon), to give 6-*amino*-9-*furfurylpurine* as needles, m. p. 191—192° (Found: C, 55·9; H, 4·4; N, 33·2. $C_{10}H_9ON_5$ requires C, 55·8; H, 4·2; N, 32·6%). Basification of the original aqueous formamide filtrates gave a further quantity (0·95 g.; m. p. 188—189°) of 6-amino-9-furfurylpurine.

6-*Amino*-9-*furfurylpurine* (*Second Method*).—6-Chloro-9-furfurylpurine (0·5 g.) was heated in a Carius tube with alcoholic ammonia (20 ml., saturated at 0°) at 135° during 5 hr. After evaporation the residue (0·4 g.) crystallised from water in prismatic needles, m. p. 191—192°, identical with 6-amino-9-furfurylpurine prepared as above.

6-*Ethoxy*-9-*furfurylpurine*.—6-Chloro-9-furfurylpurine (0·1 g.) was suspended in 2N-sodium hydroxide (1 ml.) and alcohol (1 ml.), and the mixture was heated at 40° during 10 min. A solution was formed, and later the precipitated *product* (0·1 g.) was filtered from the cooled mixture. Crystallisation from water gave the purine as prismatic needles, m. p. 118° (Found: C, 58·9; H, 5·0; N, 22·8. $C_{12}H_{12}O_2N_4$ requires C, 59·0; H, 4·95; N, 22·95%).

6-*Amino*-1′-*furfuryl*-1′ : 2′ : 3′-*triazolo*(5′ : 4′-4 : 5)*pyrimidine*.—Sodium nitrite (0·47 g.) in water (3 ml.) was added to a solution of 4 : 5-diamino-6-furfurylaminopyrimidine (1·05 g.) in acetic acid (20 ml.) at 10°. After 15 min. the solution was diluted with water, and the *triazolopyrimidine* (0·85 g.) collected. It crystallised from alcohol in needles, m. p. 237° (Found: C, 49·8; H, 3·8; N, 38·6. $C_9H_8ON_6$ requires C, 50·0; H, 3·7; N, 38·9%). A solution of the compound in dilute aqueous sulphuric acid did not form a silver salt with silver nitrate. The compound was insoluble in dilute aqueous alkali.

The triazolopyrimidine (0·5 g.) was dissolved in water (20 ml.) containing sulphuric acid (2 g.) at 45—50°; sodium nitrite (1 g.) in water (5 ml.) was added and the mixture boiled for about 3 min. The product, which separated on cooling and scratching, recrystallised from alcohol and gave 6-*hydroxy*-1′-*furfuryl*-1′ : 2′ : 3′-*triazolo*(5′ : 4′-4 : 5)*pyrimidine* as pale yellow plates, m. p. 226—227° (decomp.) (Found: C, 49·6; H, 3·5; N, 32·4. $C_9H_7O_2N_5$ requires C, 49·75; H, 3·2; N, 32·25%), soluble in N-sodium hydroxide.

I am grateful to Mr. K. Jones for technical assistance.

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ALDERLEY PARK, MACCLESFIELD, CHESHIRE. [*Received, February 3rd*, 1958.]