892. *Synthesis of* "6-*Succinoaminopurine*" [6-(1 : 2-*Dicarboxyethylamino*)*purine*].

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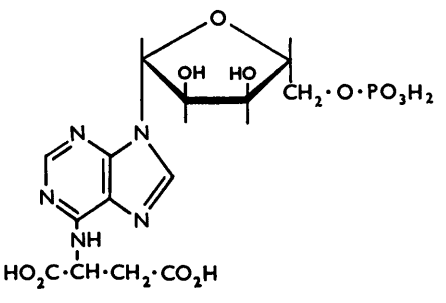
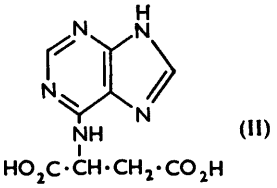
2 : 6 : 8-Trichloropurine with aspartic acid at pH 9 gives the dichloroaminopurine (III) in good yield. Removal of the two chlorine atoms with phosphine and hydrogen iodide yields the aminopurine (II), identical with a substance obtained by acid hydrolysis of a nucleotide (I) which had been produced enzymically from fumarate and adenosine-5′ phosphate.

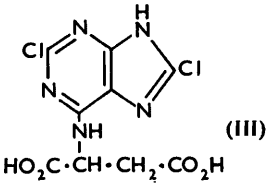
THE unusual purine nucleotide (I) was first isolated by Carter and Cohen [1] as a product of the reaction between adenosine-5′ phosphate and fumarate in the presence of an enzyme isolated from a yeast autolysate. It is also formed from inosine-5′ phosphate and aspartate in the presence of an enzyme from *Escherichia coli*,[2] and probably occupies a central position in the important conversion of inosine-5′ phosphate into adenosine-5′ phosphate.[2,3] Acid hydrolysis of the nucleotide yields "6-succinoaminopurine" [6-(1 : 2-dicarboxyethylamino)purine] (II). Since the identification of this and the original nucleotide depends largely on ultraviolet absorption spectra and chromatographic properties,[4] we considered that a synthesis of the purine (II) was desirable.



(I)



(II)



(III)

A convenient starting material for the synthesis of 6-substituted purines is 2 : 6 : 8-trichloropurine. This is readily prepared from uric acid,[5] and the 6-chlorine atom is replaced by an amino-group under moderate reaction conditions.[6] The chlorine atoms in 6-amino-2 : 8-dichloropurine have been removed by reduction with phosphonium iodide and hydrogen iodide, and in related glycosides catalytic methods have been used.[7]

2 : 6 : 8-Trichloropurine was heated in aqueous solution at pH 9 with aspartic acid and the resulting dichloro-aminopurine (III) was isolated in good yield by acidification. The absorption spectrum of this compound was similar to that of the purine (II) and its nucleotide (see Table 1). Attempts to remove the chlorine atoms catalytically were unsuccessful: it is possible that the acidic hydrogen atom at position 9 or 7 may prevent the correct orientation of the molecule and catalyst.

The two chlorine atoms were replaced by hydrogen when phosphine was passed through a suspension of the compound in hydrogen iodide. The product (II) was isolated in about 30% yield by chromatography on a Dowex-2 column. It was indistinguishable from a sample kindly provided by Dr. C. E. Carter when examined by paper chromatography

[1] Carter and Cohen, *J. Amer. Chem. Soc.*, 1955, **77**, 499.
[2] Lieberman, *ibid.*, 1956, **78**, 251.
[3] Abrams and Bentley, *ibid.*, 1955, **77**, 4179.
[4] Carter, personal communication.
[5] Davoll and Lowy, *J. Amer. Chem. Soc.*, 1951, **73**, 2936.
[6] Fischer, *Ber.*, 1897, **30**, 2226.
[7] Davoll, Lythgoe, and Todd, *J.*, 1948, 967.

in several solvent systems, and the ultraviolet absorption spectra were identical. On electrometric titration it consumed approximately two equivalents of alkali.

The pyrimidine ring in this compound and its nucleotide is somewhat unstable towards acids.[4] After hydrolysis for several hours in 0·5N-hydrochloric acid at 100°, 5-aminoglyoxaline-4-carboxyamide was detected among the products from the synthetic purine and from the sample provided by Dr. Carter, being identified by paper chromatography. It absorbed ultraviolet light on the paper and gave characteristic colours when sprayed with diazotised sulphanilic acid or nitrous acid followed by β-naphthol in dilute alkaline solution.

Experimental

2 : 6 : 8-*Trichloropurine*.—This was prepared as its ammonium salt by the method of Davoll and Lowy [5] without modification. Although the ammonium salt was reasonably stable, the free purine slowly evolved hydrogen chloride.

2 : 8-*Dichloro*-6-(1 : 2-*dicarboxyethylamino*)*purine*.—The ammonium salt of 2 : 6 : 8-trichloropurine (3·0 g., 1 mol.) and DL-aspartic acid (3·36 g., 2 mols.) were dissolved in hot water (75 ml.). Sodium hydroxide was added to pH 9, followed by sodium carbonate (1·61 g., 1·2 mols.), and the solution was refluxed for 9 hr. Concentrated hydrochloric acid was added to the cooled solution until a permanent precipitate had been formed. The mixture was kept at 0° overnight and the resulting precipitate (2·17 g., 60%) was filtered off, washed with water, and dried (P_2O_5 and KOH). The slight colour was removed (charcoal) during recrystallisation from hot water. The 2 : 8-*dichloro-compound* formed needles, decomp. 220—225° (Found : C, 34·4; H, 2·6. $C_9H_7O_4N_5Cl_2$ requires C, 33·8; H, 2·2%). The substance was homogeneous when examined by paper chromatography in several solvent systems. For R_P values see Table 2, and for ultraviolet absorption data see Table 1.

TABLE 1. *Ultraviolet absorption spectra of purine derivatives.*

	pH	$\lambda_{max.}$ (mμ)	log ε	$\lambda_{min.}$ (mμ)
Purine (III)	< 1	272·5	4·30	236
	>12	279·5	4·32	245
Purine (II) : synthetic	< 1	275	4·24	235
	>12	274	4·24	240
from Dr. Carter's data	< 1	276	4·25	235
	>12	275	—	244

6-(1 : 2-*Dicarboxyethylamino*)*purine*.—A stream of nitrogen was passed for 1 hr. through a suspension of the dichloro-purine (500 mg.) in hydriodic acid (20 ml.; freshly distilled from red phosphorus; b. p. 126°; *d* 1·7). Phosphine (from 6 g. of white phosphorus and 8 g. of sodium hydroxide in 100 ml. of water) was diluted with nitrogen, and the mixed gases were passed through a condenser (to remove some water), then through sulphuric acid, and into the purine in hydriodic acid at 70°. After 2 hr. the dark brown mixture was evaporated to dryness below 50° and the residue was dissolved in water (100 ml.). Most of the iodine was removed by continuous extraction with carbon disulphide. The disulphide reservoir contained a saturated solution of sodium arsenite to prevent volatilisation of the extracted iodine. The aqueous layer was evaporated to about half its volume below 40° to remove carbon disulphide, water was added (to 500 ml.), and the solution was adjusted to pH 4·5 with ammonia.

The purine was isolated from the above solution by chromatography on a Dowex-2 (formate form) resin column (205 ml.; 200—400 mesh). After absorption of reaction products, the column was washed with water (500 ml.) and eluted with 0·5M-formic acid. Fractions (25 ml.) were collected automatically, the product being detected by optical density at 276 mμ. It was the first substance to be eluted and was usually contained in about 200 ml. Appropriate fractions were bulked and evaporated to dryness below 40°. The resulting gum was dried at the oil-pump and then dissolved in water (10 ml.). The solution was concentrated (to 2 ml.) and acetone (10 ml.) was added, whereupon the product (57 mg.) crystallised. Concentration of the mother-liquors and addition of acetone gave a further crop (73 mg.) of less well-crystallised material (total yield 33%). This *purine* may be recrystallised with some loss from aqueous acetone. It does not melt but chars at 190° and evolves gas at 225° (Found : C, 42·5; H, 3·9. $C_9H_9O_4N_5$ requires C, 43·0; H, 3·6%). On electrometric titration with 0·01N-sodium hydroxide it consumed approx. 2 equivs. up to pH 7 (Found : equiv., 124·8. $\frac{1}{2}C_9H_9O_4N_5$ requires equiv., 125·5).

Acid Hydrolysis.—A solution of the foregoing material (3·8 mg.) in 0·5N-hydrochloric acid (0·1 ml.) was heated in a sealed tube in a boiling-water bath for 5 hr. Solvent was removed in a desiccator, and the products were examined by paper chromatography in solvent system *D* (see below). The aminoglyoxaline derivative had R_F 0·66 and was identified by comparison with an authentic sample on an adjacent track. Its presence was demonstrated by absorption of ultraviolet light and by the dark blue colour produced when the paper was sprayed with a dilute solution of diazotised sulphanilic acid in sodium hydrogen carbonate. A second paper was exposed to nitrous fumes and then sprayed with a 1% solution of β-naphthol in 1% sodium hydroxide solution. The glyoxaline gave an orange colour on the paper.

Paper Chromatography.—Ascending-front chromatography on Whatman No. 4 paper was used. The following solvent systems were used : *A*, *iso*propyl alcohol–water–ammonia (*d* 0·88) (3 : 2 : 1); *B*, *n*-butyl alcohol–acetic acid–water (2 : 1 : 1); *C*, *n*-butyl alcohol–acetic acid–water (4 : 1 : 5) (organic layer); *D*, *n*-propyl alcohol–ammonia (*d* 0·88)–water (6 : 3 : 1); *E*, 5% disodium hydrogen phosphate solution–*iso*pentyl alcohol (2 : 1). Results are in Table 2.

TABLE 2. *R_F values in solvent systems A—E.*

	A	*B*	*C*	*D*	*E*
Purine (III)	0·81	0·83	0·82	0·45	0·85
Purine (II)	0·76	0·61	0·45	0·29	0·86

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