564. *The Synthesis of 6-Dimethylamino-9-β-D-ribofuranosylpurine-5′ phosphate.*

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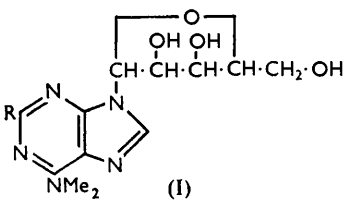
6-Dimethylamino-9-β-D-ribofuranosylpurine-5′ phosphate has been synthesised, from the nucleoside, by using *O*-benzylphosphorous *OO*-diphenylphosphoric anhydride. Ribofuranosides derived from 6-dimethylamino-8-azapurine and 6-dimethylamino-2-methylthio-8-azapurine are also described.

Although the antimetabolite theory has not led to a generally useful chemotherapeutic agent for cancer, results on experimental tumours with such compounds as 6-mercaptopurine and 8-azaguanine have stimulated searches for potent purine and pyrimidine antimetabolites. Evidence has been given that purine ribotides, the precursors of nucleic acids, are synthesised from glycinamide ribotide with the gradual building up of the



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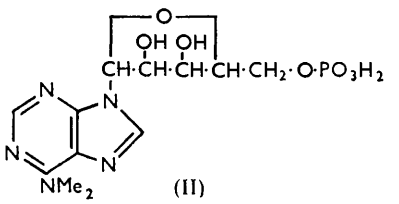

(II)

purine ring. Brown and his collaborators [2] have however shown that purines and purine nucleosides can also act as precursors. Therefore, to obtain information on whether a purine or a purine nucleoside or nucleotide would be the most effective antimetabolite we undertook to prepare a number of nucleosides and, if possible, nucleotides of known active purines. One of the purines chosen was 6-dimethylaminopurine, on the basis of the puromycin studies.

Our first objective, 6-dimethylamino-9-β-D-ribofuranosylpurine (I; R = H) was prepared *via* the 2-methylthio-compound by Kissman, Pidacks, and Baker,[3] and our synthesis, performed independently, varied only in detail. The preparation of tetra-*O*-acetyl-β-D-ribofuranose, required for the formation of the acetochloro-sugar used in the

[1] Goldthwait, Peabody, and Greenberg, *J. Amer. Chem. Soc.*, 1954, **76**, 5258; Hartman, Levenberg, and Buchanan, *ibid.*, 1955, **77**, 501; Greenberg and Spilman, *J. Biol. Chem.*, 1956, **219**, 411; Levenberg and Melnick, *Fed. Proc.*, 1956, **15**, 117.

[2] Brown, Roll, and Weinfeld, " Phosphorus metabolism," Johns Hopkins Univ. Press, Baltimore, 1952, Vol. II, p. 385.

[3] Kissman, Pidacks, and Baker, *J. Amer. Chem. Soc.*, 1954, **77**, 18.

synthesis, can present some difficulty.[3] We found that hand-separation combined with one or two recrystallisations (see below) was the least laborious method of preparing 50—100 g. batches.

6-Dimethylamino-2-methylthio-(?)9-ribofuranosyl-8-azapurine* and 6-dimethylamino-9-ribofuranosyl-8-azapurine were also synthesised, and the 2′ : 3′-*O*-benzylidene derivative of 6-dimethylamino-9-β-D-ribofuranosylpurine was prepared.

The preparation of 6-dimethylamino-9-β-D-2′ : 3′-*O*-*iso*propylideneribofuranosylpurine, with zinc chloride as catalyst, gave *ca.* 25% yields, but use of toluene-*p*-sulphonic acid (cf. Baker and Schaub [4] who used ethanesulphonic acid) led to a 71% yield, and the corresponding 2-methylthio-compound was obtained in 90% yield.

In an attempt to synthesise 6-dimethylamino-9-β-D-ribofuranosylpurine-5′ phosphate (II), dibenzyl phosphorochloridate was first used with the 2′ : 3′-*O*-*iso*propylidene and the 2′ : 3′-*O*-benzylidene derivative (cf. the phosphorylation of *iso*propylideneadenosine [5]); but, although after removal of the protecting groups a small amount of organic phosphate was detected by paper chromatography none could be isolated. *O*-Benzylphosphorous *OO*-diphenylphosphoric anhydride [6] was then used with the 2′ : 3′-*O*-*iso*propylidene derivative. The crude 5′-phosphite was converted into the phosphorochloridate, then into the benzyl hydrogen phosphate by hydrolysis, and the protecting groups were removed in the usual way (cf. the synthesis of deoxyguanosine-5′ phosphate [7]). It was essential not to hydrolyse the *iso*propylidene group (by prolonged storage in acid) until after hydrogenolysis of the benzyl group, as otherwise hydrogenolysis was inhibited and crystalline material was not obtained.

Kissman *et al.*[3] reported that 6-dimethylamino-9-β-D-ribofuranosylpurine was active against the transplanted mammary adenocarcinoma of the C_3H mouse. Studies on this compound by Dr. Grunberg in the laboratories of Hoffmann-La Roche, Inc., Nutley, New Jersey, showed possibly a slight but not an appreciable activity against the Sarcoma 180 strain of transplantable mouse tumour and the result, on a molar basis, was of the same order as for the purine. The 5′-phosphate is under investigation.

Experimental

6-Dimethylamino-2-methylthio-9-β-D-ribofuranosylpurine (I; R = MeS).—6-Dimethylamino-2-methylthiopurine [8] (16 g.), suspended in ethanol (100 ml.), was treated with aqueous 2N-sodium hydroxide solution (38 ml.). To the solution formed on warming was added a solution of mercuric chloride (22 g.) in ethanol (100 ml.). After cooling, the precipitated solid was washed with water, ethanol, and ether and dried *in vacuo* (P_2O_5) (yield 25 g., 73·5%).

This mercuric chloride complex (13·5 g.) and " Hyflo supercel " (13·5 g.) were suspended in chlorobenzene (250 ml.) and dried by distillation of half the solvent, with stirring. Acetochlororibofuranose was prepared from tetra-*O*-acetyl-β-D-ribofuranose † (12 g.) and ethereal hydrogen chloride [9] (120 ml.; saturated at 0°). The solvent was evaporated *in vacuo*, and the residual gum was dissolved in dry toluene and re-evaporated *in vacuo*; all evaporations were at low bath-temperature. The acetochlororibofuranose, dissolved in dry chlorobenzene (100 ml.), was added to the suspension of the purine compound and the mixture was stirred and heated under reflux for 3 hr. The mixture was filtered hot and the solids were extracted with hot chloroform until the extracts were colourless. The combined filtrates were evaporated *in vacuo*, and the residue was dissolved in chloroform (150 ml.). The chloroform solution was

* Although it has been proved that 6-substituted 2-methylthiopurines give 9-β-D-ribofuranosylpurines it is not yet safe to assume that substitution in similar 8-azapurines is also at the 9-position.

† When a solution of the crude product evaporates in a large dish in the air the furanose and the pyranose compounds form large crystals which are easily recognised and separated by hand. One or two recrystallisations then give pure material.

[4] Baker and Schaub, *ibid.*, 1955, **77**, 5900.
[5] Baddiley and Todd, *J.*, 1947, 650.
[6] Corby, Kenner, and Todd, *J.*, 1952, 3669.
[7] Hayes, Michelson, and Todd, *J.*, 1955, 808.
[8] Baker, Joseph, and Schaub, *J. Org. Chem.*, 1954, **19**, 631.
[9] Davoll, Lythgoe, and Todd, *J.*, 1948, 967.

washed with 30% aqueous potassium iodide (2 × 50 ml.) and water (100 ml.). The chloroform phase was dried by filtration, treated with charcoal, filtered, and evaporated *in vacuo*, yielding a yellow-brown glass (16·3 g.).

The glass was dissolved in dry methanol (25 ml.), and methanolic 5N-ammonia (200 ml.) was added. The solution was left at atmospheric temperature for 24 hr., then evaporated *in vacuo*. A yellow oil was obtained which solidified on trituration with water and, recrystallised from water (*ca.* 200 ml.), had m. p. 174—175°, $[\alpha]_D^{20}$ −43·6° (*c* 1·6 in MeOH) (3·3 g., 40%) [a second crop (0·9 g.) had m. p. 173—174°] (Found, in material dried at 110° *in vacuo*: C, 46·0; H, 5·8; N, 20·6. Calc. for $C_{13}H_{19}O_4N_5S$: C, 45·7; H, 5·6; N, 20·5%).

6-*Dimethylamino*-9-β-D-*ribofuranosylpurine* (I; R = H).—6-Dimethylamino-2-methylthiopurine mercuric chloride complex (32 g.) was treated with acetochlororibofuranose [from tetra-*O*-acetylribofuranose (28 g.)] as in the previous experiment, except that the mixture was heated for 3½ hr. After evaporation of the chloroform solution, the crude 6-dimethylamino-2-methylthio-9-β-D-2′ : 3′ : 5′-*O*-triacetylribofuranosylpurine was dissolved in methanol (1·5 l.), freshly prepared Raney nickel (*ca.* 80 g.; prepared according to Mozingo's directions [10] with the final heating at 50°) was added, and the mixture was stirred and heated under reflux for 1 hr. The nickel was removed through a pad of " Hyflo supercel," and was washed with boiling methanol (1 *l.*). The filtrates were evaporated *in vacuo*, yielding a yellow gum. (Ultraviolet absorption showed desulphurisation to be complete by the absence of a maximum at 248 mμ.) The gum was treated in methanol (300 ml.) with methanolic N-sodium methoxide (3 ml.) and refluxed for 1 hr. (If the pH was then less than 8, further sodium methoxide was added and heating continued for a further ½ hr.) The solution was evaporated *in vacuo* (crystals which came out before evaporation was complete were collected), and the residue dissolved in a few ml. of water, and this solution treated with boiling acetone. The acetone solution was evaporated *in vacuo*. The residual oil, after two evaporations *in vacuo* with acetone, solidified and the solid was collected, combined with the crystals obtained from the methanol evaporation, and recrystallised from water (*ca.* 10 ml.) and acetone (*ca.* 150 ml.). The white fluffy needles (9·8 g.) were filtered off, washed with acetone, and dried *in vacuo* (P_2O_5); they had m. p. 182—183°. Evaporation of the mother-liquors and recrystallisation of the residue yielded a further 2·3 g. with the same m. p. {total yield 57%; $[\alpha]_D^{20}$ −58·5° (*c* 2·3 in H_2O)} (Found, in material dried *in vacuo* at 110: C, 48·5; H, 5·7; N, 23·6. Calc. for $C_{12}H_{17}O_4N_5$: C, 48·7; H, 5·8; N, 23·8%).

6-*Dimethylamino*-9-(2′ : 3′-O-iso*propylidene*-β-D-*ribofuranosyl*)*purine*.—6-Dimethylamino-9-β-D-ribofuranosylpurine (9 g.), dry acetone (450 ml.), and anhydrous copper sulphate (36 g.) were stirred and toluene-*p*-sulphonic acid (36 g.) in dry acetone (200 ml.) was added. The mixture was stirred for ½ hr. The solids were removed and were washed with acetone (2 × 200 ml.). The filtrate was poured into a solution of sodium carbonate (30 g., anhyd.) in water (*ca.* 400 ml.), which was then extracted with chloroform (3 × 400 ml.). The chloroform phase was evaporated *in vacuo*, yielding a solid which, recrystallised from ethanol, gave the *product* as needles (6·8 g., 73%), m. p. 176—177° (Found, in material dried at 110° *in vacuo*: C, 53·7; H, 6·5; N, 21·0. $C_{15}H_{21}O_4N_5$ requires C, 53·7; H, 6·3; N, 20·9%).

6-*Dimethylamino*-9-β-D-*ribofuranosylpurine*-5′ *phosphate* (cf. II).—To benzyl dihydrogen phosphite (4·07 g.) in dry benzene (33 ml.) diphenyl phosphorochloridate (4·9 ml.) was added. The solution was stirred and triethylamine (3·33 ml.) in dry benzene (33 ml.) was added during *ca.* 10 min. The mixture was stirred for 1 hr. at room temperature and the triethylamine hydrochloride formed was removed. To the filtrate was added 6-dimethylamino-9-(2′ : 3′-*O*-*iso*propylidene-β-D-ribofuranosyl)purine (5 g., dried *in vacuo* at 100° over P_2O_5 for 5 hr.) and 2 : 6-lutidine (2·7 ml.). The mixture was stirred for ½ hr. at room temperature, then filtered and evaporated *in vacuo* at room temperature. The residue was dissolved in chloroform (100 ml.) and washed with water (100 ml.), saturated sodium hydrogen carbonate solution (100 ml.) and water (100 ml.). The chloroform solution was dried by filtration and evaporated *in vacuo*, at room temperature, yielding crude 6-dimethylamino-9-(2′ : 3′-*O*-*iso*propylidene-β-D-ribofuranosyl)purine-5′ benzyl hydrogen phosphite as a pale yellow oil (7·8 g.).

The phosphorochloridate was formed by stirring the oil in dry benzene (80 ml.) with *N*-chlorosuccinimide (2 g.) at room temperature for 2 hr. The resultant solution was stirred with acetonitrile (80 ml.) and saturated aqueous sodium hydrogen carbonate solution (160 ml.) for 6 hr. and kept for a further 9 hr. The aqueous phase was separated and filtered and the

[10] Mozingo, *Org. Synth.*, 1941, **21**, 15.

acetonitrile removed by evaporation *in vacuo* at <30°. Half of the residual aqueous solution was set aside and the other half was cooled in ice-water and acidified with dilute hydrochloric acid to pH *ca.* 2 and extracted with chloroform (5 × 50 ml.). The combined chloroform extracts were dried by filtration and evaporated *in vacuo* at room temperature. The gummy residue (2·2 g.) was immediately dissolved in ethanol (100 ml.), and water (100 ml.) was added. The solution was treated with charcoal and the filtered solution was hydrogenated at atmospheric pressure over palladium oxide (0·5 g.), and 10% palladium–charcoal (0·5 g.) [previously reduced in 50% ethanol (50 ml.)]; 100 ml. of hydrogen were absorbed in 2 hr. The catalyst was removed and the filtrate evaporated *in vacuo*. The residue was dissolved in water (3 ml.) and boiled for *ca.* 2 min. to remove the *iso*propylidene group. After cooling, acetone was added to turbidity and the solution was set aside at 0° for 3 days during which crystals were deposited. The crystals (0·6 g., 19·5%) were collected, washed with acetone, and dried *in vacuo* over P_2O_5; they had m. p. 223° (decomp.). Recrystallisation from water (15 ml.) and acetone yielded the *phosphate* (0·5 g.), m. p. 225° (decomp.), $[\alpha]_D^{20}$ −51° (*c* 1·98 in H_2O), $\lambda_{max.}$ 268 mμ (ε 18,300). The compound showed a single spot of R_F 0·39 (ultraviolet-absorbent and phosphorus-containing) on a paper chromatogram, with propan-1-ol–ammonia (*d* 0·88)–water (60 : 30 : 10) as solvent ascending on Whatman No. 1 paper for 17 hr. at room temperature (Found, in material dried *in vacuo* at 110°: C, 38·9; H, 5·1; N, 18·8; P, 8·1. $C_{12}H_{18}O_7N_5P$ requires C, 38·4; H, 4·8; N, 18·7; P, 8·3%).

6-*Dimethylamino*-2-*methylthio*-8-*azapurine*.—4 : 5-Diamino-6-dimethylamino-2-methylthiopyrimidine (10 g.) was partially dissolved in 2M-sodium acetate (400 ml.), 2N-hydrochloric acid (25 ml.), and acetic acid (5 ml.) at 80°. Sodium nitrite (20 g.) in water (200 ml.) was added, and the mixture kept at 95° for ½ hr., then cooled, and filtered. The solid was washed with water and crystallised from ethanol (*ca.* 500 ml.) (charcoal). 6-*Dimethylamino*-2-*methylthio*-8-*azapurine* crystallised in colourless needles (8·5 g.), m. p. 263° [the mother-liquors gave a second crop (0·8 g.), m. p. 262°] (Found, in material dried *in vacuo* at 110°: C, 40·1; H, 4·9; N, 40·4. $C_7H_{10}N_6S$ requires C, 40·0; H, 4·8; N, 40·0%).

6-*Dimethylamino*-2-*methylthio*-(?)9-β-D-*ribofuranosyl*-8-*azapurine*.—The 6-dimethylamino-2-methylthio-8-azapurine mercuric chloride complex (prepared in a similar way to the complex described above) (12 g.) and acetochlororibofuranose (from 9·5 g. of tetra-*O*-acetyl-β-D-ribofuranose) were caused to react by the method previously described. Deacetylation of the crude tri-*O*-acetylribosyl compound with methanolic ammonia yielded a solid *purine* on trituration with water; recrystallised twice from water this had m. p. 146·5—148° (yield 49·5%) (Found, in material dried *in vacuo* at 100°: C, 41·5; H, 5·4; N, 24·7. $C_{12}H_{18}O_4N_6S$ requires C, 42·0; H, 5·3; N, 24·6%).

6-*Dimethylamino*-9-β-D-*ribofuranosyl*-8-*azapurine*.—Dimethylamino-2-methylthio-9-β-D-ribofuranosyl-8-azapurine (400 mg.) was stirred in ethanol (100 ml.) with Raney nickel (*ca.* 3 g.) under reflux for 1 hr. The mixture was filtered hot through " Hyflo supercel," and the solids were extracted with boiling ethanol (250 ml.). The filtrates were evaporated *in vacuo*. The residue (150 mg.) crystallised on trituration with ether (m. p. 199—200°). Recrystallisation from a little water–ethanol gave a *product*, m. p. 216° (70 mg., 21%) (Found, in material dried *in vacuo* at 110°: C, 44·5; H, 5·7; N, 28·2. $C_{11}H_{16}O_4N_6$ requires C, 44·6; H, 5·4; N, 28·4%).

6-*Dimethylamino*-9-(2′ : 3′-O-*benzylidene*-β-D-*ribofuranosyl*)*purine*.—Zinc chloride (750 mg.), benzaldehyde (3·7 ml.; dry), and 6-dimethylamino-9-β-D-ribofuranosylpurine (290 mg.) were shaken for 24 hr., in which dissolution was effected. The solution was poured into dry ether (50 ml.), and the precipitate was collected, washed with dry ether, dissolved in " Ethyl cellosolve " (4·3 ml.), and treated with aqueous 2N-sodium hydroxide (3·2 ml.). After 10 min., the solid was removed and was washed with hot " Ethyl cellosolve." The filtrate was evaporated *in vacuo*. On trituration with water, the resulting oil crystallised. The *compound* (155 mg.) washed with water and dried *in vacuo* (P_2O_5), had m. p. 159—162°. From the filtrate and washings, by evaporation, starting material (80 mg.) was obtained. The product was recrystallised twice from ethanol, the m. p. rising to 172° (yield 100 mg., 34%) (Found, in material dried *in vacuo* at 110°: C, 59·4; H, 5·7; N, 17·8. $C_{19}H_{21}O_4N_5$ requires C, 59·5; H, 5·8; N, 18·3%).

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