609. *Chemistry of the Vitamin B_{12} Group. Part II.* Synthesis of* 5 : 6-*Dimethyl*-1-α-D-*ribofuranosylbenziminazole.*

By A. W. JOHNSON, G. W. MILLER, J. A. MILLS, and A. R. TODD.

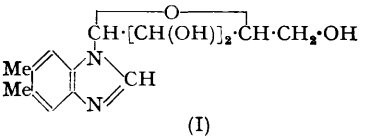
Various synthetic routes to glycosylbenziminazoles have been examined and condensation of the free bases with *O*-acetylglycosyl halides has been shown to yield a mixture of the α- and β-anomers of the *N*-glycosyl compounds. 5 : 6-Dimethyl-1-α-D-ribofuranosylbenziminazole, a degradation product of vitamin B_{12}, has been synthesised by this method.

DEGRADATION of vitamin B_{12} with mineral acid yields, among other products, 5 : 6-dimethylbenziminazole (Brink and Folkers, *J. Amer. Chem. Soc.*, 1949, **71**, 2951; 1950, **72**, 4442; Beavan, Holiday, Johnson, Ellis, Mamalis, Petrow, and Sturgeon, *J. Pharm. Pharmacol.*, 1949, **1**, 957), its 1-α-D-ribofuranosyl derivative (I) (Brink *et al.*, *J. Amer. Chem. Soc.*, 1950, **72**, 1866; 1952, **74**, 2856) and the 2′(or 3′)-phosphate of (I) (Buchanan, Johnson, Mills, and Todd, *J.*, 1950, 2845). A synthesis of (I) has been described by the Merck group of workers (Holly *et al.*, *J. Amer. Chem. Soc.*, 1950, **72**, 1866; 1952, **74**, 4521); 4 : 5-dimethyl-2-nitro-*N*-(5′-*O*-trityl-D-ribofuranosyl)aniline (II) was hydrogenated, and then cyclised with *iso*propyl formimidate hydrochloride and the triphenylmethyl group was finally removed by hydrolysis, yielding (I).

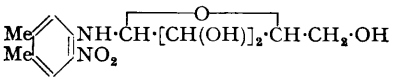
The anomeric compound, 5 : 6-dimethyl-1-β-D-ribofuranosylbenziminazole, was synthesised by the same route save that the reduction and cyclisation steps were carried out starting with the diacetyl derivative of (II). It is of some interest that small amounts of the α-ribosyl compound were formed in addition to the β-compound; rather similar

* Part I, *J.*, 1950, 2845.

effects of acetylation of intermediates on the course of the cyclisation step in purine nucleoside syntheses have been reported from this laboratory (Kenner, Todd, *et al.*, *J.*, 1946, 852; 1948, 2265; 1949, 1613) and have been ascribed to chelation. The configuration at the glycoside carbon in these ribosylbenziminazoles was assigned on the basis of periodate oxidation studies and the optical rotations of the glycosyl derivatives and their



(I)



(II)

picrates. The α-configuration of the ribosylbenziminazole obtained from vitamin B_{12} is surprising, in view of the uniform β-configuration of all the natural purine and pyrimidine ribonucleosides.

Our own work in this field, which was completed before the appeareance of any experimental details of the American syntheses, led to an independent synthesis of (I), and in view of the difficulties encountered in all efforts to synthesise glycofuranosylbenziminazoles it seems desirable to place our results on record. The formal resemblance of (I) to the purine nucleosides naturally suggested the application of synthetic methods which had been successful in that series (for review see Kenner, *Fortsch. Chemie Org. Naturstoffe*, 1951, **8**, 96). These were of two main types : (1) the conversion of a mono-*N*-glycosyl-*o*-phenylenediamine into a benziminazole by a process introducing one more carbon atom, and (2) the reaction of a preformed benziminazole with an *O*-acetylglycosyl halide. The synthesis of glycopyranosyl compounds by methods of type (1) is relatively straightforward (Part I, *loc. cit.*; Mamalis, Petrow, and Sturgeon, *J. Pharm. Pharmacol.*, 1950, **2**, 491, 503, 512, 579; Holly *et al.*, *loc. cit.*) but is much less satisfactory for glycofuranosyl compounds. The synthesis of (I) by Holly *et al.* (*loc. cit.*) is indeed of this type, but the overall yields reported by them were both low and variable. Our experience was similar and as we were unable to effect any material improvements we abandoned this route.

Methods of type (2), employing a performed benziminazole, have the advantage of directness and are attractive in the case of 5 : 6-dimethylbenziminazole, whose symmetrical structure removes the chief drawback to such methods, *viz.*, ambiguity as regards position of the entering sugar residue. In Part I (*loc. cit.*) we described a synthesis of 1-β-D-glucopyranosyl-5 : 6-dimethylbenziminazole from the silver salt of 5 : 6-dimethylbenziminazole and acetobromoglucose, but the yield was very poor. Other workers (Mamalis, Petrow, and Sturgeon, *loc. cit.*; Weygand, Wacker, and Wirth, *Z. Naturforsch.*, 1951, **66**, 25; Weygand and Wirth, *Chem. Ber.*, 1952, **85**, 1000) have made a number of glycosylbenziminazoles in the same way, but only in reactions using silver 5 : 6-dichlorobenziminazole (Weygand, Wacker, and Wirth, *loc. cit.*) were really satisfactory yields obtained. The poor results are probably attributable largely to the heterogeneous nature of the initial reactions. Our attempts to improve matters by using a mixture of the benziminazole with silver oxide, or by substituting zinc, cadmium, or thallium derivatives were unsuccessful. In the course of these studies we confirmed Davoll and Lowy's observation (*J. Amer. Chem. Soc.*, 1951, **73**, 5781) that chloromercuri-derivatives of benziminazole are more satisfactory than silver derivatives in such condensations, although considerable darkening usually occurred under the conditions recommended by them (boiling xylene); both Davoll and Lowy (*loc. cit.*) and Weygand and Wirth (*loc. cit.*) prepared the β-anomer of (I) by this method. In all condensations of metal derivatives of benziminazoles with tetra-*O*-acetylglucosyl bromide the occurrence of a Walden inversion has been assumed (cf. Howard, *J.*, 1950, 1045) and the products have accordingly been formulated as 1-tetra-*O*-acetyl-β-D-glucosylbenziminazoles.

In a further attempt to improve this type of synthesis of *N*-glucosylbenziminazoles the reaction between acetohalogeno-sugars and metal-free benziminazoles was investigated. This reaction is analogous to that normally employed for the synthesis of pyrimidine nucleosides (cf. Howard, Lythgoe, and Todd, *J.*, 1947, 1052; Kenner, *loc. cit.*, p. 108),

starting with acetohalogeno-sugars and 2 : 4-diethoxypyrimidine and proceeding by elimination of ethyl halide from the quaternary salt initially formed. In the case of benziminazoles hydrogen bromide is, of course, liberated, but this can be removed as a salt by working with excess of the base. The reaction between tetra-*O*-acetylglucosyl bromide and benziminazole was carried out in dioxan solution, and the crude 1-D-tetra-*O*-acetylglucosylbenziminazole was deacetylated with mineral acid. Excess of benziminazole was removed by chromatography on alumina, and the glycosyl material subjected to chromatography on charcoal–Hyflo supercel with ethanol as eluant.

By this means 1-β-D-glucopyranosylbenziminazole was obtained, identical with that previously described (Buchanan *et al.*, Mamalis *et al.*, and Davoll and Lowy, *locc. cit.*), as well as an isomeric glucosyl compound which from its optical rotation was identified as the corresponding 1-α-D-glucopyranosylbenziminazole and was produced in about 1/5 of the amount of the β-isomer. The α- and the β-isomer were also separated by fractional crystallisation of the picrates. Although there have been several reports of the preparation of glycosides from *cis*-acetohalogeno-sugars without inversion (or possibly with a double inversion) at the 1-carbon atom of the sugar (Howard, *loc. cit.*) there have been but few reports of this phenomenon in the *N*-glycosyl series; Davoll and Lythgoe (*J.*, 1949, 2526), however, isolated both isomers of 3' : 4'-diacetyl-2'-deoxy-D-ribopyranosyltheophylline by reaction of the silver salt of the base with an acetohalogenoribose. Reaction of 5 : 6-dimethylbenziminazole with acetobromoglucose yielded similarly both forms of the *N*-D-glucopyranosyl derivative, and the α-form, not previously described, was further characterised as its picrate.

For the preparation of the *N*-D-ribofuranosyl compounds, tri-*O*-acetyl-D-ribofuranosyl chloride was first prepared. The tetra-*O*-acetyl-D-ribofuranose required was obtained from 1 : 2 : 3-tri-*O*-acetyl-5-tritylribose (Howard, Lythgoe, and Todd, *J.*, 1947, 1052; Bredereck and Hoepfner, *Ber.*, 1948, **81**, 51) or by direct acetylation of D-ribose. The latter process, studied by Zinner (*Ber.*, 1950, **83**, 153), has now been made less capricious by use of dioxan as solvent and a small quantity of pyridine as catalyst, although the yields obtained were then lower.

Condensation of tri-*O*-acetyl-D-ribofuranosyl chloride with 5 : 6-dimethylbenziminazole was carried out as in the glucose series, and the crude ribosyl mixture was chromatographed as before. The two isomers were separated, the β- (10%) being more abundant than the required α-isomer (2%). The latter was identical with the nucleoside derived from vitamin B_{12} (Brink and Folkers, *J. Amer. Chem. Soc.*, 1952, **74**, 2856). When tri-*O*-acetylribofuranosyl bromide was used (cf. Howard, Lythgoe, and Todd, *loc. cit.*), its additional reactivity was counteracted by its instability and the yield of the α-ribosyl compound was not improved. The use of acetonitrile as solvent likewise offered no advantage.

Being a rather weak base (pK_a 5·53), benziminazole may not be very effective in promoting base-catalysed side reactions such as the elimination of hydrogen halide from *O*-acylglycosyl halides to produce glycoseens (cf. Maurer, *Ber.*, 1929, **62**, 232) or 1 : 6-anhydro-sugars (cf. Micheel, *ibid.*, p. 687). Nevertheless, reactions other than the formation of *N*-glycosyl compounds always occurred, and little or no unchanged tetra-*O*-acetylglycosyl halide was normally present at the end of the reaction. In one experiment, tri-*O*-acetyl-1 : 6-anhydroglucose was provisionally identified on a paper chromatogram of the non-basic components of the reaction mixture.

The reaction of the free benziminazole bases and *O*-acylglycosyl halides compares favourably with the reactions involving the heavy metal derivatives of the benziminazoles, especially in ease of manipulation, and the yields obtained are of the same order as those reported from the condensations using the mercury derivatives (Davoll and Lowy, *loc. cit.*).

Experimental

Rotations refer to pyridine solutions, unless otherwise stated.

1-D-*Glucopyranosylbenziminazoles*.—Benziminazole (20 g., 2·2 mols.) and tetra-*O*-acetylglucosyl bromide (32 g., 1 mol.) were dissolved in dioxan (80 c.c.) at 100° and kept at this temperature for 3½ hr., during which crystalline benziminazole hydrobromide separated. Xylene

(300 c.c.) was added to the solution which then was cooled (ice), and solids were removed by filtration. The acetyl groups were removed from the product by heating the xylene solution at 100° with successive quantities of N-hydrochloric acid (3 × 30 c.c.) for 30 min. each and the combined aqueous layers were then made alkaline with aqueous ammonia (*d* 0·88) and evaporated to dryness under reduced pressure. The dark residue was extracted with boiling ethanol (3 × 150 c.c.), and the combined ethanolic extracts were dried by azeotropic distillation with benzene (50 c.c.). The dried solution was brought on to a column of alumina (8 × 2½") and washed exhaustively with ethanol to remove unchanged benziminazole. The progress of the separation was followed by examining the acidified eluate in ultra-violet light, benziminazole being fluorescent. Elution with water rapidly removed the glucosyl derivative from the column and when this eluate was concentrated under reduced pressure to 50 c.c. and cooled at 0° overnight crystalline 1-β-D-glucopyranosylbenziminazole, m. p. 140—141°, separated. Recrystallisation from water gave the pure monohydrate (4·9 g., 21% based on tetra-*O*-acetylglucosyl bromide), m. p. 141—142°, $[\alpha]_D^{15}$ −28° (*c* 2) (Found: Loss at 125° in a high vacuum, 5·6. Calc. for $C_{13}H_{16}O_5N_2,H_2O$: 6·0%). When boiled with dry *iso*propanol (20 c.c. per g.) the hydrate dissolved and the solution quickly deposited the anhydrous product as a fine powder, m. p. 210—212°. This afforded a picrate, m. p. 145—148°, $[\alpha]_D^{15}$ −18° (*c* 2), and a tetra-*O*-acetyl derivative separating from benzene as fine needles, m. p. 152—153°, $[\alpha]_D^{15}$ −27° (*c* 2 in $CHCl_3$). These values are in good agreement with the published data on 1-β-D-glucopyranosylbenziminazole (Mamalis, Petrow, and Sturgeon, *J. Pharm. Pharmacol.*, 1950, **2**, 593; Davoll and Lowy, *J. Amer. Chem. Soc.*, 1951, **73**, 5781).

The aqueous mother-liquors from the β-D-glucopyranosyl compound were brought on to a column (9 × 2½") of charcoal (" Karbak " grade)–Hyflo supercel (1 : 1; 300 g.), and the column washed with water until the eluate, which contained mainly non-nitrogenous impurities, no longer reduced ammoniacal silver nitrate. The eluant was then changed to ethanol (some batches of charcoal required the addition of 1% of pyridine to the ethanol for elution of the glucoside), and the eluate collected in two main fractions (2 l. each). Each fraction was taken to dryness under reduced pressure and the colourless residue dissolved in a small quantity of water and treated with successive portions of saturated aqueous picric acid. From the first fraction the following picrates were obtained (*c* = 1): $[\alpha]_D^{19}$ +90° (1·55 g.), +80° (0·1 g.), +50° (1 g.), +8° (0·5 g.); and from the second eluate fraction: $[\alpha]_D^{19}$ +47° (2·1 g.) and +23° (0·85 g.). The picrate fractions with $[\alpha]_D^{19}$ > +80° (1·65 g.) were combined and recrystallised from water to give long feathery needles (1·5 g., 3·7% based on tetra-*O*-acetylglucosyl bromide), m. p. 125—135°, $[\alpha]_D^{19}$ +93° (*c* 1·2). The *picrate* appeared to be hydrated but the m. p. range was not decreased after drying (Found: C, 43·0; H, 3·9; N, 13·1; loss at 100° *in vacuo* for 24 hr., 3·5. $C_{19}H_{19}O_{12}N_5,H_2O$ requires C, 43·3; H, 4·0; N, 13·3; H_2O, 3·4%). On periodate titration the picrate reacted with 2·02 mols. of oxidant.

The picrate (100 mg.) was decomposed by treating a methanolic solution with small quantities of freshly prepared Dowex 2 ion-exchange resin (1 g.) in the hydroxide form. When the colour of the supernatant liquid had been discharged, the orange resin was separated and the clear solution evaporated to yield 1-α-D-*glucopyranosylbenziminazole* (52 mg.) as a colourless solid which crystallised from ethanol as needles, m. p. 178—179°, $[\alpha]_D^{15}$ +170° (*c* 1·8) (Found, on a sample dried at 105° in a high vacuum: C, 55·6; H, 6·0; N, 10·1. $C_{13}H_{16}O_5N_2$ requires C, 55·7; H, 5·8; N, 10·0%). Titration with sodium periodate gave a value of 2·0 mols. of reagent consumed by each mol. of the product.

1-D-*Glucopyranosyl*-5 : 6-*dimethylbenziminazoles*.—5 : 6-Dimethylbenziminazole (10·5 g., 2·2 mols.) and tetra-*O*-acetylglucosyl bromide (13·5 g., 1 mol.) were heated in dry dioxan (32 c.c.) at 100° for 3 hr. The product was then treated as in the previous experiment; xylene (200 c.c.) was added and the filtered solution hydrolysed at 100° with N-hydrochloric acid (3 × 30 c.c.). The combined aqueous layers were made alkaline with aqueous ammonia (*d* 0·88), and the unchanged 5 : 6-dimethylbenziminazole was removed by chromatography on alumina as before. The brown aqueous solution of glucosyl derivatives, contaminated with unchanged glucose, was treated on charcoal (" Karbak ")–Hyflo supercel (1 : 1; 300 g.) as before. The alcoholic eluate was collected in 5 fractions (1 l. each). Evaporation of fractions 1—3 gave colourless feathery crystals of 1-β-D-glucopyranosyl-5 : 6-dimethylbenziminazole, which after crystallisation from absolute ethanol had m. p. 248—250° (2·0 g., 20% based on tetra-*O*-acetylglucosyl bromide) undepressed by an authentic specimen (Buchanan, Johnson, Mills and Todd, and Davoll and Brown, *locc. cit.*). It consumed 2·01 mols. of sodium periodate.

Fractions 4 and 5 gave a colourless residue which after crystallisation from water or aqueous alcohol formed needles, m. p. 176°, $[\alpha]_D^{19}$ +171° (*c* 1·2) (0·52 g., 5·1% based on tetra-*O*-

acetylglucosyl bromide) (Found, on a sample dried at 100° over P_2O_5 *in vacuo* for 2 days: C, 57·95; H, 6·7; N, 9·4. $C_{15}H_{20}O_5N_2$ requires C, 58·4; H, 6·5; N, 9·1%). The corresponding *picrate*, crystallised from aqueous ethanol, had $[\alpha]_D^{19}$ +101° (*c* 1) (Found: C, 45·8; H, 4·4; N, 12·4. $C_{21}H_{23}O_{12}N_5,H_2O$ requires C, 45·4; H, 4·5; N, 12·6%). The m. p. was indefinite and varied with the rate of heating but on very slow heating a value m. p. 205° was obtained, presumably of the anhydrous form. The glycosyl derivative consumed 2 mols. of sodium periodate; the picrate of the oxidised product, after drying *in vacuo* for 2 days at room temperature over P_2O_5, had m. p. 185—186°, undepressed in admixture with the picrate of the product obtained from the periodate oxidation of 5 : 6-dimethyl-1-α-D-ribofuranosylbenziminazole (Brink and Folkers, *J. Amer. Chem. Soc.*, 1952, **74**, 2856, give m. p. 183—185·5°).

Tetra-O-acetyl-D-ribofuranose.—D-Ribose (5 g.) was heated with dry dioxan (80 c.c.) and pyridine (0·8 c.c.) on the steam-bath until dissolved. Acetic anhydride (19·2 c.c., 6 mols.) was added dropwise to the stirred hot solution during 1¼ hr. Heating was continued for another 2¼ hr. and the solvent removed at 50° (bath-temp.)/15 mm. The residual yellowish oil was cooled and diluted with ice-water (40 c.c.). A seed crystal of tetra-*O*-acetyl-D-ribofuranose was added and after 2 hr. at 0° the crystalline solid (6·5 g.) was separated, washed, and dried. It then had m. p. 69—75° but after crystallisation from *iso*propanol (100 c.c.) (charcoal) pure tetra-*O*-acetylribofuranose (2 g.), m. p. 81—82°, was obtained. A further quantity (1 g.), m. p. 81—82°, was isolated from the *iso*propanolic mother-liquors after evaporation to half volume under reduced pressure and seeding. Further evaporation and seeding gave a mixture of the furanose and the pyranose form. The yield of tetra-*O*-acetyl-D-ribofuranose obtained decreased when larger batches (*e.g.*, 20 g.) of D-ribose were used. Tri-*O*-acetyl-D-ribofuranosyl chloride was obtained from tetra-*O*-acetyl-D-ribofuranose essentially as described by Zinner (*loc. cit.*).

1-D-*Ribofuranosyl*-5 : 6-*dimethylbenziminazoles.*—5 : 6-Dimethylbenziminazole (7·5 g., 2·2 mols.) in dry dioxan (100 c.c.) at 100° was added to the tri-*O*-acetyl-D-ribofuranosyl chloride syrup (1 mol.) prepared from tetra-*O*-acetyl-D-ribofuranose (7·5 g.), and the mixture heated at 100° for 3 hr. The product was treated as in the above preparations of the D-glucopyranosyl compounds; xylene (200 c.c.) was added and the filtered solution repeatedly hydrolysed with N-hydrochloric acid at 100° (3 × 30 c.c.). The combined aqueous layers were made alkaline with aqueous ammonia (*d* 0·88), and the unchanged 5 : 6-dimethylbenziminazole removed by chromatography on alumina (8 × 2½ cm.). The brown aqueous solution of ribosyl and other carbohydrate derivatives was adsorbed on a column of charcoal ("Karbak")–Hyflo supercel (1 : 1; 300 g.), and washed exhaustively with water to remove salts and a good deal of the non-nitrogenous material. The ribosides were eluted with ethanol–pyridine (9 : 1), and the eluate was collected in 7 fractions (1 l. each). Each fraction was diluted with successive portions of water (100 c.c.) and then evaporated to dryness at reduced pressure until all of the pyridine was removed. The residues were each taken up in water (20 c.c.) and then cooled at 0°. Fractions 4—7 yielded colourless solids which were collected, combined and converted into the picrate. After crystallisation from water this had m. p. 175° and it was re-converted into 5 : 6-dimethyl-1-β-D-ribofuranosylbenziminazole by treatment with Dowex 2 resin in the hydroxide form as described below for the α-isomer. The free base, recrystallised from water, had m. p. 197—200°, $[\alpha]_D^{19}$ −44° (*c* 1) (0·65 g.; 10% based on tetra-*O*-acetylribofuranose), and consumed 1·0 mol. of sodium periodate. These values agree with those given by Holly, Shunk, Peel, and Folkers (*loc. cit.*).

Fractions 1, 2, and 3 from the chromatogram were severally treated with excess of aqueous picric acid. The precipitated picrates were separated, washed, and dried and each had m. p. 210°, $[\alpha]_D^{19}$ +9° (*c* 1). The combined picrates (463 mg.) were crystallised from water, giving 5 : 6-dimethyl-1-α-D-ribofuranosylbenziminazole picrate (Found: C, 47·3; H, 4·2; N, 14·0. Calc. for $C_{20}H_{21}N_5O_{11}$: C, 47·3; H, 4·2; N, 13·8%).

The picrate consumed 0·92 mol. of sodium periodate; the dialdehyde picrate so formed was crystallised from aqueous ethanol; it had m. p. 185—186°, undepressed on admixture with the picrate of the periodate oxidation product of 1-α-D-glucopyranosyl-5 : 6-dimethylbenziminazole.

Dowex 2 ion exchange resin in the hydroxide form (1 g.) was added to a warm solution of the picrate of 5 : 6-dimethyl-1-α-D-ribofuranosylbenziminazole (0·46 g.) in methanol (20 c.c.). The resin was separated and washed with boiling methanol, and the combined methanolic filtrates were taken to dryness. The residual oil (243 mg., 96%) on cooling at 0° or on trituration with ether gave a colourless solid, m. p. 190—195°, which was crystallised first from acetone and then from water, to give the pure α-D-ribofuranosylbenziminazole as colourless needles,

m. p. 198°, $[\alpha]_D^{19}$ +14° (*c* 1) (Found : C, 60·4; H, 6·8; N, 10·0. Calc. for $C_{14}H_{18}O_4N_2$: C, 60·4; H, 6·5; N, 10·1%). The *iso*propylidene derivative of the glycoside was also used for the purification (Holly *et al.*, *loc. cit.*).

Grateful acknowledgment is made to the D.S.I.R. for a maintenance grant (to G. W. M.) and to the Australian National University for a scholarship (to J. A. M.).

UNIVERSITY CHEMICAL LABORATORY, CAMBRIDGE. [*Received, June* 16*th*, 1953.]