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Vitamin B_{12}. XIX. Synthesis of the 1-D-Ribosides of 5,6-Dimethylbenzimidazole

BY FREDERICK W. HOLLY, CLIFFORD H. SHUNK, ELIZABETH W. PEEL, JOSEPH J. CAHILL, JOE B. LAVIGNE AND KARL FOLKERS

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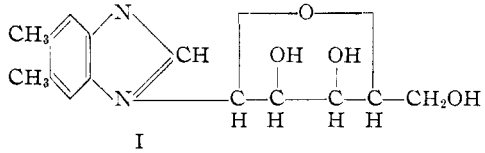
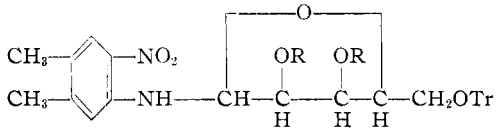
Synthetic methods are described for preparation of the four 1-D-ribosides of 5,6-dimethylbenzimidazole: 1-α-D-ribofuranosyl-5,6-dimethylbenzimidazole, 1-β-D-ribofuranosyl-5,6-dimethylbenzimidazole, 1-α-D-ribopyranosyl-5,6-dimethylbenzimidazole and 1-β-D-ribopyranosyl-5,6-dimethylbenzimidazole.

The isolation of α-ribazole, 1-α-D-ribofuranosyl-5,6-dimethylbenzimidazole (I), from vitamin B_{12} has been described[1,2]; in addition a synthesis of α-ribazole and a synthesis of β-ribazole, 1-β-D-ribofuranosyl-5,6-dimethylbenzimidazole, have been reported.[1] Details of these syntheses and preparation of the two corresponding anomeric ribopyranosides of 5,6-dimethylbenzimidazole are described in this paper.



I

A synthesis of α-ribazole was accomplished by hydrogenation of 2-nitro-4,5-dimethyl-N-(5'-trityl-D-ribofuranosyl)-aniline (II), reaction of the reduced product with an alkyl formimino ether hydrochloride, and acid hydrolysis of the reaction products. The 2-nitro-4,5-dimethyl-N-(5'-trityl-

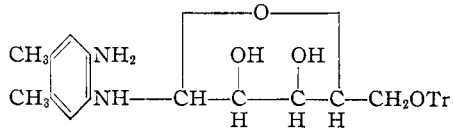


Tr = $(C_6H_5)_3C$
II, R = H
VII, R = CH_3CO

D-ribofuranosyl)-aniline (II) was prepared by allowing 2-nitro-4,5-dimethylaniline to react with 5-trityl-D-ribofuranose in refluxing benzene solution with acetic acid as a catalyst. A preparation of the nitroriboside (II) by tritylation of 2-nitro-4,5-dimethyl-N-D-ribosylaniline has been reported.[3]

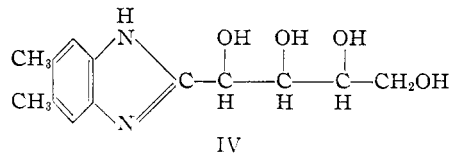
Hydrogenation of the nitroriboside (II) in methanol solution over a palladium catalyst yielded a colorless solution which was refluxed with ethyl- or isopropylformimino ether hydrochloride for two hours. The mixture was heated with dilute hydrochloric acid to hydrolyze the trityl group and α-ribazole was isolated as a crystalline picrate. To prepare the free base, an aqueous suspension of the picrate at *ca.* *p*H 2 was extracted continuously with chloroform until the picric acid was removed. Concentration of the neutralized aqueous solution to a small volume yielded crystalline α-ribazole. The over-all yield of α-ribazole from 2-nitro-4,5-dimethyl-N-(5'-trityl-D-ribofuranosyl)-aniline (II) has been about 6%. Close attention to experimental details has been found necessary to obtain reproducible results.

The hydrogenation was usually completed in not more than three hours and the solution containing the hydrogenated material, after removal of the catalyst, was used immediately in the next step. The structure of the reduced product is assumed to be the corresponding aminoriboside (III).

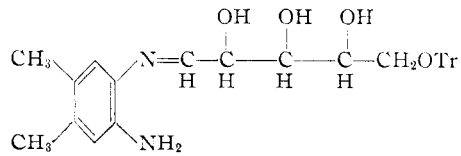


III

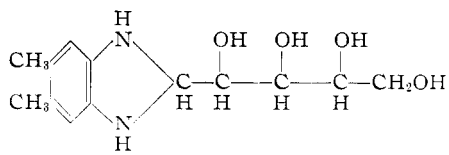
Attempts were made to isolate and characterize the reduced product. Removal of the solvent yielded a nearly colorless residue which was not obtained in a crystalline form. Analytical data determined on this material were in fair agreement with structure III, but purity was not established. Attempts to form crystalline derivatives were unsuccessful, possibly due to the lability of the glycosidic linkage. The trityl ether appeared to survive the hydrogenation without undergoing hydrogenolysis, as indicated by the formation of triphenylcarbinol when the material was hydrolyzed; no triphenylmethane was isolated. Since in successful preparations of α-ribazole the reduced material has been used immediately in methanol solution, and in view of the relatively low yield of α-ribazole obtained, it is not possible to assign with certainty a structure to the hydrogenated product which participates in the condensation step to yield α-ribazole. From certain runs, 2-D-ribo-5,6-di-



IV



V



VI

(1) N. G. Brink, F. W. Holly, C. H. Shunk, E. W. Peel, J. J. Cahill and K. Folkers, THIS JOURNAL, **72**, 1866 (1950).
(2) N. G. Brink and K. Folkers, *ibid.*, **74**, 2856 (1952).
(3) R. Kuhn and R. Ströbele, *Ber.*, **70**, 773 (1937).

methylbenzimidazole (IV) rather than α-ribazole was the only product isolated. This product might be formed by an intramolecular reaction of the aminoriboside (III), reacting in its open-chain tautomeric form (V). Addition of the amino group to the carbon–nitrogen double bond would yield a dihydrobenzimidazole (VI) which would be expected to dehydrogenate to the 2-ribo derivative (IV).

A similar sequence of reactions has been postulated to explain the formation of 2-methylbenzimidazole from *o*-phenylenediamine and acetaldehyde.[4]

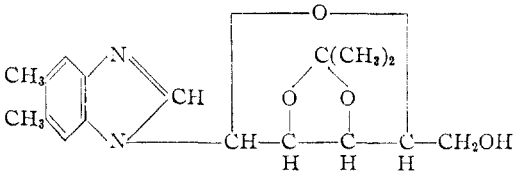
From the above sequence of reactions no β-ribazole has been isolated. A synthesis of β-ribazole also utilized 2-nitro-4,5-dimethyl-N-(5′-trityl-D-ribofuranosyl)-aniline (II) as the starting product. Acetylation of this riboside in pyridine and acetic anhydride gave 2-nitro-4,5-dimethyl-N-(2′,3′-diacetyl-5′-trityl-D-ribofuranosyl)-aniline (VII) which was purified by chromatography over alumina and was obtained as an orange amorphous product. This compound was hydrogenated in benzene solution over a palladium catalyst, and the material in benzene solution was allowed to react with ethylformimino ether hydrochloride at room temperature. After hydrolysis of the reaction product, crystalline β-ribazole picrate was isolated; from the filtrate a low yield of α-ribazole picrate was obtained. Crystalline β-ribazole was prepared by conversion of the picrate to the free base as described above for α-ribazole. From some preparations crystalline β-ribazole separated before formation of the picrate.

While the picrates of α- and β-ribazole are crystalline products, they have been, in certain cases, difficult to obtain in a high degree of purity. Final purification has been accomplished by conversion of the picrates to the free bases, formation of the isopropylidene derivatives, and subsequent acid hydrolysis of these products to α- and β-ribazole. Although inversion of configuration about the glycosidic carbon atom during either the formation or the hydrolysis of these isopropylidene derivatives seems most unlikely, it is of interest that in the case of the unsubstituted ribosides α-ribazole is the anomer of higher positive rotation, while the isopropylidene derivative prepared from β-ribazole has the more positive rotation. The nomenclature which we have employed for the isopropylidene derivatives of α- and β-ribazole (VIIIa) and (VIIIb), therefore, constitutes an exception to the usual convention[5] for the designation of configuration of the two anomers.

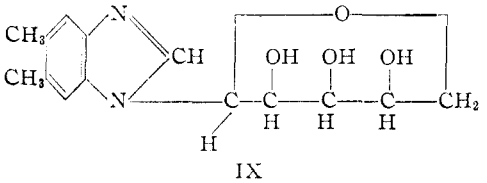
1-α-D-Ribopyranosyl-5,6-dimethylbenzimidazole (IX) was synthesized by hydrogenation of 2-nitro-4,5-dimethyl-N-(2′,3′,4′-triacetyl-D-ribopyranosyl)-aniline (X) over a palladium catalyst, reaction of the reduced product with ethyl formimino ether hydrochloride in benzene solution, followed by hydrolysis of the reaction mixture. The ribopyranoside was isolated as a crystalline picrate. Isolation of a ribopyranoside, rather than a ribofuranoside, from this sequence of reactions indicates that the triacetate (X) contains a pyranose ring.

(4) G. McCoy and A. R. Day, THIS JOURNAL, **65**, 2159 (1943).

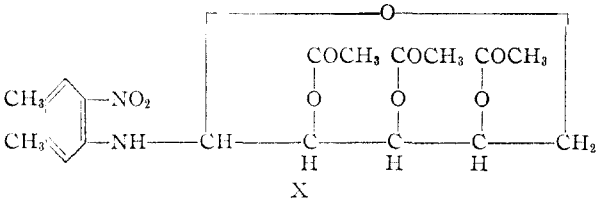
(5) C. S. Hudson, *ibid.*, **31**, 66 (1909).



VIIIa, prepared from α-ribazole
VIIIb. prepared from β-ribazole

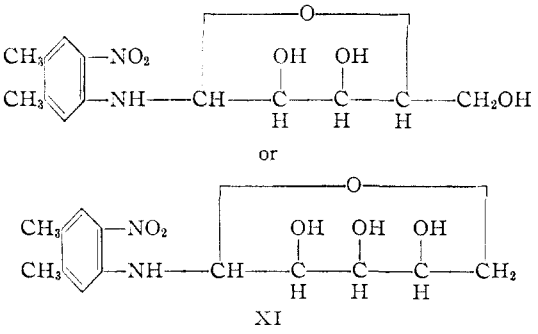


IX



X

The anomeric ribopyranoside, 1-β-D-ribopyranosyl-5,6-dimethylbenzimidazole,[6] was synthesized from 2-nitro-4,5-dimethyl-N-(D-ribosyl)-aniline (XI) by two alternate procedures. In the first method the nitroriboside (XI) was hydrogenated over a palladium–Darco catalyst and the reduced material was allowed to react with isopropylformimino ether hydrochloride in methanol solution. The ribopyranoside was isolated from the reaction mixture as a crystalline picrate which was converted to the crystalline 1-β-D-ribopyranosyl-5,6-dimethylbenzimidazole. Although in this instance a pyranosylbenzimidazole was produced from the nitroriboside (XI), the ring structure of this nitroriboside, which may tautomerize in solution, is uncertain.[7]



XI

In the second procedure, 2-nitro-4,5-dimethyl-N-(2′,3′,4′-triacetyl-D-ribopyranosyl)-aniline (X) was hydrogenated over a palladium–Darco catalyst in ethyl acetate. The hydrogenated product was allowed to react with potassium dithioformate in aqueous alcohol at 0°. A precipitate that formed was removed by filtration and was treated with

(6) This riboside has been prepared by reaction of the silver salt of 5,6-dimethylbenzimidazole with 1-α-bromo-2,3,4-triacetyl-D-ribopyranose and also by ring closure of 2-amino-4,5-dimethyl-N-(2′,3′,4′-triacetyl-D-ribopyranosyl)-aniline with ethyl orthoformate. G. Cooley, B. Ellis, P. Mamalis, V. Petrow and B. Sturgeon, *J. Pharm. Pharmacol.*, **2**, 579 (1950).

(7) For a discussion of the isomerism of these glycosides, see P. Mamalis, V. Petrow and B. Sturgeon, *ibid.*, **2**, 491 (1950).

ethanolic sodium ethoxide. From the reaction mixture, the picrate of 1-β-D-ribopyranosyl-5,6-dimethylbenzimidazole was isolated. The above procedure is an adaptation of a method described[8] for synthesis of purine nucleosides.

1-β-D-Glucopyranosyl-5,6-dimethylbenzimidazole[9] was desired for use in the structural determination of α-ribazole.[2] This glucoside was synthesized by ring closure of 2-amino-4,5-dimethyl-N-(2',3',4',6'-tetraacetyl-D-glucopyranosyl)-aniline with isopropylformimino ether hydrochloride and also by a reaction of the silver salt of 5,6-dimethylbenzimidazole with α-acetobromoglucose.

Experimental[10]

All melting points were determined on a Kofler micro block.

2-Nitro-4,5-dimethyl-N-(5'-trityl-D-ribofuranosyl)-aniline (II).—A solution of 100 g. of 5-trityl-D-ribose[11a] and 42.4 g. of 2-nitro-4,5-dimethylaniline[11b] in 1400 ml. of dry benzene containing 14.5 ml. of glacial acetic acid was refluxed for eight hours in a flask equipped with a continuous water separator. The theoretical amount of water was collected. The solution was concentrated to 200 ml. under reduced pressure. Toluene (200 ml.) was added and the solution was concentrated to dryness to remove the acetic acid. The resulting residue was dissolved in 250 ml. of 40% petroleum ether (b.p. 30–60°) in benzene and chromatographed on acid-washed aluminum oxide[12] (2800 g.) as rapidly as possible to prevent decomposition. A column 43 cm. long and 10 cm. in diameter was used. The chromatography required 1.5 hours. Elution with 40% petroleum ether in benzene gave 2-nitro-4,5-dimethyl-N-tritylaniline, which, after it was recrystallized from ethanol, melted at 235–240°.

Anal. Calcd. for $C_{27}H_{24}N_2O_2$: C, 79.38; H, 5.92; N, 6.85. Found: C, 78.77; H, 5.73; N, 6.87.

The eluant was changed to benzene and 2-nitro-4,5-dimethylaniline was obtained. Pressure was applied to speed the elution. The 2-nitro-4,5-dimethyl-N-(5'-trityl-D-ribofuranosyl)-aniline (II) was then obtained with 5% ethanol in benzene. Concentration of this fraction to constant weight *in vacuo* yielded 103 to 117 g. of a fluffy brittle material. A sample was rechromatographed for an analytical sample, $[\alpha]^{23}D$ +24° (*c* 2 in pyridine).

Anal. Calcd. for $C_{32}H_{32}N_2O_6$: C, 71.09; H, 5.96; N, 5.18. Found: C, 71.20; H, 5.93; N, 5.23.

1-α-D-Ribofuranosyl-5,6-dimethylbenzimidazole Picrate (α-Ribazole Picrate).—2-Nitro-4,5-dimethyl-N-(5'-trityl-D-ribofuranosyl)-aniline (113 g.) was hydrogenated in 3 l. of methanol over 17 g. of palladium–Darco catalyst (5% palladium). After about 75% of the theoretical amount of hydrogen had been absorbed, the reduction became very slow. At this point (after about three hours) the reduction was stopped and the catalyst was filtered. Isopropylformimino ether hydrochloride[13] (65 g.) was added immediately and the resulting red solution was refluxed for two hours. For best results, it was found necessary to carry out the chromatography, the reduction, and the reaction with the formimino ether hydrochloride without interruption. The solution was concentrated to dryness *in vacuo* and the residue was extracted with chloroform (750 ml.). The chloroform extract was concentrated to dryness under reduced pressure and the residual oil was heated for one hour with 150 ml. of ethanol, 150 ml. of water and 30 ml. of hydrochloric acid (sp. gr. 1.19). Triphenylcarbinol separated during this treatment. The mixture was concentrated to dryness *in vacuo* to remove most of the hydrochloric acid.

The dark colored residue was partitioned between water (750 ml.) and chloroform (750 ml.). The aqueous solution was washed with chloroform, 250 ml. of chloroform was added, and the mixture was adjusted to *p*H 10 with 30% sodium hydroxide with shaking. A dark colored oil separated at the interface. The oil was dissolved in dilute hydrochloric acid, chloroform was added, and the mixture was made alkaline as before with sodium hydroxide. The aqueous layers were combined and extracted with three portions of chloroform (250 ml. each) to remove 5,6-dimethylbenzimidazole. The aqueous solution was filtered and acidified to *p*H 2 with hydrochloric acid. Aqueous picric acid was added until further addition gave no more precipitate. The mixture was warmed on the steam-bath and then left at room temperature overnight. The picrate was collected and recrystallized from ethanol giving 8.40 g. of material melting at 216–217°. The aqueous and alcoholic filtrates were concentrated to obtain second crops. These were combined and recrystallized from ethanol giving an additional 4.90 g. of material melting at 210–212°. Two recrystallizations of the first crop from ethanol gave α-ribazole picrate melting at 218–220°, $[\alpha]^{23}D$ +9° (*c* 4 in pyridine). The compound consumed 1 mole of periodate per mole.

Anal. Calcd. for $C_{20}H_{21}N_5O_{11}$: C, 47.34; H, 4.17; N, 13.80. Found: C, 47.55; H, 4.28; N, 13.74.

1-α-D-Ribofuranosyl-5,6-dimethylbenzimidazole (α-Ribazole) (I).—α-Ribazole picrate (7.4 g., m.p. 219–220°) was suspended in 500 ml. of water containing 15 ml. of 2.5 *N* hydrochloric acid and continuous extraction with chloroform was carried out until a colorless aqueous solution was obtained. The solution was concentrated to 100 ml. under reduced pressure and neutralized with sodium hydroxide. The crystals that separated on cooling were recrystallized from 50 ml. of water to give 3.58 g. of α-ribazole, m.p. 198–199°, $[\alpha]^{23}D$ +14° (*c* 0.9 in pyridine).

Anal. Calcd. for $C_{14}H_{18}N_2O_4$: C, 60.42; H, 6.52; N, 10.07. Found: C, 60.43; H, 6.39; N, 10.10.

When α-ribazole picrate of lower melting point (*i.e.*, 214–216°) was used for the preparation of α-ribazole, material with a lower melting point (185–194°) was obtained. It was difficult to obtain pure α-ribazole from this material by recrystallization. Purification was achieved by preparation of the 2',3'-isopropylidene derivative and subsequent acid hydrolysis to give pure α-ribazole.

1-α-D-(2',3'-Isopropylideneribofuranosyl)-5,6-dimethylbenzimidazole (VIIIa).—Three and one-half grams of α-ribazole melting at 191–196° was suspended in 200 ml. of acetone. The suspension was cooled in an ice-bath and 8.0 ml. of sulfuric acid (sp. gr. 1.84) was added slowly with swirling. This was left at room temperature for one hour The resulting solution was cooled and added slowly to a stirred cold solution of 32 g. of sodium carbonate in 100 ml. of water. The mixture was concentrated to one-half volume under reduced pressure to remove the acetone and the aqueous suspension was extracted with chloroform. The chloroform solution was washed with water, dried over sodium sulfate, filtered and evaporated to dryness under reduced pressure. Crystallization of the residue from acetone gave 2.59 g. of 1-α-D-(2'-3'-isopropylideneribofuranosyl)-5,6-dimethylbenzimidazole (VIIIa) melting at 181–181.5°, $[\alpha]^{23}D$ −76° (*c* 1.9 in chloroform).

Anal. Calcd. for $C_{17}H_{22}N_2O_4$: C, 64.12; H, 6.97; N, 8.80. Found: C, 64.29; H, 7.03; N, 8.84.

Treatment of 1 g. of 1-α-D-(2',3'-isopropylideneribofuranosyl)-5,6-dimethylbenzimidazole with 10 ml. of 0.4 *N* hydrochloric acid for 15 minutes at 100° followed by neutralization with sodium hydroxide and cooling gave 0.8 g. of α-ribazole melting at 198–200°.

2-Nitro-4,5-dimethyl-N-(5'-trityl-2',3'-diacetyl-D-ribofuranosyl)-aniline (VII).—To a solution of 98 g. of 2-nitro-4,5-dimethyl-N-(5'-trityl-D-ribofuranosyl)-aniline in 250 ml. of pyridine at 0° was added 150 ml. of acetic anhydride. After the solution had remained at 0° for 15 hours, 200 ml. of ethanol was added; a cooling bath was used to prevent the

(8) J. Baddiley, B. Lythgoe and A. R. Todd, *J. Chem. Soc.*, 318 (1944).

(9) Subseqtent to this work syntheses of 1-β-D-glycopyranosyl-5,6-dimethylbenzimidazole have been reported: (a) J. Davoll and G. B. Brown, THIS JOURNAL, **73**, 5781 (1951); (b) P. Mamalis, V. Petrow and B. Sturgeon, *J. Pharm. Pharmacol.*, **2**, 503 (1950); (c) J. G. Buchanan, A. W. Johnson, J. A. Mills and A. R. Todd, *J. Chem. Soc.*, 2845 (1950); (d) F. Weygand, A. Wacker and F. Wirth, *Z. Naturforsch.*, **66**, 25 (1951).

(10) We are indebted to Mr. Richard Boos and his associates for the microanalyses.

(11) (a) H. Bredereck, M. Köthnig and E. Berger, *Ber.*, **73**, 956 (1940); (b) E. Noelting, A. Braun and E. Thesmar, *ibid.*, **34**, 2242 (1901).

(12) Clark, Johnson and Robinson, "The Chemistry of Penicillin," Princeton University Press, Princeton, New Jersey, 1949, p. 84.

(13) J. W. Cornforth, and R. H. Cornforth, *J. Chem. Soc.*, 96 (1947).

temperature from rising above 30°. The solution was concentrated *in vacuo;* the residue was dissolved in a small volume of benzene–petroleum ether (1–1) and chromatographed on 2.5 kg. of acid-washed aluminum oxide. Elution with ethanol–petroleum ether (1–1) yielded 87 g. of 2-nitro-4,5-dimethyl-N-(5′-trityl-2′,3′-diacetyl-D-ribofuranosyl)-aniline (VII) as a yellow residue $[\alpha]^{20}D$ +60° (*c* 2.77 in pyridine).

Anal. Calcd. for $C_{36}H_{36}N_2O_8$: C, 69.21; H, 5.81; N, 4.48. Found: C, 68.92; H, 5.64; N, 4.90.

Material from various preparations did not give identical physical constants; for example, from another preparation an amorphous product showed a rotation of $[\alpha]^{20}D$ +68° (*c* 2.7 in pyridine). These preparations were, however, satisfactory for a synthesis of β-ribazole.

1-β-D-Ribofuranosyl-5,6-dimethylbenzimidazole (β-Ribazole).—A solution of 32 g. (0.05 mole) of 2-nitro-4,5-dimethyl-N-(2′,3′-diacetyl-5′-trityl-D-ribofuranosyl)-aniline was hydrogenated over 4 g. of palladium–Darco catalyst (containing 5% palladium) in 400 ml. of benzene; 0.15 mole of hydrogen was absorbed during a 24-hour period. The catalyst was removed by filtration and the filtrate was freeze-dried. A 10.9-g. sample of the reduction product, presumably 2-amino-4,5-dimethyl-N-(2′,3′-diacetyl-5′-trityl-D-ribofuranosyl)-aniline, and 28 g. of ethylformimino ether hydrochloride was added to 500 ml. of benzene. The mixture was stirred at room temperature for 23 hours, filtered, and the filtrate was concentrated *in vacuo*. The residue was dissolved in 100 ml. of aqueous hydrochloric acid to give a solution of *p*H 1–2 and the solution was extracted with chloroform. The aqueous layer was adjusted to *p*H 8–9 with sodium hydroxide and was extracted with chloroform. The white crystalline precipitate that separated during the extraction was collected and washed with water. From the aqueous filtrate a second crop was obtained to give a total of 1.4 g. of crude β-ribazole (1-β-D-ribofuranosyl-5,6-dimethylbenzimidazole), m.p. 160–170°. This material was recrystallized from water to give 400 mg. of β-ribazole, m.p. 190–192°, $[\alpha]^{28}D$ −44° (*c* 3 in pyridine).

Anal. Calcd. for $C_{14}H_{18}N_2O_4$: C, 60.42; H, 6.52; N, 10.07. Found: C, 60.31; H, 6.45; N, 10.28.

1-β-D-(2′,3′-Isopropylideneribofuranosyl)-5,6-dimethylbenzimidazole (VIIIb).—A suspension of impure β-ribazole (1.66 g.), m.p. 182–190°, in 100 ml. of acetone was cooled in an ice-bath and 4 ml. of sulfuric acid (sp. gr. 1.84) was added. The mixture was stirred until the solid had dissolved and was then kept at room temperature for five hours. The solution was cooled and added slowly to a cold stirred solution of 16 g. of sodium carbonate in 100 ml. of water. The acetone was removed *in vacuo* and the resulting aqueous suspension was extracted with chloroform. The chloroform extract was washed with water, dried over sodium sulfate, filtered and evaporated under reduced pressure leaving a solid which was recrystallized by dissolving it in 50 ml. of refluxing acetone, concentrating to 15 ml. by distillation, and cooling. 1-β-D-(2′,3′-Isopropylideneribofuranosyl)-5,6-dimethylbenzimidazole (VIIIb) (1.09 g.) melting at 188–191° was obtained. Recrystallization of the product from acetone raised the melting point to 191–192°, $[\alpha]^{23}D$ −28° (*c*, 1.8 in chloroform).

Anal. Calcd. for $C_{17}H_{22}N_2O_4$: C, 64.12; H, 6.97; N, 8.80. Found: C, 64.02; H, 6.70; N, 9.10.

Conversion of 1-β-D-(2′,3′-Isopropylideneribofuranosyl)-5,6-dimethylbenzimidazole to β-Ribazole Picrate.—1-β-D-(2′,3′-Isopropylideneribofuranosyl)-5,6-dimethylbenzimidazole (35 mg.) was suspended in 1.5 ml. of 1 *N* hydrochloric acid and the mixture was heated on the steam-bath for one-half hour. The solution was neutralized with aqueous sodium hydroxide and cooled. The precipitate was collected, washed with water and dried to give 20 mg. of β-ribazole, m.p. 190–192°. The picrate was prepared by suspending the material in 1 ml. of water, adding 2 ml. of a saturated aqueous solution of picric acid, warming and allowing the solution to cool. The solid that separated was collected and washed with water to give 24 mg. of β-ribazole picrate, m.p. 173–175°. Recrystallization of the product from water raised the melting point to 175–177°, $[\alpha]^{23}D$ −24° ± 2° (*c* 2 in pyridine). The compound consumed 1.1 mole of periodate per mole, constant at 1.5 hours.

Anal. Calcd. for $C_{20}H_{21}N_5O_{11}$: C, 47.34; H, 4.17; N, 13.80. Found: C, 47.58; H, 4.00; N, 13.92.

1-α-D-Ribopyranosyl-5,6-dimethylbenzimidazole Picrate.—A solution of 1.4 g. of 2-nitro-4,5-dimethyl-N-(2′,3′,4′-triacetyl-D-ribopyranosyl)-aniline[3] in 50 ml. of ethyl acetate was hydrogenated over 1 g. of palladium–Darco catalyst (5% palladium) until absorption of hydrogen ceased. The catalyst was removed by filtration and the filtrate was concentrated *in vacuo* to an oil. The oil was dissolved in 70 ml. of benzene, 250 mg. of ethylformimino ether hydrochloride was added, and the mixture was stirred at 25° for 16 hours.

The mixture was filtered, and the filtrate was concentrated *in vacuo* to an oil which was heated at 80° for two hours in 50 ml. of 5% hydrochloric acid containing sufficient methanol to dissolve the oil. The solution was concentrated *in vacuo* and the residue was dissolved in water. The solution was extracted with chloroform, adjusted to *p*H 2 with 2.5 *N* hydrochloric acid, and filtered to give a clear, colorless solution. An aqueous solution of picric acid was added until no more precipitate formed. The yellow crystalline precipitate was collected on a filter and recrystallized from methanol–water to give 50 mg. of 1-α-D-ribopyranosyl-5,6-dimethylbenzimidazole picrate, m.p. 185–188° (wet at 161°); $[\alpha]^{25}D$ −4.4° ± 4.4° (*sic*) (*c* 0.23 in pyridine); two moles of periodate was consumed per mole of compound.

Anal. Calcd. for $C_{20}H_{21}N_5O_{11}$: C, 47.34; H, 4.17; N, 13.80. Found: C, 46.88; H, 3.81; N, 13.75.

1-β-D-Ribopyranosyl-5,6-dimethylbenzimidazole. A. Formiminoether Method.—2-Nitro-4,5-dimethyl-N-(D-ribopyranosyl)-aniline (14.6 g.) in 300 ml. of methanol was hydrogenated over 3 g. of a palladium–Darco catalyst (5% palladium). The reduction was complete in 1.75 hours. After removal of the catalyst, 15 g. of isopropylformimino ether hydrochloride was added and the solution was refluxed for two hours. The method used for the preparation of the picrate of α-ribazole was followed, and there was obtained 5.5 g. of a picrate melting at 205–207° after one recrystallization from methanol. A second recrystallization from ethanol gave 4.4 g. of material melting at 207–208°.

The above picrate was suspended in 100 ml. of water containing 6 ml. of 2.5 *N* hydrochloric acid and continuous extraction with chloroform was carried out until the aqueous solution was colorless. Aqueous sodium hydroxide (6 ml. of 2.5 *N*) was added and the solution was cooled in an ice-bath. The crystalline product that separated was collected and recrystallized from water giving 0.22 g. of material, m.p. 210–238°. Two more recrystallizations from water gave 0.12 g. of 1-β-D-ribopyranosyl-5,6-dimethylbenzimidazole melting at 250–253°, $[\alpha]^{27}D$ −75° (*c* 0.4 in pyridine). The compound consumed two molecular equivalents of periodate (constant after two hours).

Anal. Calcd. for $C_{14}H_{18}N_2O_4$: C, 60.42; H, 6.52; N, 10.07. Found: C, 60.75; H, 6.88; N, 9.88.

The picrate of 1-β-D-ribopyranosyl-5,6-dimethylbenzimidazole, m.p. 215–217°, was prepared in the usual manner. Recrystallization from water raised the melting point to 218–200°, $[\alpha]^{27}D$ −29° (*c* 0.8 in pyridine).

Anal. Calcd. for $C_{20}H_{21}N_5O_{11}$: C, 47.34; H, 4.17; N, 13.80. Found: C, 47.71; H, 4.34; N, 13.74.

B. Dithioformate Method.—2-Nitro-4,5-dimethyl-N-triacetylribopyranosylaniline (X) (2.0 g.) was reduced in ethyl acetate using a palladium–Darco catalyst (5% palladium). After removal of the catalyst, the filtrate was concentrated *in vacuo* and the residue was dissolved in 25 ml. of ethanol. The ice-cold solution was added to an ice-cold aqueous solution of potassium dithioformate (4.0 g.) in 25 ml. of water. The mixture was kept in an ice-bath for 20 hours after which the material that had separated was collected and dried under reduced pressure.

The above material was added to 25 ml. of absolute ethanol containing the sodium ethoxide obtained from 0.03 g. of sodium. The solution was refluxed for 2.75 hours. Hydrogen sulfide was evolved and the solution became dark colored. After being kept at 0° overnight, the solution was concentrated under reduced pressure. The resulting oil was partitioned between water and chloroform. The aqueous layer was washed with three portions of chloroform and was concentrated under reduced pressure to an oil. Trituration with ethanol (10 ml.) gave a solid which was removed. The filtrate was concentrated under reduced pressure, the resulting oil was dissolved in water and a saturated aqueous solution of picric acid (25 ml.) was added. The precipitate that separated was collected and dried under re-

duced pressure to give 0.14 g. of material, m.p. 160–190°. This was triturated with 7 ml. of hot ethanol and filtered. The insoluble material melted at 200–207°. The filtrate, when cooled, yielded 0.05 g. of crystals melting at 205–207°. Recrystallization of the latter from ethanol gave the picrate of 1-β-D-ribopyranosyl-5,6-dimethylbenzimidazole as small needle-like prisms melting at 218–221°. When mixed with the picrate of the pyranoside prepared by method A, the melting point was not depressed.

2-Thioformamido-4,5-dimethyl-N-triacetylribopyranosylaniline.—In one thioformylation crystalline 2-thioformamido-4,5-dimethyl-N-triacetylribopyranosylaniline was obtained. The diamine obtained from the reduction of 2.25 g. of 2-nitro-4,5-dimethyl-N-triacetylribopyranosylaniline was dissolved in 25 ml. of ethanol. The solution was cooled in an ice-bath and was added to the cold aqueous solution of potassium dithioformate prepared by dissolving 8.0 g. of potassium dithioformate in 25 ml. of water. One milliliter of 2.5 *N* hydrochloric acid was added and the solution was filtered in a nitrogen atmosphere. An additional 10 ml. of ethanol was added to give a clear solution which was kept at 0° for two days. The yellow crystalline precipitate that had separated was collected, m.p. 67–73°, wt. 0.90 g. The material was recrystallized by dissolving it in warm ethanol and adding an equal volume of water; the solution slowly deposited 0.50 g. of 2-thioformamido-4,5-dimethyl-N-triacetylribopyranosylaniline, m.p. 95–98°.

Anal. Calcd. for $C_{20}H_{26}N_2O_7S$: C, 54.78; H, 5.98; N, 6.39. Found: C, 54.51; H, 6.07; N, 6.61.

2-Nitro-4,5-dimethyl-N-(2′,3′,4′,6′-tetraacetyl-D-glucopyranosyl)-aniline.[3,9b]—To a solution of 60 g. of 2-nitro-4,5-dimethyl-N-(D-glucopyranosyl)-aniline in 300 ml. of pyridine at 5°, 150 ml. of acetic anhydride was added gradually and the solution was left at 5° for five days. The excess acetic anhydride was decomposed by the addition of 250 ml. of ethanol to the cold solution. The solution was cooled in an ice-bath for three hours, concentrated *in vacuo* to a volume of 200 ml., and water was added until crystallization of a yellow product occurred. The mixture was cooled, the precipitate was collected on a filter, washed twice with methanol, and dried *in vacuo* to give 80 g. of 2-nitro-4,5-dimethyl-N-(2′,3′,4′,6′-tetraacetyl-D-glucopyranosyl)-aniline, m.p. 139–147°. A 3.3-g sample was recrystallized from ethanol–benzene to give 3.2 g. of product, m.p. 132–135°, $[\alpha]^{23}D$ −64.3° (*c* 2.1 in pyridine).

Anal. Calcd. for $C_{22}H_{28}N_2O_{11}$: C, 53.22; H, 5.68; N, 5.64. Found: C, 53.59; H, 5.84; N, 6.02.

1-β-Glucopyranosyl-5,6-dimethylbenzimidazole Picrate.[9]

A.—A solution of 10 g. (0.02 mole) of 2-nitro-4,5-dimethyl-N-(2′,3′,4′,6′-tetraacetyl-D-glucopyranosyl)-aniline in 200 ml. of benzene was hydrogenated over 5 g. of a palladium–Darco catalyst (5% palladium) at 25° for four hours at about 40 p.s.i.; 0.06 mole of hydrogen was absorbed. The catalyst was removed by filtration and 9 g. of isopropylformimino ether hydrochloride was added to the filtrate. The mixture was shaken for 17 hours at 25°. A white solid was removed by filtration and was washed with benzene. The filtrate was concentrated *in vacuo* to an oil which was refluxed in a solution of 20 ml. of ethanol, 40 ml. of water, and 6 ml. of concentrated hydrochloric acid for 20 hours. The mixture was concentrated to a small volume and was extracted with chloroform; the aqueous layer was adjusted to *p*H 10 with 2.5 *N* sodium hydroxide and was extracted with chloroform. The aqueous layer was adjusted to *p*H 1–2 with concentrated hydrochloric acid and a saturated aqueous solution of picric acid was added. A crystalline picrate separated and was recrystallized from a mixture of ethanol, methanol, water and dioxane to give 2.6 g. of 1-β-D-glucopyranosyl-5,6-dimethylbenzimidazole picrate, m.p. 235–236°, $[\alpha]^{23}D$ −16° (*c* 1.25 in pyridine). (From the filtrate 2.3 g. of the picrate, m.p. 235–236°, was obtained.)

Anal. Calcd. for $C_{21}H_{23}N_5O_{12}$: C, 46.93; H, 4.31. Found: C, 46.69; H, 4.16.

B.—The silver salt of 5,6-dimethylbenzimidazole was prepared by the procedure described[14] for preparation of the silver salt of benzimidazole. A mixture of 1.5 g. of the silver salt of 5,6-dimethylbenzimidazole and 2.4 g. of α-acetobromoglucose in 100 ml. of xylene was refluxed for two hours. The mixture was filtered hot, the silver bromide was washed with xylene and the filtrate was evaporated *in vacuo* to a small volume. Addition of petroleum ether to the solution yielded a solid product, presumably 1-(2′,3′,4′,6′-tetraacetyl-β-D-glucopyranosyl)-5,6-dimethylbenzimidazole, which was hydrolyzed by refluxing for two hours under nitrogen with a 5% solution of hydrochloric acid containing 20% ethanol. The solution was cooled, adjusted to *p*H 9 with aqueous sodium hydroxide, extracted three times with chloroform, adjusted to *p*H 1–2 with aqueous hydrochloric acid, and extracted once with chloroform. Addition of picric acid to the aqueous solution yielded a yellow crystalline precipitate, which was recrystallized from a mixture of ethanol and water to give 160 mg. of 1-β-D-glucopyranosyl-5,6-dimethylbenzimidazole picrate, m.p. 227–230° (dec.); $[\alpha]^{23}D$ −16° (*c*, 5 in pyridine). A sample recrystallized for analysis melted at 235–238°, with softening at 233°.

Anal. Calcd. for $C_{21}H_{23}N_5O_{12}$: C, 46.93; H, 4.31; N, 13.03. Found: C, 47.33; H, 4.60; N, 13.24.

2-D-Ribo-5,6-dimethylbenzimidazole (IV).—A solution of 21.5 g. of 2-nitro-4,5-dimethyl-N-(5′-trityl-D-ribofuranosyl)-aniline (II) in 500 ml. of methanol was hydrogenated over 2 g. of a palladium–Darco catalyst (5% palladium). The solution became colorless and the catalyst was removed by filtration. A 375-ml. aliquot of the solution was refluxed with 69 g. of ethylformimino ether hydrochloride for 1.5 hours. To the reaction mixture was added 30 ml. of concentrated hydrochloric acid in 177 ml. of water and the resulting solution was refluxed for two hours. The solution was concentrated to dryness *in vacuo*, the residue was suspended in water, and the insoluble material was removed by filtration. The aqueous solution was adjusted to *p*H 2 and extracted three times with chloroform. Aqueous sodium hydroxide was added and a white precipitate formed. The product was collected on a filter and recrystallized from methanol to give 7 g., m.p. 231–233°. A picrate prepared from this material melted at 210–212°. Mixture of the free base and of the picrate with authentic samples[15] of 2-D-ribo-5,6-dimethylbenzimidazole and 2-D-ribo-5,6-dimethylbenzimidazole picrate melted without depression.

2-Amino-4,5-dimethyl-N-(5′-trityl-2′,3′-diacetyl-D-ribofuranosyl)-aniline.—A solution of 5.0 g. (0.008 mole) of 2-nitro-4,5-dimethyl-N-(5′-trityl-2′,3′-diacetyl-D-ribofuranosyl)-aniline (VII) in 100 ml. of benzene was hydrogenated over 2 g. of a palladium–Darco catalyst (5% palladium) for three hours at 25°; 0.024 mole of hydrogen was absorbed. The catalyst was filtered and the filtrate was "freeze-dried" to give a white powder, $[\alpha]^{24}D$ +10.7° (*c* 2.8 in pyridine).

Anal. Calcd. for $C_{36}H_{38}N_2O_6$: C, 72.71; H, 6.44; N, 4.71. Found: C, 72.75; H, 6.51; N, 4.84.

RAHWAY, NEW JERSEY

(14) E. Bamberger and J. Lorenzen, *Ann.*, **273**, 269 (1893).

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