ingaldehyde and 0.83 g. (0.004 mole) of 2-hydroxy-3-methoxy-5-propylacetophenone, and 6 ml. of an aqueous potassium hydroxide solution (25 g. of pellets, 75 g. of water) added. The mixture was heated in a closed flask for 24 hours at 70°, with frequent shaking at first and occasional shaking during the remaining time. The reaction mixture was dissolved in water, acidified with dilute hydrochloric acid, shaken with petroleum ether to remove the bulk of the unreacted phenone, and the aqueous solution drained from the precipitated orange resin. The resin was dissolved in ether, the ether solution shaken with several portions of 30% sodium bisulfite solution, and the ether solution dried. The ether was evaporated and the residue dissolved by boiling with several portions of petroleum naphtha. The combined naphtha extracts were cooled in a refrigerator and the solvent, containing some residual phenone, was decanted. The resinous residue was crystallized from 50% aqueous ethanol, yielding 0.20 g. of orange crystals sintering at 68° and fusing to a viscous resin at about 73°. The product, apparently a hydrate, was dried at 105° and recrystallized from petroleum naphtha to give orange needles, m.p. sinters 110°, melts 114–116°.

Anal. Calcd. for $C_{21}H_{24}O_5$: C, 67.73; H, 6.50. Found: C, 68.03; H, 6.58.

Acknowledgment.—The author wishes to thank Dr. F. E. Brauns, Institute of Paper Chemistry, for the sample of spruce "native lignin," Dr. K. Freudenberg, Heidelberg University, for the sample of enzymatically polymerized coniferyl alcohol and Mr. A. W. Goos, Cliffs–Dow Chemical Company, for the distillate containing 2,6-dimethoxy-4-propylphenol.

MADISON 5, WISCONSIN RECEIVED OCTOBER 12, 1951

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF MERCK & CO., INC.]

Vitamin B_{12}. XVIII. The Degradation of Vitamin B_{12} to 1-α-D-Ribofuranosyl-5,6-dimethylbenzimidazole

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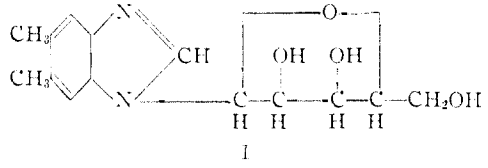
RECEIVED FEBRUARY 7, 1952

Acid hydrolysis of vitamin B_{12} yielded a product which has been identified as 1-α-D-ribofuranosyl-5,6-dimethylbenzimidazole. It was characterized as a 1-pentofuranosyl-5,6-dimethylbenzimidazole by periodate oxidation studies, which led to the isolation of a crystalline α-(5,6-dimethylbenzimidazole-1)-α′-hydroxymethyldiglycolic aldehyde derivative, and which suggested the α-configuration at carbon atom one of the ribose moiety of the glycoside.

The preparations of 1-α-D-ribofuranosyl-5,6-dimethylbenzimidazole (α-ribazole) (I) from vitamin B_{12} and by synthesis have been communicated.[1] Details of the isolation of the degradation product



I

from an acid hydrolysate of vitamin B_{12} and its characterization as a 1-pentofuranosyl-5,6-dimethylbenzimidazole are described herein. The final characterization of the degradation product as α-ribazole resulted from its identification with synthetic α-ribazole.[2] The assignment of the α-configuration to the degradation product followed from a comparison of the optical rotations of synthetic α- and β-ribazoles.[2] A direct correlation of β-ribazole with 1-β-D-glucopyranosyl-5,6-dimethylbenzimidazole is described below.

Hydrolysis of vitamin B_{12} in 6 *N* hydrochloric acid at 150° for 20 hours gave 5,6-dimethylbenzimidazole,[3] an observation which was shortly confirmed by others.[4] When the hydrolysis was carried out at a somewhat lower temperature, 120°, and the hydrolysate treated to yield a basic fraction isolated by continuous chloroform extraction, the absorption spectrum of the crude product suggested the presence of a different substituted benzimidazole. When a solution of the previously obtained 5,6-dimethylbenzimidazole was made alkaline, an absorption maximum appeared at 2470 Å., the magnitude of the absorption of this new peak being less, however, than those of the two principal maxima at 2810 and 2880 Å. With the basic fraction from the hydrolysis done at 120°, the new peak which appeared at 2500 Å. when the solution was made alkaline was higher than the two original absorption peaks. The crude product was only sparingly soluble in ether; and the ether-insoluble material was indicated to contain carbohydrate by the color test involving dehydration to furfural or its derivatives.[5] This same material yielded a crystalline picrate which melted at 212–214°, and hence was not identical with 5,6-dimethylbenzimidazole picrate (m.p. 273–275°). The crystalline picrate also gave a positive carbohydrate test.[5]

Subsequent hydrolyses of vitamin B_{12} done in 6 *N* hydrochloric acid at 120° afforded mixtures of the benzimidazole glycoside picrate and 5,6-dimethylbenzimidazole picrate. However, when the hydrolysis was carried out overnight at a temperature of 100°, splitting of the glycoside was negligible, and the glycoside picrate could be readily isolated in pure form. The picrate was dextrorotatory and had an ultraviolet absorption spectrum in acidic ethanol solution with maxima at 2760 Å. (E_M 10,950), 2850 Å. (E_M 10,600) and 3590 Å. (E_M 13,000). Analyses indicated that the composition of the picrate corresponded to the formula $C_{14}H_{18}N_2O_4 \cdot C_6H_3N_3O_7$. Removal of picric acid by chloroform extraction of a dilute aqueous hydrochloric acid solution of the picrate yielded the amorphous

(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) F. W. Holly, C. H. Shunk, E. W. Peel, J. J. Cahill, J. B. Lavigne and K. Folkers, *ibid.*, in press.

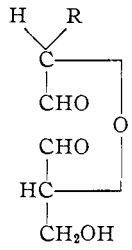
(3) N. G. Brink and K. Folkers, *ibid.*, **72**, 4442 (1950).

(4) E. R. Holliday and V. Petrow, *J. Pharm. Pharmacol.*, **1**, 734 (1949); G. R. Beaven, E. R. Holliday, E. A. Johnson, B. Ellis, P. Mamalis, V. Petrow and B. Sturgeon, *ibid.*, **1**, 957 (1949).

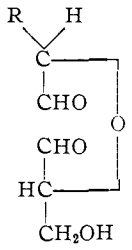
(5) F. Feigl, "Qualitative Analysis by Spot Tests," Third English Edition, Elsevier, New York, N. Y., 1946, p. 410.

hydrochloride. The free base was obtained by dissolving the hydrochloride in water, making the solution weakly alkaline, and extracting continuously with chloroform.

The amorphous free base prepared from the picrate *via* the hydrochloride reacted with approximately 0.7 mole of periodate per mole. The crystalline glycoside picrate consumed 0.92 mole of periodate per mole. This latter oxidation yielded a crystalline picrate of m.p. 180–185° and $[\alpha]^{23}D$ +24 ± 4°. The product was unstable, its melting point decreased upon recrystallization, and satisfactory analytical results were not obtained. From the method of preparation, it was formulated to be an α-(5,6-dimethylbenzimidazole-1)-α′-hydroxymethyldiglycolic aldehyde picrate (II, R = 5,6-dimethylbenzimidazole-1). Similar periodate oxidation of a synthetic model compound, 1-β-D-glucopyranosyl-5,6-dimethylbenzimidazole picrate[2] with two equivalents of periodate led not to the above dialdehyde picrate, but to a presumably anomeric picrate (III) of m.p. 141–144° and specific rotation of $[\alpha]^{23}D$ −20 ± 2°. This product too was unstable, and its melting point decreased upon recrystallization.



II



III

The consumption by the glycoside derived from vitamin B_{12} of one mole of periodate permitted the assignment of a furanoid ring structure to the pentose moiety. The lack of identity of the oxidation products II and III suggested an α-configuration about the glycosidic carbon atom,[6] leading to a structure of the degradation product as a 1-α-pentofuranosyl-5,6-dimethylbenzimidazole.

In order to determine which of the four possible pentoses was combined with 5,6-dimethylbenzimidazole in the glycoside, experiments to hydrolyze the glycosidic linkage and identify the sugar were done. The conditions used in the hydrolytic degradation of vitamin B_{12} to the benzimidazole glycoside demonstrated the stability of the N-glycosidic linkage, which was not noticeably cleaved by treatment with strong mineral acid for several hours at 100°, and which was only partially broken by similar hydrolysis at 120°. Indeed, hydrolytic conditions which sufficed to cleave the glycosidic linkage in the degradation product caused such extensive decomposition of the pentose that extensive degradative experiments to identify the carbohydrate moiety were not undertaken. It is well known that purine nucleosides are readily hydrolyzable by acids to give a pentose and a base, whereas pyrimidine nucleosides cannot be hydrolyzed with dilute acids. Hydrolysis of compounds of the latter class under more drastic conditions gives only the base, the sugar being destroyed.[7] It is evident that the behavior of the glycosidic linkage of the degradation product toward acid hydrolysis is the opposite of what might have been expected from the reported properties of the purine and pyrimidine glycosides and the obvious structural resemblance between the degradation product and the purine nucleosides.

The final identification of the vitamin B_{12} degradation product as 1-α-D-ribofuranosyl-5,6-dimethylbenzimidazole was accomplished by comparing the picrate with a synthetic specimen.[2] The synthetic picrate melted at 212–214° and showed a rotation of $[\alpha]^{23}D$ +9.1 ± 1°, as compared with the corresponding values of 212–214° and $[\alpha]^{23}D$ +9.9 ±1.6° for the compound obtained from vitamin B_{12}. A mixture of the materials melted without depression, and the absorption spectra were identical. The synthetic α-ribazole picrate consumed one mole of periodate and gave a crystalline α-(5,6-dimethylbenzimidazole-1)-α′-hydroxymethyldiglycolic aldehyde picrate which was identical with the corresponding derivative of the picrate of the degradation product on the basis of melting point, mixed melting point and specific rotation.

It is of interest that synthetic β-ribazole picrate on periodate oxidation gave a product which appeared to be identical with the corresponding derivative from 1-β-D-glycopyranosyl-5,6-dimethylbenzimidazole picrate described above. This confirms the assignments of configuration about the glycosidic carbon atoms of α- and β-ribazoles, which were originally made on the basis of the specific rotations.

Experimental Part

Acid Hydrolysis of Vitamin B_{12}.—A solution of vitamin B_{12} in 4 ml. of 6 *N* hydrochloric acid was heated in a sealed tube at 120° for eight hours. The solution was then filtered, concentrated to dryness, and the residue dissolved in 4 ml. of water containing 3 drops of 1 *N* hydrochloric acid. This acidic solution was extracted continuously with chloroform for 36 hours. The aqueous solution was then adjusted to *p*H 10 by the addition of sodium hydroxide solution, and extracted continuously with chloroform for 12 hours. The chloroform extract of the basic solution was evaporated to dryness, leaving a colorless oil which weighed 3.5 mg.

A portion of this oily basic fraction showed in 0.01 *N* ethanolic hydrochloric acid solution an absorption spectrum characterized by maxima at 2775 Å. ($E^{1\%}_{1\,cm.}$ 222) and 2850 Å. ($E^{1\%}_{1\,cm.}$ 222). When the solution was made 0.01 *N* in sodium hydroxide, the spectrum showed maxima at 2500 Å. ($E^{1\%}_{1\,cm.}$ 210), 2825 Å. ($E^{1\%}_{1\,cm.}$ 152) and 2890 Å. ($E^{1\%}_{1\,cm.}$ 154).

The remainder of the basic fraction was leached with 2 ml. of ether, and the ether extract discarded. Part of the ether-insoluble material which remained was heated with oxalic and sulfuric acids and the vapors tested for furfural or a related aldehyde with *o*-dianisidine according to Feigl.[5] The test was strongly positive. Another portion of the ether-insoluble basic fraction upon solution in water and treatment with saturated aqueous picric acid solution gave a crystalline picrate, m.p. 211–214°. After recrystallization from aqueous ethanol, the product melted at 212–214°. The crystalline picrate also gave a positive Feigl carbohydrate test.

Preparation of 1-α-D-Ribofuranosyl-5,6-dimethylbenzimidazole from Vitamin B_{12}.—A solution of 86 mg. of vitamin B_{12} in 5 ml. of 6 *N* hydrochloric acid was heated at 100° for 18 hours. The solution was then evaporated to dryness *in*

(6) *Cf.* B. Lythgoe and A. R. Todd, *J. Chem. Soc.*, 592 (1944).

(7) P. A. Levene and L. W. Bass, "Nucleic Acids," Chemical Catalog Co. (Reinhold Publ. Corp.), New York, N. Y., 1931, p. 144-145.

vacuo and the residue was dissolved in 8 ml. of water and 0.4 ml. of 1 *N* hydrochloric acid. This solution was extracted continuously with chloroform for eight hours. The aqueous solution was then made alkaline (*p*H 9–10) with sodium hydroxide solution and extracted with a fresh portion of chloroform for 16 hours. The chloroform extract of the alkaline aqueous solution was filtered and evaporated to dryness. The resulting 10.2-mg. residue was extracted with four 3-ml. portions of boiling ether and the combined ethereal extracts were concentrated to a volume of 0.5 ml. The white, amorphous precipitate which had formed during the concentration was separated from the supernatant solution, dried and dissolved in 0.5 ml. of water. The addition of 1 ml. of a saturated aqueous solution of picric acid resulted in the immediate formation of a crystalline precipitate. After two hours, the mixture was centrifuged, and the precipitate was washed twice with water and dried *in vacuo*. The yield of 1-α-D-ribofuranosyl-5,6-dimethylbenzimidazole picrate was 7.0 mg. It melted at 212–214°, and was recrystallized from aqueous ethanol to give a product of m.p. 213–214°.

Anal. Calcd. for $C_{14}H_{18}N_2O_4 \cdot C_6H_3N_3O_7$: C, 47.34; H, 4.17; N, 13.80; picric acid, 45.3. Found: C, 47.52; H, 3.92; N, 14.07; picric acid, 45.9 (spectrophotometric).

The picrate had a specific rotation $[\alpha]^{23}D$ +9.9 ± 1.6° (*c* 2.4 in pyridine). Its ultraviolet absorption spectrum in acidic ethanol solution showed maxima at 2760 Å. (E_M 10,950), 2850 Å. (E_M 10,600) and 3590 Å. (E_M 13,000).

A solution of 6 mg. of 1-α-D-ribofuranosyl-5,6-dimethylbenzimidazole picrate in 4.5 ml. of water containing 0.2 ml. of 1 *N* hydrochloric acid was extracted for two hours with chloroform. The colorless aqueous solution was then lyophilized, yielding 3.5 mg. of oily α-ribazole hydrochloride. This was in turn dissolved in water (3 ml.), and the solution brought to *p*H 8 with sodium hydroxide, and extracted continuously for six hours with chloroform. Evaporation of the chloroform extract gave 2.8 mg. of white, amorphous α-ribazole free base.

Periodate Oxidations.—One and two-tenths milligrams of amorphous α-ribazole (0.0043 millimole) was dissolved in 2.00 ml. of water and 0.50 ml. of 0.0207 *M* sodium periodate solution was added. After 45 minutes, a 1.00-ml. aliquot portion was withdrawn from the reaction solution and analyzed for unreacted periodate by addition of sodium bicarbonate and potassium iodide and titration with 0.0100 *N* arsenite solution. A volume of 0.600 ml. of reagent was required, corresponding to a consumption of periodate of 0.67 mole per mole of α-ribazole. Subsequent titrations after 80 and 170 minutes gave values for periodate consumption of 0.65 and 0.67 mole per mole, respectively. No change in the *p*H of the reaction solution could be detected during the oxidation.

A 0.771-mg. (0.00152 millimole) sample of α-ribazole picrate was dissolved in 1.8 ml. of pure methanol, and 0.20 ml. of 0.0207 *M* aqueous sodium periodate solution was added. After the solution had stood at room temperature for one hour, bicarbonate and iodide were added; the mixture then consumed 0.550 ml. of 0.0100 *N* arsenite solution. This corresponds to a periodate consumption of 0.00139 millimole, or 0.92 mole per mole of α-ribazole picrate.

In another experiment, 5.1 mg. (0.010 millimole) of α-ribazole picrate was dissolved in 4 ml. of methanol and the solution was treated with 0.27 ml. of 0.0447 *M* sodium periodate solution (0.012 millimole). After having stood overnight, the solution was diluted with 2 ml. of water and concentrated *in vacuo* to a volume of about 2 ml. Another 2 ml. of water was added, and the solution concentrated to a volume of 1 ml. and allowed to stand at 5° overnight. The yellow crystals which had separated were collected by centrifugation, washed twice with water, and dried *in vacuo*. The product weighed 4.2 mg. and melted at 180–185° (dec.). One recrystallization from aqueous ethanol failed to change the melting point. The material showed a specific rotation of $[\alpha]^{23}D$ +24 ± 4° (*c* 0.58 in pyridine).

Periodate Oxidation of Synthetic α-Ribazole Picrate.—A 31-mg. quantity of synthetic α-ribazole picrate[2] was oxidized with periodate exactly as described above for the vitamin B_{12} degradation product. Recrystallization of the resulting compound from aqueous ethanol gave a picrate which melted at 183–186° and had a rotation of $[\alpha]^{23}D$ +20 ± 4° (*c* 5.5 in pyridine). Upon admixture with the periodate oxidation product (m.p. 180–185°) of "natural" α-ribazole picrate, the material melted at 183–185.5°.

Anal. Calcd. for $C_{20}H_{19}N_5O_{11}$: N, 13.86. Found: N, 13.08.

Periodate Oxidation of 1-β-D-Glucopyranosyl-5,6-dimethyl-benzimidazole Picrate.—A solution of 9.6 mg. of 1-β-D-glucopyranosyl-5,6-dimethylbenzimidazole picrate[2] in 5 ml. of methanol was treated with 0.77 ml. of 0.0533 *M* sodium periodate solution (2.3 moles per mole of picrate). The reaction mixture stood at room temperature overnight and was then worked up by the addition of 1 ml. of water and concentration *in vacuo* to a volume of 1 ml. The crystalline product which separated was collected, washed twice with water and dried *in vacuo*. It weighed 6.4 mg., melted at 141–144° and had a specific rotation of $[\alpha]^{23}D$ −20 ± 2° (*c* 1.0 in pyridine). Recrystallization of the picrate from aqueous ethanol lowered the melting point to 136–141°.

Periodate Oxidation of 1-β-D-Ribofuranosyl-5,6-dimethylbenzimidazole Picrate.—Oxidation of 2.4 mg. of synthetic β-ribazole picrate[2] in the manner described above gave a product in the form of yellow platelets. It melted at 132–136°, and no depression of melting point was observed with a mixture of this product and the oxidation product of 1-β-D-glycopyranosyl-5,6-dimethylbenzimidazole picrate described above.

Acknowledgment.—The authors wish to thank Mr. R. N. Boos and his associates for the microanalyses reported here. They are indebted to Dr. N. R. Trenner and co-workers for some of the determinations of specific rotation and ultraviolet absorption.

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