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

Studies on Carcinolytic Compounds. V. 6,7-Dimethyl-9-[1′-(5-desoxy-D-ribityl)]-isoalloxazine

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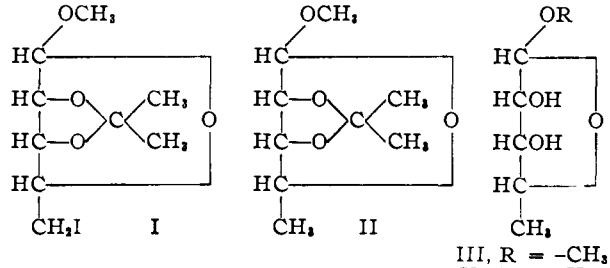
RECEIVED OCTOBER 11, 1954

6,7-Dimethyl-9-[1′-(5′-desoxy-D-ribityl)]-isoalloxazine has been synthesized as a new analog of riboflavin for biological and nutritional studies. Condensation of 5-desoxy-D-ribose with 3,4-xylidine, followed by reduction, gave N-(5′-desoxy-D-ribityl)-3,4-dimethylaniline. Reaction of this compound with diazotized aniline followed by reaction of the resulting N-(5′-desoxy-D-ribityl)-2-phenylazo-4,5-dimethylaniline with barbituric acid yielded 6,7-dimethyl-9-[1′-(5′-desoxy-D-ribityl)]-isoalloxazine.

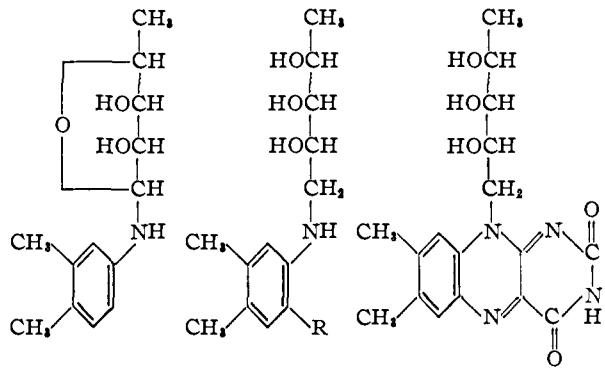
Riboflavin is known to be a constituent of two coenzymes. Riboflavin mononucleotide is the 5′-phosphoric acid ester of riboflavin. The other coenzyme, riboflavin adenine dinucleotide, is a molecule in which adenylic acid and riboflavin-5′-phosphate are united as a pyrophosphate. Since both coenzymes contain the riboflavin moiety as the 5′-phosphoric acid ester, it was of interest to prepare and to test biologically a riboflavin analog in which the 5′-position of the ribityl group is blocked. Consequently, a synthesis of 6,7-dimethyl-9-[1′-(5′-desoxy-D-ribityl)]-isoalloxazine (5′-desoxyriboflavin) was devised.

In the synthesis used for the preparation of 5′-desoxyriboflavin, 5-desoxy-D-ribose was prepared and condensed with 3,4-xylidine. This compound was then hydrogenated and converted to the desired isoalloxazine by reactions similar to those used for a synthesis of riboflavin.

The method used for the preparation of 5-desoxy-D-ribose is a modification of the procedure[1] used for the preparation of 5-desoxy-D-xylose. Methyl 2,3-isopropylidene-5-iodo-D-ribofuranoside (I) was prepared by the method of Levene and Stiller.[2] Hydrogenation of this compound over a platinum catalyst in an alkaline medium gave methyl 2,3-isopropylidene-5-desoxy-D-ribofuranoside (II). It was found that the isopropylidene group could be hydrolyzed selectively by treatment with 0.12 *N* sulfuric acid in refluxing methanol. The resulting methyl 5-desoxy-D-ribofuranoside (III) was treated with aqueous 0.4 *N* sulfuric acid at 90° to yield 5-desoxy-D-ribose (IV). In the preparation of 5-desoxy-D-ribose, it is not necessary to isolate methyl 2,3-isopropylidene-5-desoxy-D-ribofuranoside nor methyl 5-desoxy-D-ribofuranoside. Complete hydrolysis of the product II obtained by hydrogenation of the 5-iodo compound I gave 5-desoxy-D-ribose in 88% yield.



I, II; III, R = $-CH_3$; IV, R = $-H$



V; VI, R = —H; VII, R = —N=N—C_6H_5; VIII

The melting point and the specific rotation of the phenylosazone of 5-desoxy-D-ribose prepared by the above method were found to be in good agreement with the values reported by Micheel[3] for this derivative of 5-desoxy-D-ribose which he obtained by ozonization and hydrolysis of diacetyldigitoxoseen.

The condensation of 5-desoxy-D-ribose with 3,4-xylidene was carried out in ethanol at room temperature giving N-(5′-desoxy-D-ribosyl)-3,4-dimethylaniline (V). Hydrogenation of this compound over Adams platinum oxide catalyst yielded N-(5′-desoxy-D-ribityl)-3,4-dimethylaniline (VI).

The reactions used for the conversion of N-(5′-desoxy-D-ribityl)-3,4-dimethylaniline to 5′-desoxyriboflavin (VIII) are similar to those which were devised for a synthesis of riboflavin.[4] N-(5′-Desoxy-D-ribityl)-3,4-dimethylaniline was coupled with diazotized aniline, and the resulting N-(5′-desoxy-D-ribityl)-2-phenylazo-4,5-dimethylaniline (VII) reacted with barbituric acid giving 6,7-dimethyl-9-[1′-(5′-desoxy-D-ribityl)]-isoalloxazine (VIII).

5′-Desoxyriboflavin has been studied by Dr. David Hendlin and Mrs. Elaine Dillberger of the Microbiology Department for riboflavin activity and for riboflavin inhibition using *Lactobacillus casei* as the test organism. We acknowledge their report that this compound showed no riboflavin activity. It was active, however, as an inhibitor with an inhibition index of 150 and the inhibition appeared to be competitive.

We thank Dr. Gladys A. Emerson of the Merck Institute for Therapeutic Research for testing 5′-

(1) P. A. Levene and J. Compton, *J. Biol. Chem.*, 111, 325 (1935).
(2) P. A. Levene and E T. Stiller, *ibid.*, 106, 421 (1934).
(3) F. Micheel, *Ber.*, 63, 347 (1930).
(4) M. Tishler, K. Pfister, R. D. Babson, K. Ladenburg and A. J Flemming, THIS JOURNAL, 69, 1487 (1947).

desoxyriboflavin for possible enhancement of the rate of regression of lymphosarcoma (6C3H-ED) transplants in C_3H mice maintained on a riboflavin deficient diet. In two out of three tests, 5′-desoxyriboflavin appeared to show a low order of activity. The regression of these transplants on a riboflavin deficient diet seems to be established.[5] It was not feasible to further test 5′-desoxyriboflavin in our laboratories. We are indebted to Dr. K. Sugiura and Dr. C. Chester Stock of the Sloan-Kettering Institute for Cancer Research for testing it against the 6C3H-ED lymphosarcoma. The first test was negative, but the animals were on a normal diet. Another test with 5′-desoxyriboflavin was negative in the case of a riboflavin deficient diet which was supplemented for seven days with 0.2 mg./kg. of riboflavin. Another test involving a riboflavin deficient diet was negative.

Although it is possible that 5′-desoxyriboflavin, a microbial inhibitor, has weak activity under limited conditions for lymphosarcoma regression, it is evident that this compound is not significantly active on either a normal or riboflavin deficient diet.

Experimental[6,7]

Methyl 2,3-Isopropylidene-D-ribofuranoside.—Methyl 2,-3-isopropylidene-D-ribofuranoside prepared by the method of Levene and Stiller[8] was used without purification by distillation.

Methyl 2,3-Isopropylidene-5-*p*-toluenesulfonyl-D-ribofuranoside.—The method of Levene and Stiller[2] was used with modifications. To a solution of 204 g. of crude methyl 2,3-isopropylidene-D-ribofuranoside in 250 ml. of pyridine was added 286 g. of *p*-toluenesulfonyl chloride. After cooling in an ice-bath to moderate the reaction, the solution was left at room temperature overnight. The mixture was heated on the steam-bath for one hour and then cooled. Water (35 ml.) was added, and after one hour the mixture was partitioned between water and chloroform. The chloroform solution was washed with successive portions of cold 0.1 *N* sulfuric acid, water, dilute sodium hydroxide and water. After drying over sodium sulfate the chloroform solution was concentrated *in vacuo* to a sirup. The sirup was dissolved in 500 ml. of absolute ethanol and cooled. The methyl 2,3-isopropylidene-5-*p*-toluenesulfonyl-D-ribofuranoside that crystallized was collected and washed with ethanol yielding 138 g. of product melting at 83.5–84.5° (reported[2] m.p. 83–84°). A second crop of 25 g., m.p. 82–83°, gave an over-all yield of 31% from ribose.

Methyl 2,3-Isopropylidene-5-iodo-D-ribofuranoside (I).—Methyl 2,3-isopropylidene-5-*p*-toluenesulfonyl-D-ribofuranoside was converted to the 5-iodo compound by treatment with sodium iodide in acetone.[2]

Methyl 2,3-Isopropylidene-5-desoxy-D-ribofuranoside (II).—A solution of 10.0 g. (0.032 mole) of methyl 2,3-isopropylidene-5-iodo-D-ribofuranoside in 100 ml. of methanol and 14.4 ml. (0.036 mole) of 2.5 *N* sodium hydroxide was hydrogenated at room temperature and atmospheric pressure using 0.10 g. of Adams platinum oxide catalyst. When the absorption of hydrogen had ceased the solution was neutralized with carbon dioxide and filtered. The filtrate was distilled using a short column, until the distillation temperature reached 72°. The remaining solution was then extracted with four portions of chloroform. The extracts were combined and concentrated under reduced pressure (water-pump) with a bath temperature below 50° to yield 5.0 g. of methyl 2,3-isopropylidene-5-desoxy-D-ribofuranoside as an oil. A portion was distilled for analysis, b.p. 66° (9 mm.), $[\alpha]^{25}D$ −109° (*c* 2, absolute ethanol).

Anal. Calcd. for $C_9H_{16}O_4$: C, 57.43; H, 8.57. Found: C, 57.58; H, 8.95.

Methyl 5-Desoxy-D-ribofuranoside (III).—A solution of 15.9 g. (0.085 mole) of methyl 2,3-isopropylidene-5-desoxy-D-ribofuranoside in 90 ml. of methanol and 40 ml. of 0.4 *N* sulfuric acid was refluxed for three hours. Further heating did not change the rotation of the solution. The solution was neutralized with barium carbonate, filtered and concentrated under reduced pressure with the bath temperature being kept below 50°. The residual oil was dried *in vacuo* over phosphorus pentoxide giving 11.8 g. of a yellow oil which was distilled at 83–88° (0.3 mm.) yielding 8.2 g. of methyl 5-desoxy-D-ribofuranoside, $[\alpha]^{25}D$ −76° (*c* 2, absolute ethanol). The compound was crystalline but low melting and very hygroscopic.

Anal. Calcd. for $C_6H_{12}O_4$: C, 48.64; H, 8.17. Found: C, 48.66; H, 8.14.

5-Desoxy-D-ribose (IV). A.—A solution of 5.5 g. (0.037 mole) of methyl 5-desoxy-D-ribofuranoside in 50 ml. of 0.4 *N* sulfuric acid was heated at 90° for 1.5 hours. Longer heating did not change the rotation of the solution. The cooled solution was neutralized with barium carbonate, treated with decolorizing charcoal and filtered. The filtrate was evaporated to an oil *in vacuo* at 50° and the residue was extracted with methanol. The extract was filtered and concentrated as before. The resulting oil was dried over phosphorus pentoxide giving 4.7 g. (94%) of 5-desoxy-D-ribose, $[\alpha]^{25}D$ 11° (*c* 4, water).

Anal. Calcd. for $C_5H_{10}O_4$: C, 44.77; H, 7.52. Found: C, 44.62; H, 7.34.

B.—Methyl 2,3-isopropylidene-5-iodo-D-ribofuranoside (7.44 g., 0.024 mole) was reduced in 100 ml. of methanol containing 9.5 ml. of 2.5 *N* sodium hydroxide as previously described. The solution was neutralized with carbon dioxide and the catalyst was removed by filtration. The filtrate was added to 500 ml. of water and the solution was distilled until 400 ml. of distillate had been collected. Ten milliliters of 2 *N* sulfuric acid was added to the distillate and the solution was refluxed for 1.5 hours and then distilled to a volume of 50 ml. The cooled residue was neutralized with barium carbonate and worked up as in A, yielding 2.8 g. (88%) of 5-desoxy-D-ribose, $[\alpha]^{25}D$ 12.0 (*c* 3, water). This material was used for the preparation of N-(5′-desoxy-D-ribosyl)-3,4-dimethylaniline.

The phenylosazone of 5-desoxyribose was prepared by the method used by Micheel,[8] m.p. 175–177°, $[\alpha]^{25}D$ −61° (*c* 2, ethanol–pyridine, 3:2). The same compound prepared from 5-desoxy-D-arabinose is reported[8] to melt at 172–174°, $[\alpha]D$ −65° (*c* 0.65, ethanol–pyridine, 3:2).

N-(5′-Desoxy-D-ribosyl)-3,4-dimethylaniline (V).—A solution of 2.80 g. (0.023 mole) of 3,4-dimethylaniline in 10 ml. of ethanol was added to a solution of 2.48 g. (0.0185 mole) of 5-desoxy-D-ribose in 15 ml. of ethanol. On standing at room temperature for three hours a crystalline precipitate separated. After cooling at 5° overnight the crystals were collected and washed with cold ethanol and with ether giving 2.54 g. (58%) of N-(5′-desoxy-D-ribosyl)-3,4-dimethylaniline, m.p. 135–136°, $[\alpha]^{25}D$ 95° (*c* 2, pyridine).

Anal. Calcd. for $C_{13}H_{19}NO_4$: C, 65.80; H, 8.07; N, 5.90. Found: C, 66.10; H, 8.14; N, 5.84.

N-(5′-Desoxy-D-ribityl)-3,4-dimethylaniline (VI).—A suspension of N-(5′-desoxy-D-ribosyl)-3,4-dimethylaniline (7.98 g.) in 550 ml. of methanol was hydrogenated with 0.25 g. of Adams platinum oxide catalyst at room temperature and three atmospheres pressure. The absorption of hydrogen ceased after about 15 hours. The catalyst was filtered off and the filtrate was concentrated *in vacuo* with the temperature being kept below 50°. The resulting semi-crystalline mixture was triturated with acetone and the crystals were collected. The crude N-(5′-desoxy-D-ribityl)-3,4-dimethylaniline, 4.30 g. (53%), m.p. 139–143°, was not purified further but was used directly in the next step. A sample for analysis was prepared from a similar run by recrystallization from isopropyl alcohol, m.p. 146–148°, $[\alpha]^{25}D$ −37° (*c* 0.4, pyridine).

Anal. Calcd. for $C_{13}H_{21}NO_4$: C, 65.24; H, 8.85; N, 5.85. Found: C, 64.80; H, 8.71; N, 6.04.

N-(5′-Desoxy-D-ribityl)-2-phenylazo-4,5-dimethylaniline (VII).—A solution of 2.13 g. (0.023 mole) of aniline in 6.25

(5) H. C. Stoerk and G. A. Emerson, *Proc. Soc. Exp. Biol. and Med.*, **70**, 703 (1949).

(6) Melting points were determined on a Kofler micro hot-stage.

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

(8) P. A. Levene and E. T. Stiller, *J. Biol. Chem.*, **104**, 299 (1934).

ml. of 12 *N* hydrochloric acid and 15 ml. of water was cooled to 0°. A total of 1.60 g. of solid sodium nitrite was added at such a rate that the temperature of the solution did not exceed 3°. After the sodium nitrite had been added the solution was kept at 0° for one-half hour.

To a suspension of 4.30 g. (0.018 mole) of N-(5'-desoxy-D-ribityl)-3,4-dimethylaniline in 40 ml. of water was added 6.1 ml of 12 *N* hydrochloric acid and 6.06 g. of sodium acetate. The mixture was cooled to −5° and the solution of diazotized aniline prepared above was added. The resulting solution was stirred at −5° for 1.5 hours and then at 0 to 5° for 1.5 hours. After warming to 20° a solution of 5.72 g. of sodium acetate in 50 ml. of water was added at such a rate that the *p*H remained at 3 to 3.5 and the temperature at 17 to 20°. After stirring the resultant mixture for 14 hours the insoluble product was collected and washed with water. The product was recrystallized from 200 ml. of 60% ethanol and dried over phosphorus pentoxide giving 5.04 g. (81%) of N-(5'-desoxy-D-ribityl)-2-phenylazo-4,5-dimethylaniline melting at 174–176°.

Anal. Calcd. for $C_{19}H_{25}N_3O_3$: C, 66.45; H, 7.34; N, 12.24. Found: C, 66.32; H, 7.26; N, 11.71.

5'-Desoxyriboflavin (VIII).—N-(5'-Desoxy-D-ribityl)-2-phenylazo-4,5-dimethylaniline (4.98 g., 0.015 mole) and barbituric acid (3.05 g., 0.024 mole) were added to 43 ml. of butanol and 8 ml. of acetic acid. The resulting mixture was refluxed with stirring for 2.25 hours. After cooling in ice for one hour the insoluble material was collected and washed with butanol. The solid was then triturated with water at 80° for one-half hour, filtered and washed with methanol. The product was recrystallized by dissolving it in 25 ml. of hot 6 *N* hydrochloric acid, treating with decolorizing charcoal, filtering and diluting the filtrate with 50 ml. of hot water. On cooling, 5'-desoxyriboflavin (6,7-dimethyl-9-[1'-(5'-desoxy-D-ribityl)]-isoalloxazine), crystallized. The product (2.98 g., 57%) melted with decomposition at 282–283°. The analytical sample was recrystallized as above, m.p. 283–285° dec., $[\alpha]^{23}D$ 60° (*c* 1, 6 *N* hydrochloric acid). The compound has absorption maxima at 2230 mμ (*E* 33,400), 2670 mμ (*E* 33,800) and 3710 mμ (*E* 10,600).

Anal. Calcd. for $C_{17}H_{20}N_4O_5$: C, 56.66; H, 5.60; N, 15.56. Found: C, 56.83; H, 5.62; N, 15.27.

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, PURDUE UNIVERSITY]

An Aldotriouronic Acid from Hemicellulose-B of Corn Cob[1,2]

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RECEIVED OCTOBER 7, 1954

Continued investigation of the acidic oligosaccharides isolated from a partial hydrolysate of corn cob hemicellulose-B suggests that one is *O*-α-D-glucopyranosyluronic acid-(1 → 4)-*O*-β-D-xylopyranosyl-(1 → 4)-D-xylose.

Absorption of the acidic oligosaccharides from a partial hydrolysate of corn cob hemicellulose-B on Amberlite IR-4B resin has led to the isolation of four aldobiouronic acids and one aldotriouronic acid. Three of the aldobiouronic acids have been characterized.[3,4] The aldotriouronic acid is described here.

Two additional procedures for the isolation of acidic oligosaccharides from polysaccharide partial hydrolysates have been investigated in an attempt to find the most rapid and convenient. Displacement from a carbon column[5] gave no clear-cut separation between acidic di- and trisaccharides nor could a satisfactory resolution of a mixture of neutral and acidic oligosaccharides be effected. The method now preferred involves precipitation of the acidic oligosaccharides as barium salts, followed by removal of barium ions on a column of IR-120 resin and separation of the free acids on a cellulose column.

The aldotriouronic acid thus isolated reduces boiling Fehling solution and is very resistant to complete hydrolysis. In the hydrolysate from the trisaccharide are found xylose, glucuronic acid and an aldobiouronic acid, which corresponds in chromatographic position to the mixture of 2-*O*-(α-D-glucopyranosyluronic acid)-D-xylose and 4-*O*-(α-D-glucopyranosyluronic acid)-D-xylose previously characterized.[4] The neutral trisaccharide produced by reduction of the methyl glycoside methyl ester of the aldotriouronic acid by lithium aluminum hydride[6–8] hydrolyzes to D-glucose and D-xylose only. Methylation of the neutral trisaccharide followed by hydrolysis yields two sugar derivatives which on paper chromatography correspond to 2,3-di-*O*-methyl-D-xylose and 2,3,4,6-tetra-*O*-methyl-D-glucose. On bromine oxidation of the aldotriouronic acid with subsequent methylation and hydrolysis, 2,3-di-*O*-methyl-D-xylose and a methylated uronic acid derivative are detected as the only reducing units on paper chromatography. Methylation of the trisaccharide, first with dimethyl sulfate and sodium hydroxide and later with methyl iodide and silver oxide, gives a fully methylated derivative which hydrolyzes very readily with formic acid to 2,3-di-*O*-methyl-D-xylose and 2,3,4-tri-*O*-methyl-D-glucuronic acid. These compounds are separated by paper chromatography and converted to crystalline derivatives.

This evidence characterizes the aldotriuronic acid as a linear molecule in which D-glucuronic acid is the non-reducing end-group. The stability of the molecule during the initial depolymerization of hemicellulose-B and its resistance to hydrolysis when isolated suggest that the central D-xylosyl unit exists in the pyranose form. The remote possibility of a 1 → 5 linkage between the central and reducing xylosyl units is not eliminated.

On the basis of optical rotatory considerations, the α-D configuration was assigned to the glycosidic linkage in the 4-*O*-(D-glucopyranosyluronic acid)-

(1) Journal Paper No. 820 of the Purdue Agricultural Experiment Station.

(2) Paper presented before the Division of Carbohydrate Chemistry at the 126th Meeting of the American Chemical Society in New York, N. Y., September, 1954.

(3) R. L. Whistler, H. E. Conrad and L. Hough, THIS JOURNAL, **76**, 1668 (1954).

(4) R. L. Whistler and L. Hough, *ibid.*, **75**, 4918 (1953).

(5) R. L. Whistler and D. F. Durso, *ibid.*, **72**, 677 (1950).

(6) B. Lythgoe and S. Trippett, *J. Chem. Soc.*, 1983 (1950).

(7) M. Abdel Akher and F. Smith, *Nature*, **166**, 1037 (1950).

(8) S. Roseman, THIS JOURNAL, **74**, 4467 (1952).