as before and the crude aglycone treated with pyridine and benzoyl chloride. The dibenzoate was worked up as described by Buzas, v. Euw and Reichstein and chromatogramed on 2.5 g. of prepared alumina. From the fractions eluted with benzene containing 10 to 40% chloroform there was obtained 31.4 mg. of a benzoate that crystallized from methanol (in which it is very insoluble) and then melted at 223–224°. It was taken up in a little methanol and chloroform, filtered, and the solvent evaporated until crystallization started. Ether was added to complete the crystallization. The melting point was 178–180°, with no double melting point as reported by Buzas, *et al.*, and $[\alpha]D$ +32.0° (acetone). The sulfuric acid color test was very weak: colorless turning pink (1 min.), blue (5 min.) and blue-green (30 min.).

Anal. Calcd. for $C_{37}H_{40}O_9$: C, 70.68; H, 6.41. Found: C, 70.47, 70.52; H, 6.55, 6.42.

Buzas, *et al.*, report for sarverogenin dibenzoate a double melting point 178–184° → 192°, $[\alpha]D$ +31.5°, sulfuric acid color test red becoming violet and developing a blue rim and finally dark blue.

Sarveroside was hydrolyzed to sarverogenin and converted to sarverogenin benzoate as described above. The melting point was 178–182°, $[\alpha]D$ +30° (acetone).

The two samples of sarverogenin benzoate from sarveroside and from Substance 761 were mixed and finely ground in a mortar. The melting point behavior of this mixture was observed on the same cover slip with finely ground portions of the two samples of sarverogenin benzoate and all melted in the same way at the same temperature. When finely ground, sarverogenin benzoate began to melt at 170°, then resolidified and melted at 228–234°.

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

Synthesis of Lyxoflavin

BY DOROTHEA HEYL, EDITH C. CHASE, FRANK KONIUSZY AND KARL FOLKERS

L-Lyxoflavin has been synthesized by a route which is different from the one previously described. Calcium D-galacturonate is reduced to calcium L-galactonate, and this product is oxidized to L-lyxose. 3,4-Xylidine and L-lyxose are reductively coupled to form N-L-lyxityl-4,5-dimethylaniline, which is converted to N-L-lyxityl-2-phenylazo-4,5-dimethylaniline. Reaction of the latter compound with barbituric acid yields L-lyxoflavin.

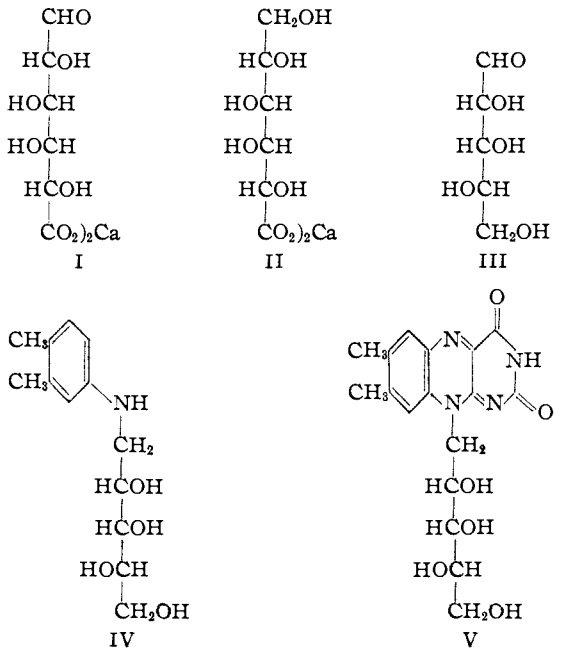
Synthetic L-lyxoflavin has shown growth-promoting or vitamin-like activity[1] in a rat assay for unidentified vitamins in liver and other source materials.

The isolation of lyxoflavin from human myocardium has been described.[2] It was shown that the "natural pentose-flavin" had properties which were identical with those of synthetic L-lyxoflavin. The method[2] of synthesis had previously been used for the synthesis of D-lyxoflavin.[3] This method involved the condensation of 2-carbethoxyamino-4,5-dimethylaniline with D- and L-lyxose to form 1-N-D- and 1-N-L-lyxityl-2-carbethoxyamino-4,5-dimethylaniline. Saponification of the urethans, and condensation of the resulting diamines with alloxan gave D- and L-lyxoflavin.

We have synthesized L-lyxoflavin by a different method. A satisfactory synthesis of L-lyxose combined with an advantageous procedure for the preparation of the flavin, permits lyxoflavin to be made available in sufficient quantities for adequate biological study. D-Galacturonic acid, which is commercially available from citrus pectin, is the source of L-lyxose.

Calcium D-galacturonate (I) was hydrogenated over a Raney nickel catalyst to give calcium L-galactonate (II). Sirupy L-lyxose (III) was prepared by oxidation[4] of calcium L-galactonate with hydrogen peroxide and ferric acetate.

L-Lyxose may be obtained in crystalline form after purification of the crude product by passage through ion exchange columns.[5] The use of pure lyxose for reductive condensation with 3,4-xylidine results in a nearly quantitative yield of N-L-lyxityl-4,5-dimethylaniline (IV); however, the crude sirupy lyxose may be used for the reductive condensation, and the yield of the xylidine derivative (IV) appears to represent the purity of the crude L-lyxose. N-L-Lyxityl-4,5-dimethylaniline coupled readily with diazotized aniline to form N-L-lyxityl-2-phenylazo-4,5-dimethylaniline, and this compound was converted to L-lyxoflavin (V) by direct condensation with barbituric acid.



(1) Emerson and Folkers, THIS JOURNAL, **73**, 2398 (1951).
(2) Pallares and Garza, *Arch. Biochem.*, **22**, 63 (1949).
(3) Karrer, Salomon, Schöpp, Benz and Becker, *Helv. Chim. Acta*, **18**, 908 (1935).
(4) Hockett and Hudson, THIS JOURNAL, **56**, 1632 (1934).
(5) Fletcher, Diehl and Hudson, *ibid.*, **72**, 4546 (1950).

The reactions used for the conversion of the N-L-lyxityl-4,5-dimethylaniline to L-lyxoflavin are identical with those which were devised for a synthesis[6] of riboflavin.

Experimental[7]

Calcium L-Galactonate.[8]—A mixture of 100.0 g. of α-D-galacturonic acid (Eastman Kodak Co.) and 17.5 g. of calcium hydroxide was added slowly, with stirring, to 500 ml. of water. The final solution was filtered and the hydrogenation was carried out in the presence of 6 g. of Raney nickel catalyst at 90° and about 100 atmospheres. After removal of the catalyst, the solution was concentrated under reduced pressure until crystallization had begun. The mixture was chilled, and the resulting crystalline product was collected on a filter and washed with water. After drying over phosphorus pentoxide at 50°, the calcium L-galactonate pentahydrate weighed 107.7 g.; yield 88%.

L-Lyxose.—Crude, sirupy L-lyxose was prepared by the method of Hockett and Hudson.[4]

N-L-Lyxityl-4,5-dimethylaniline.—A solution of 23.7 g. of crude, sirupy L-lyxose in 100 ml. of methanol was treated with a solution of 19 g. of 3,4-xylidine in 50 ml. of methanol, and hydrogenation was carried out at about 100 atmospheres and at 90–100° in the presence of 6 g. of Raney nickel catalyst. The crystals present when the bomb was opened were dissolved by warming, and the catalyst was removed. The crystals which separated on cooling were collected on a filter and dried; weight 16.3 g.; m.p. 147–148°. A second fraction, weighing 4.1 g., raised the total yield to 51%. When the reaction is carried out with pure lyxose, the yield is almost quantitative. The yield in this experiment represents the purity of the crude L-lyxose.

Anal. Calcd. for $C_{13}H_{21}NO_4$: C, 61.15; H, 8.29; N, 5.49. Found: C, 61.43; H, 8.00; N, 5.43.

N-L-Lyxityl-2-phenylazo-4,5-dimethylaniline.—A solution of 8 g. of aniline in a mixture of 23.5 ml. of 12 *N* hydrochloric acid and 55 ml. of water was cooled to 0°. Solid sodium nitrite was added in small portions at such a rate that the temperature of the solution did not exceed 3°, until 6 g. had been added. The solution was kept at 0° for one-half hour.

A suspension of 17.7 g. of N-L-lyxityl-4,5-dimethylaniline in 140 ml. of water was treated with 23 ml. of 12 *N* hydrochloric acid and 22.8 g. of anhydrous sodium acetate, and the mixture was cooled to −5°. The solution of diazotized aniline was added to this suspension. The resulting solution was stirred at −9 to −5° for one hour and at 0° for two hours. After warming to 20°, the stirred solution was treated with a solution of 21.5 g. of anhydrous sodium acetate in 175 ml. of water at such a rate that the *p*H remained approximately 3, and the temperature 17–20°. The resulting mixture was stirred at 22–25° for 17 hours. The crude N-L-lyxityl-2-phenylazo-4,5-dimethylaniline was collected on a filter, washed with two 70-ml. portions of water, and dried to constant weight in a vacuum oven at 50–60°. The material weighed 26 g.

L-Lyxoflavin.—A solution of the N-L-lyxityl-2-phenylazo-4,5-dimethylaniline from the experiment described above in 150 ml. of *n*-butanol was added to 26.7 ml. of glacial acetic acid containing 13.8 g. of barbituric acid, and the mixture was stirred and refluxed for five hours. After cooling and stirring in an ice-bath for an hour, the mixture was filtered. The solid material was slurried in 160 ml. of water at 80° for one-half hour and, after cooling to 70°, the solid was collected on a filter and washed with water and then methanol. The dark, crude material was dissolved in a mixture of 60 ml. of concentrated hydrochloric acid and 20 ml. of water. After two extractions with ether, the aqueous solution was freed from ether by a current of air and was then treated with 7 ml. of 30% hydrogen peroxide. After standing for about ten minutes, the solution was filtered through a layer of super-cel and poured into 700 ml. of boiling water. Cooling for several hours at 5° caused the precipitation of 14.0 g. of L-lyxoflavin (54% based on 17.7 g. of N-L-lyxityl-4,5-dimethylaniline). Before drying, the material had been washed thoroughly with water and methanol. After two recrystallizations from concentrated hydrochloric acid, 30% hydrogen peroxide and water, as described above, the orange needles melted at 283–284° (dec.); $\alpha^{23}D$ −49° ± 3° (*c*, 0.26 in 0.05 *N* sodium hydroxide). The analytical sample was dried over phosphorus pentoxide at 100°.

Anal. Calcd. for $C_{17}H_{20}N_4O_6$: C, 54.25; H, 5.36; N, 14.89. Found: C, 54.38; H, 5.39; N, 15.20.

RAHWAY, N. J. RECEIVED APRIL 2, 1951

(6) Tishler, Pfister, Babson, Ladenburg and Fleming, *ibid.*, **69**, 1487 (1947).

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

(8) This procedure is similar to ones described by Isbell, *J. Research Natl. Bur. Standards*, **33**, 45 (1944).

[CONTRIBUTION NO. 232 FROM THE RESEARCH LABORATORIES OF HOFFMANN-LA ROCHE, INC.]

The Preparation of Dehydro-L-ascorbic Acid and its Methanol Complex. Some Reactions of Dehydro-L-ascorbic Acid[1]

BY B. PECHERER

An improved procedure for the oxidation of L-ascorbic acid to dehydro-L-ascorbic acid by means of chlorine has been developed. A new substance, the crystalline equimolecular complex of dehydro-L-ascorbic acid and methanol, has been obtained and some of its reactions described.

Dehydro-L-ascorbic acid has been the subject of numerous studies since the elucidation of the structure of vitamin C. Since the substance defied all attempts to isolate it in crystalline form, all of the work was done on freshly prepared solutions of the oxidized vitamin. Finally in 1948, Kenyon and Munro[2] succeeded in isolating crystalline dehydro-L-ascorbic acid by the appropriate treatment[3] of a product prepared by the method of Hirst[3] and confirmed its constitution.[4]

When a large quantity of dehydro-L-ascorbic acid was needed in this laboratory, a study was made of various methods for its preparation. In the first series of experiments,[5] L-ascorbic acid was treated in aqueous solution with oxidants selected to give as

(1) Presented before the Division of Biological Chemistry at the 118th Meeting of the American Chemical Society, Chicago, Illinois, September 4, 1950.

(2) J. Kenyon and M. Munro, *J. Chem. Soc.*, 158 (1948).

(3) Unpublished work of E. L. Hirst quoted by E. M. Crook and E. J. Morgan, *Biochem. J.*, **38**, 10 (1944).

(4) For example, its reduction to ascorbic acid by hydrogen sulfide, the formation of the 2,4-dinitrophenylosazone of dehydro-L-ascorbic acid, identical with that obtained directly from ascorbic acid, and the formation of 5,6-diacetyl-dehydro-L-ascorbic acid. The extreme insolubility in cold water is surprising.

(5) These were carried out before the work of Kenyon and Munro was published and are described in the Experimental Section.