[CONTRIBUTION FROM THE DEPARTMENT OF PHYSIOLOGY AND VITAL ECONOMICS, UNIVERSITY OF ROCHESTER SCHOOL OF MEDICINE AND DENTISTRY]

The Synthesis of 6-Ethyl-9-(D-1′-ribityl)-isoalloxazine[1]

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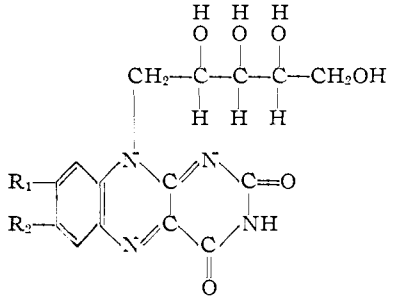
A new riboflavin analog, 6-ethyl-9-(D-1′-ribityl)-isoalloxazine, has been synthesized and found to have 3% of the activity of riboflavin for the growth of *L. casei*.

As a continuation of this Laboratory's program of the synthesis and study of the metabolism of riboflavin analogs,[2-6] a new homolog, 6-ethyl-9-(D-1′-ribityl)-isoalloxazine (I), has been synthesized and found to have 3% of the activity of riboflavin (II) for *L. casei*.



	R_1	R_2		R_1	R_2
I	H–	C_2H_5–	IV	CH_3–	H–
II	CH_3–	CH_3–	V	CH_3–	C_2H_5–
III	H–	CH_3–	VI	C_2H_5–	C_2H_5–

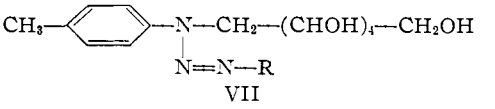
Previous workers[7] have shown that at limiting concentrations 6-methyl-9-(D-1′-ribityl)-isoalloxazine (III), 7-methyl-9-(D-1′-ribityl)-isoalloxazine (IV) and 6-ethyl-7-methyl-9-(D-1′-ribityl)-isoalloxazine (V) have approximately 3.6, 21 and 93%, respectively, of the activity of riboflavin for *L. casei*.[8]

During recent years, it has been claimed[9,10] that a methyl group must be present in either the 6- or 7-position of a ribitylisoalloxazine for the compound to have riboflavin activity. Since 6,7-diethyl-9-(D-1′-ribityl)-isoalloxazine[2,3] (VI) is 100% and 6-ethyl-9-(D-1′-ribityl)-isoalloxazine is 3% as active as riboflavin for *L. casei*, the methyl group in either the 6- or 7-position can no longer be considered to be a structural requirement for the replacement of riboflavin in the nutrition of this organism.

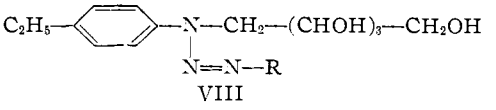
The 6-ethyl analog was synthesized by two methods. By the first method 4-ethyl-2-nitroaniline-N-D-riboside was catalytically reduced to 2-(D-ribitylamino)-5-ethylaniline. This *o*-phenylenediamine was not isolated but was used directly in the condensation with alloxan to produce 6-ethyl-9-(D-1′-ribityl)-isoalloxazine, by essentially the same procedure used by Lambooy[2] for the synthesis of diethyl riboflavin.

The synthesis by the second method consisted of condensing 1-(D-ribitylamino)-2-*p*-tolylazo-4-ethylbenzene with barbituric acid. This method yielded insufficient quantities of flavin suitable for analytical purposes. However, the small amount of flavin obtained had the identical R_f value as crystalline 6-ethyl-9-(D-1′-ribityl)-isoalloxazine when chromatographed on paper in a water:butanol:acetic acid system.

The difficulty in the second method of synthesis was due to inadequate and inconsistent yields of 1-(D-ribitylamino)-2-*p*-tolylazo-4-ethylbenzene. It is of interest to note that it has been claimed[11] that in order for diazonium salts to couple in the *o*-position of substituted anilines, the aniline derivative must have substituents in both the *m*- and *p*-positions. The basis for this belief has been that *p*-toluenеglucamine reacts with a diazonium chloride to give VII.



VII



VIII

Our inability to isolate suitable quantities of 1-(D-ribitylamino)-2-*p*-tolylazo-4-ethylbenzene may have been due to the presence of compound VIII. That the desired coupling did take place to some extent is indicated by the formation of small amounts of the desired flavin as indicated by the chromatographic means described above.

Experimental

4-Ethyl-2-nitroaniline-N-D-riboside.—D-Ribose, 1.50 g., and 2.00 g. of 4-ethyl-2-nitroaniline[12] were treated by the general procedure for the preparation of ribosides as described by Berger and Lee.[13] The riboside, in the form of yellow needles, was obtained in yields of 2.2–2.4 g. (74–80%); m.p. 190.5–194.5° dec., $[\alpha]^{23}D$ −107.5° (0.5% pyridine).

Anal. Calcd. for $C_{13}H_{18}O_6N_2$: C, 52.3; H, 6.1; N, 9.4. Found: C, 52.6; H, 6.1; N, 9.3.

6-Ethyl-9-(D-1′-ribityl)-isoalloxazine. From 4-Ethyl-2 nitroaniline-N-D-riboside.—The synthesis of the flavin was

(1) Supported in part by a research grant from the National Institutes of Health, United States Public Health Service, Grant No. G-3326-C.

(2) J. P. Lambooy, THIS JOURNAL, **72**, 5225 (1950).

(3) J. P. Lambooy, *J. Biol. Chem.*, **188**, 549 (1951).

(4) H. V. Aposhian and J. P. Lambooy, *Proc. Soc. Exp. Biol. and Med.*, **78**, 197 (1951).

(5) J. P. Lambooy and H. V. Aposhian, *J. Nutrition*, **41**, 539 (1952).

(6) E. E. Haley and J. P. Lambooy, to be published.

(7) E. E. Snell and F. M. Strong, *Enzymologia*, **6**, 186 (1939).

(8) The activities of the above homologs were computed from the titration values which appeared in reference 7.

(9) R. J. Williams, R. E. Eakins, E. Beerstecher, Jr., and W. Shive, "The Biochemistry of B Vitamins," Reinhold Publ. Corp., New York, N. Y., 1950.

(10) H. R. Rosenberg, "Chemistry and Physiology of the Vitamins," Interscience Publishers, Inc., New York, N. Y., 1945.

(11) P. Karrer and H. I. Meerwein, *Helv. Chim. Acta*, **19**, 264 (1936).

(12) J. P. Lambooy and E. E. Haley, THIS JOURNAL, **74**, 1087 (1952).

(13) L. Berger and J. Lee, *J. Org. Chem.*, **11**, 84 (1946).

based on the method of Kuhn and Strobele.[14] To 250 ml. of 72% alcohol containing 0.83 g. of primary sodium borate was added 5.95 g. of 4-ethyl-2-nitroaniline-N-D-riboside. The mixture was reduced at 30 atmospheres of hydrogen for 6 hours at 70–80° using 5 g. of catalyst in the form of palladium and trace amounts of zinc and calcium hydroxides on calcium carbonate. Ascorbic acid, 200 mg., was added. The catalyst was removed and the solution evaporated to dryness under reduced pressure in an atmosphere of nitrogen. The residue was treated repeatedly with absolute alcohol followed by evaporation. The dry residue was dissolved in 60 ml. of glacial acetic acid and to it was added a suspension of 4.2 g. of boric acid and 4.2 g. of alloxan in 60 ml. of glacial acetic acid. The mixture was shaken for 40 minutes at 55–60° and placed in the dark for 72 hours. After evaporating to dryness as above, the product was dissolved in 37.5 ml. of hydrochloric acid.[15] Superoxol, 2 ml., was added, and after 25 minutes the solution was filtered and 37.5 ml. of water added to the filtrate. When placed in the refrigerator, yellow crystalline platelets were slowly deposited to yield 2.2 g. (29%) of 6-ethyl-9-(D-1′-ribityl)-isoalloxazine. For analytical purposes, the product was dissolved in water and extracted twice with ether. The aqueous phase was then evaporated to dryness under reduced pressure. The dry residue was triturated with 50 ml. of warm (40–50°) alcohol and filtered. The insoluble material was recrystallized from boiling alcohol to yield 6-ethyl-9-(D-1′-ribityl)-isoalloxazine, m.p. 284–285° dec.

Anal. Calcd. for $C_{17}H_{20}O_6N_4$: C, 54.3; H, 5.4; N, 14.9. Found: C, 54.3; H, 5.5; N, 15.4.

4-Ethylaniline-N-D-riboside.—D-Ribose, 1.50 g., and 2 ml. of *p*-ethylaniline[16] were treated as described above[13] except that no catalyst was used. The riboside, in the form of white needles, was obtained in yields of 2.1–2.5 g. (80–91%), m.p. 130–134° dec.

Anal. Calcd. for $C_{13}H_{19}O_4N\cdot\frac{1}{4}H_2O$[17]: C, 60.6; H, 7.6; N, 5.4. Found: C, 60.4; H, 7.5; N, 5.4.

It was found that if sulfuric acid was used as a catalyst as described,[13] the riboside isolated contains one-half mole of water of crystallization.

Anal. Calcd. for $C_{13}H_{19}O_4N\cdot\frac{1}{2}H_2O$: C, 59.5; H, 7.7; N, 5.3. Found: C, 59.7; H, 7.6; N, 5.4.

4-Ethyl-N-(1′-D-ribityl)-aniline.—Three grams of 4-ethylaniline-N-D-riboside dissolved in 150 ml. of absolute alcohol was hydrogenated at 60 p.s.i. at 70° for 6 hours using 0.6 g. of Raney nickel catalyst. After removing the catalyst and cooling the filtrate, white needles were precipitated. The product was recrystallized from alcohol to yield 2.6 g. (86%) of 4-ethyl-N-(1′-D-ribityl)-aniline, m.p. 138–138.5°.

Anal. Calcd. for $C_{13}H_{21}O_4N$: C, 61.2; H, 8.3; N, 5.5. Found: C, 61.4; H, 8.4; N, 6.0.

1-(D-Ribitylamino)-2-*p*-tolylazo-4-ethylbenzene.—The synthesis of the tolylazo compound was based on the method of Tishler, *et al.*[18] A mixture of 4.7 g. of *p*-toluidine, 80 ml. of acetic acid, 10 ml. of water and 9.0 ml. of concentrated hydrochloric acid was diazotized by the addition of 2.8 g. of sodium nitrite. After the nitrite was consumed, 5.1 g. of 4-ethyl-N-(1′-D-ribityl)-aniline was added, followed by the addition of a solution of 2.9 g. of sodium hydroxide in 12 ml. of water. The mixture was aged at 8–10° for 2 hours. A longer period of aging did not increase the yield. The mixture was diluted with 500 ml. of ether and shaken with water. The ether layer was washed with water, 10% sodium bicarbonate and again with water. After the removal of the ether, the residue was suspended in hot benzene and filtered. The benzene insoluble material was recrystallized from 50% alcohol to yield 0.55 g. of red needles, m.p. 130–131°. This was the largest amount of material obtained from a number of attempts involving the above procedure. It is regrettable that due to large losses during the recrystallization of this material a sample of suitable purity for analysis was not obtained.

Anal. Calcd. for $C_{20}H_{27}O_4N_3$: C, 64.3; H, 7.3. Found: C, 64.9; H, 7.8.

6-Ethyl-9-(D-1′-ribityl)-isoalloxazine. From 1-(D-Ribitylamino)-2-*p*-tolylazo-4-ethylbenzene.—A mixture of 0.52 g. of 1-(D-ribitylamino)-2-*p*-tolylazo-4-ethylbenzene, 0.31 g. of barbituric acid, 3.9 ml. of dioxane and 0.74 ml. of acetic acid was treated by the procedure of Tishler, *et al.*,[18] to yield 0.05 g. of a yellow solid. On recrystallizing from hot water, yellow needles were deposited. This material when chromatographed on paper in a water:butanol:acetic acid system[19] has an R_f value of 0.43 as does crystalline 6-ethyl-9-(D-1′-ribityl)-isoalloxazine.

Biological Data.—The microbiological assay of this material was done by the usual procedure.[20] When the acid production by *L. casei* grown in the presence of 2.0–6.0 micrograms of 6-ethyl-9-(D-1′-ribityl)-isoalloxazine per 10 ml. of culture medium is compared to the acid produced in the presence of 0.05–0.15 microgram of riboflavin per 10 ml. of medium, it was found that 6-ethyl-9-(D-1′-ribityl)-isoalloxazine has approximately 3.0% of the activity of riboflavin for this organism.

6-Ethyl-(D-1′-ribityl)-isoalloxazine, when present in concentrations up to 9000 times that of riboflavin, did not antagonize riboflavin utilization by *L. casei*.

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(14) R. Kuhn and R. Strobele, *Ber.*, **70**, 773 (1927).

(15) R. Pasternak and E. V. Brown, U. S. Patent 2,324,800; *C. A.*, **38**, 221 (1944).

(16) E. L. Cline and E. E. Reid, THIS JOURNAL, **49**, 3150 (1927).

(17) Reference 13 states that many ribosides of this sort crystallize with variable amounts of water of crystallization.

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(19) J. L. Crammer, *Nature*, **161**, 349 (1948).

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