gram, was done by the micro Somogyi method.[8] Galactose and arabinose were found in the approximate molar ratio 7:1.

Examination of the Non-acidic Reducing Sugars.—The gum acid (1.5 g.) was heated with N sulfuric acid (30 cc.) for 17 hours and then neutralized with IR-4B resin. The resin was filtered off and the solution concentrated to a sirup, which was chromatographed on a column of cellulose, using half-saturated aqueous butanol. The first fraction gave L-arabinose (0.07 g.), m.p. and mixed m.p. 154–157° after recrystallization from methanol–acetone, $[\alpha]^{20}D$ +109 ± 4° (c 0.6). The other fraction gave D-galactose (0.46 g.), m.p. and mixed m.p. 165–167° after recrystallization from methanol $[\alpha]^{20}D$ +83 ± 3° (c 1.7). Minute traces of two other materials which corresponded to fucose and rhamnose on the chromatogram were obtained, but they were not present in sufficient amount to be identified.

The Uronic Acid.—The gum acid (2.1 g.) in 2 N sulfuric acid (25 cc.) was heated on a boiling water-bath for 16 hours. The neutralized ($BaCO_3$) and filtered hydrolysate was poured into ethanol, and the precipitated barium salt collected (centrifuge). This salt (0.4 g.), after drying, was refluxed with 2% methanolic hydrogen chloride (10 cc.) for 6 hours after which it was neutralized (Ag_2CO_3), filtered and evaporated, leaving the methyl ester methyl glycoside as a sirup (0.4 g.). The glycoside was treated with methanolic ammonia and allowed to stand for several days. Evaporation yielded a sirup which crystallized on trituration with absolute ethanol. Recrystallization of this from ethanol gave the amide of methyl-4-*O*-methyl-α-D-glucuronoside, m.p. and mixed m.p. 232–235°, $[\alpha]^{25}D$ +150° (c 0.6).

Partial Hydrolysis.—The gum acid in N sulfuric acid was heated at 100° for 6 hours and the neutralized ($BaCO_3$) hydrolysate chromatographed in solvent (a) for 72 hours. An aldobiuronic acid spot with R_{Ga} (relative to galactose) 0.51 corresponded in position to the spot for 6-*O*-β-(4-*O*-methyl-D-glucuronosyl)-D-galactose from gum myrrh.[4] A spot corresponding to 4-*O*-α-(4-*O*-methyl-D-glucuronosyl)-D-galactose was not detected. There was a strong single spot of R_{Ga} 0.18, which might have been an aldotriuronic acid.

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(8) M. Somogyi, *J. Biol. Chem.*, **195**, 19 (1952).

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Isolation and Characterization of 6,7-Dimethyl-9-(2′-hydroxyethyl)-isoalloxazine as a Bacterial Fermentation Product of Riboflavin

BY H. T. MILES AND E. R. STADTMAN

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Bacteria that catalyze the anaerobic decomposition of riboflavin have been obtained in a partially purified state by selective (serial) enrichment on a growth medium containing riboflavin as the major carbon source. During fermentation, the riboflavin, which is present in the growth medium mainly as an insoluble suspension, is converted to a less soluble green substance (1–3 days) which is subsequently converted into a red substance and ultimately (4–8 weeks) into an orange compound.

In order to characterize the latter compound, an experiment was set up as follows: 500 ml. of medium containing 5.0 g. of riboflavin, 500 mg. of yeast extract and mineral salts[1] were inoculated with 5.0 ml. of a partially purified enrichment culture of the riboflavin decomposing bacteria. The mixture was incubated for several weeks at 37° under strictly anaerobic conditions. During this time, the typical color changes described above took place, and most of the riboflavin disappeared. The orange precipitate which had accumulated was filtered and washed in turn with water, methanol and ether. The precipitate was dissolved in 4 N potassium hydroxide giving a dark green solution, which was centrifuged to remove bacterial cell debris.

Part of the green solution was treated with 10 N sulfuric acid to give a gelatinous green precipitate, which on filtering on a Büchner funnel overnight became orange. In order to obtain crystalline material, the remainder of the green, basic solution was shaken with ethyl acetate, slow hydrolysis of which led to a gradual acidification and resulted after several hours in the deposition of a crop of orange crystals. The compound had an absorption spectrum very similar to that of riboflavin (λ_{max} at 222, 268, 260 and 445) and had a yellow fluorescence in ultraviolet light, but could be readily separated from riboflavin by paper chromatography using as solvent system butanol–acetic acid–water (4:1:5, upper layer); the fermentation product had an R_f value of 0.45 and riboflavin 0.20.[2]

Treatment of the substance with periodic acid showed negligible periodate consumption by arsenite titration,[3] indicating that no adjacent hydroxyl groups were present.

The infrared spectrum showed a band at 3.11 μ, suggesting that at least one hydroxyl group was present.

The compound was acetylated with acetic anhydride and pyridine at 0°. A chloroform-soluble product was obtained which was separable from the parent compound by paper chromatography in the butanol–acetic acid–water or a butanol–pyridine–water solvent system (3:4:5). R_f values of the parent compound and of its acetate were 0.45 and 0.63, respectively, in the former solvent system and 0.66 and 0.83 in the latter.[2] The acetylated derivative lacked the 3.11 μ band in the infrared and had an additional ester carbonyl band at 5.75 μ; other bands were at 5.83, 5.98, 6.32 and 6.45. The infrared spectrum of the parent compound had bands at 5.78, 5.96, 6.32 and 6.45 μ.

The analyses indicated one hydroxyl group on a two-carbon side chain attached to the flavin ring. On the basis of the foregoing the most probable structure is 6,7-dimethyl-9-(2′-hydroxyethyl)-isoalloxazine.

Anal.[4] Calcd. for $C_{14}H_{14}N_4O_3$: C, 58.73; H, 4.93; N, 19.57. Found: C, 58.25, 58.33; H, 4.58, 4.35; N, 20.31, 20.27. Calcd. for the acetate, $C_{16}H_{16}N_4O_4$: C, 58.53; H, 4.91; N, 17.07; Ac, 13.1. Found: C, 58.82, 58.29, 58.36; H, 4.72, 4.82, 5.09; N, 16.72, 16.42; Ac, 12.24, 12.34.

A sample of this compound, recently prepared from riboflavin, was obtained through the courtesy of Dr. H. G. Petering[5] and found to have an infrared spectrum superimposable with that of the bacterial fermentation product, confirming their identity. The spectra of the acetates were likewise superimposable.

Isolation of the Green Intermediate.—As mentioned above, during the fermentation of riboflavin, substances were observed having colors other than the characteristic yellow of flavins. Both red and green compounds were precipitated from the solution early in the fermentation.

The light green substance which accumulates as a transient intermediate was isolated by the following fermentation procedure: 5.0 g. of riboflavin was suspended in 25 ml. of water, and the suspension was sealed in a cellophane bag. The cellophane bag was then placed in one liter of medium containing mineral salts and yeast extract. The medium was inoculated with riboflavin decomposing bacteria and was incubated at 37° for several weeks. Under these conditions, the riboflavin suspension is physically separated from the rest of the bacterial culture. As dissolved riboflavin diffused through the cellophane membrane in to the surrounding medium, it was converted by the bacteria to the green intermediate, which crystallized out. The cellophane membrane thus served to maintain separation of the unused

(1) One hundred ml. of growth medium contained KH_2PO_4, 0.65 g.; K_2HPO_4, 0.175 g.; $MgSO_4 \cdot 7H_2O$, 20 mg.; $CaSO_4 \cdot 2H_2O$, 1 mg.; $FeSO_4 \cdot 7H_2O$, 0.5 mg.; $MnSO_4 \cdot 4H_2O$, 0.25 mg.; $NaMoO_4 \cdot 2H_2O$, 0.25 mg.; and $Na_2S \cdot 9H_2O$, 30 mg.

(2) The numerical values varied slightly in different runs.

(3) E. L. Jackson, "Organic Reactions," Vol. 2, John Wiley and Sons, Inc., New York, N. Y., 1944, p. 361.

(4) The microanalyses were performed by Clark Microanalytical Laboratory of Urbana, Illinois.

(5) H. H. Fall and H. G. Petering, Abstracts of Papers, 126th Meeting of the American Chemical Society, Sept. 1954, New York, N. Y., p. 37c.

riboflavin from the green fermentation product. The latter compound was then easily isolated by filtration and was washed with methanol and acetone. The compound (600 mg.) thus obtained remained green in the dry state, but upon exposure to air in the presence of water it was readily oxidized to an orange substance which from chromatographic analysis appears to be identical with 6,7-dimethyl-9-(2′-hydroxyethyl) isoalloxazine.

Kuhn and Ströbele[6] reported that one mole of half-reduced flavin could form quinhydrone-like complexes with one mole of flavin (chloroflavin, light green) with one mole of half-reduced flavin (verdoflavin, dark green) or with one mole of dihydroflavin (rhodoflavin, red).

In view of the ready air oxidation to yellow flavin of the green and red substances observed during the fermentation, it appeared possible that these substances are molecular complexes of the kind observed by Kuhn and Ströbele.

Evidence in support of this hypothesis was obtained by showing that when 100 mg. of the green compound (equivalent to 175 μM of presumed complex) was suspended in 2.0 ml. of water and shaken in air for 4 hours in a Warburg respirometer at 26°, 40 μM of oxygen was consumed. No further oxygen uptake occurred even after shaking overnight. The observed oxygen uptake corresponds to 91% of the theoretical uptake expected from a molecular complex composed of one mole of half-reduced and one mole of oxidized flavin. Further evidence in support of the above hypothesis was obtained by showing that when an alkaline solution of the purified bacterial flavin is treated under anaerobic conditions with a solution of sodium hydrosulfite, a dark green precipitate is formed, corresponding to the verdoflavin reported by Kuhn. Addition of concentrated acid caused formation of a bright red solution (rhodoflavin), which, on dilution with water and gradual admission of air became light green (chloroflavin).

It appears very probable, therefore, that the red and green precipitates observed early in the fermentations are in fact quinhydrone-like complexes of partially reduced 6,7-dimethyl-9-(2′-hydroxyethyl)-isoalloxazine.

(6) R. Kuhn and R. Ströbele, *Ber.*, **70**, 753 (1937).

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The Iodination of Tyrosine and its Derivatives

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Early attempts to iodinate tyrosine in various alkaline media were generally unsatisfactory. In recent years, however, tyrosine has been converted into 3,5-diiodotyrosine in good yields by reaction with iodine monochloride[2] and with iodine and ethylamine.[3] Bauer and Strauss[4] reported that iodine did not react with 3-nitrotyrosine in alkaline solutions or in the presence of mercuric oxide. With iodine monochloride, they obtained 3-iodo-5-nitrotyrosine in small yield.

A new method was developed recently for the iodination of phenols and aromatic ethers.[5] This method involved the reaction of the aromatic compound with iodine and hydrogen peroxide in the presence of a strong mineral acid. As part of a research program which has had to be abandoned, this method was used very satisfactorily in the iodination of tyrosine derivatives. Good yields of monoiodo derivatives were obtained from 3-nitrotyrosine and *o*-methyltyrosine and of 3,5-diiodotyrosine from tyrosine.

Experimental[6]

3,5-Diiodotyrosine.—Powdered iodine (2.8 g.) was suspended in a solution of tyrosine (2.0 g.) in glacial acetic acid (14.0 cc.) and 36 *N* hydrochloric acid (8.0 cc.). Thirty per cent. hydrogen peroxide solution was then added in small portions with shaking during five minutes until the iodine color had disappeared, the temperature of the reaction being maintained at 60–65°. Approximately 1.3 cc. of the hydrogen peroxide solution was required. The yellow solution was cooled and diluted successively with water (10 cc.), 0.880 *M* ammonia solution (9.0 cc.), and 10% sodium hydrogen sulfite solution (5 cc.). 0.880 *M* ammonia solution was then added dropwise until crystallization began. 3,5-Diiodotyrosine separated in flat, almost colorless needles. It was collected, washed with water and alcohol and air-dried, m.p. 198° (lit. 201° (cor.)[2a]) (3.4 g., 71%).

3-Iodo-5-nitrotyrosine.—Concentrated nitric acid (3.0 cc.) was added to a suspension of 3-nitrotyrosine (6.0 g.) and powdered iodine (3.4 g.) in 95% alcohol (45 cc.). Thirty per cent. hydrogen peroxide solution was then added in 0.5-cc. portions until the color of iodine had disappeared. 4.0 cc. of hydrogen peroxide solution was required and the heat of the reaction maintained the temperature at 45–50° throughout the addition. The reaction mixture was heated to 70° for five minutes, diluted with water (30 cc.) and treated with concentrated ammonia, added dropwise, until a heavy yellow solid separated. After standing at 0° for two hours, the solid was collected, washed with a small quantity of water and alcohol and heated under reflux with alcohol (30 cc.) for ten minutes. On cooling, the crystalline solid was collected. It was dissolved in hot dilute hydrochloric acid and precipitated with ammonia (5.6 g., 60%, m.p. 220°). For analysis the 3-iodo-5-nitrotyrosine was recrystallized from water and separated in golden-yellow needles, m.p. 224–226° dec. (lit. 225–226°).

Monoiodo-*o*-methyltyrosine.—Concentrated sulfuric acid (1.0 cc.) and finely powdered iodine (1.48 g.) were added to a suspension of *o*-methyltyrosine hydrogen sulfate[7] (3.40 g.) in alcohol (15 cc.). The reaction mixture was maintained at 50° and treated slowly with 30% hydrogen peroxide solution (1.4 cc.) during ten minutes. The temperature was raised to 65° for five minutes, the solution then was diluted with water (10 cc.) and adjusted to *p*H 7.5 with concentrated ammonia when crystallization of the product began. Ten per cent. aqueous sodium hydrogen sulfite (3.0 cc.) was added and the mixture was cooled. The white crystalline mass was collected, washed with alcohol, and air-dried (3.12 g., 84%, m.p. 220–221°). Recrystallized from water, the monoiodo-*o*-methyltyrosine separated in colorless needles, m.p. 222°.

Anal. Calcd. for $C_{10}H_{12}INO_3 \cdot {}^1/_2H_2O$: C, 36.3; H, 4.0; I, 38.5; N, 4.2. Found: C, 36.3; H, 4.0; I, 39.2; N, 4.4.

(1) U. S. Department of Agriculture, Pasadena, California.

(2) (a) P. Block, Jr., and G. Powell, THIS JOURNAL, **65**, 1430 (1943); (b) E. T. Borrows, J. C. Clayton and B. A. Hems, *J. Chem. Soc.*, 5185 (1949).

(3) J. H. Barnes, E. T. Borrows, J. Folks, B. A. Hems and A. G. Long, *ibid.*, 2824 (1950).

(4) (a) H. Bauer and E. Strauss, *Ber.*, **68B**, 1108 (1935); (b) **69**, 245 (1936).

(5) (a) L. Jurd, *Australian J. Sci. Research*, **2A**, 595 (1949); (b) **3A**, 587 (1950).

(6) All melting points are uncorrected.

(7) L. D. Behr and H. T. Clarke, THIS JOURNAL, **54**, 1630 (1932).

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The Chemistry of Nitroacetic Acid and its Esters. III. The Synthesis of Tryptamine from Ethyl-α-nitro-β-(3-indole)-propionate[1]

BY DOUGLAS A. LYTTLE AND DAVID I. WEISBLAT

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Ethyl α-nitro-β-(3-indole)-propionate (I) is a key intermediate in our synthesis of *dl*-tryptophan from ethyl nitroacetate[2a] or ethyl nitromalonate[2b] and

(1) D. I. Weisblat and D. A. Lyttle, U. S. Patent 2,616,896.

(2) (a) D. A. Lyttle and D. I. Weisblat, THIS JOURNAL, **69**, 2118 (1947); (b) **71**, 3079 (1949).