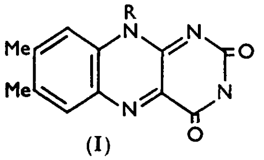
# 75. *Studies in Relation to Biosynthesis. Part X.* A Synthesis of Lumichrome from Non-benzenoid Precursors.*

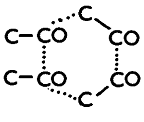
By A. J. Birch and C. J. Moye.

Self-condensation of butane-2 : 3-dione yields 5-acetyltetrahydro-2-hydroxy-2 : 5-dimethyl-3-oxofuran (III) which with 4 : 5-diaminouracil gives a substance (V) or (VI) which is converted by the action of alkali into lumichrome (VII). Some possible implications of these reactions in the biosynthesis of riboflavin are discussed.

In earlier Parts of this series [1,2] the biosynthesis of certain natural aromatic compounds from acetic acid by head-to-tail linkage was discussed. An obviously different scheme is represented by the dimethylbenzene rings of riboflavin (I; R = ribityl) and ribazole. This structure could be formally derived from a head-to-head and head-to-tail linkage of acetic acid units as shown in (II). In fact a probable route would involve two mols. of diacetyl, derived from four mols. of pyruvic acid.

We have accordingly examined the self-condensation of diacetyl in the presence of alkali, a reaction reported [3] to give a crystalline compound $C_8H_{12}O_4$ for which several


(I)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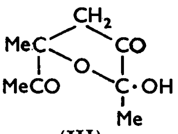
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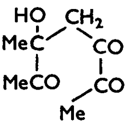
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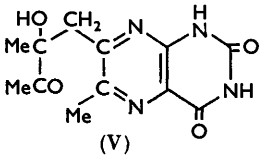
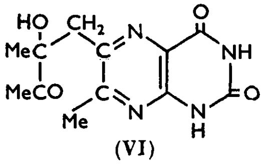
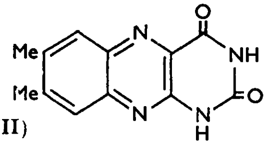
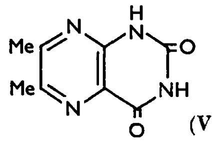
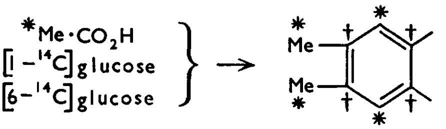
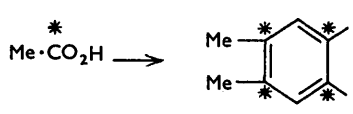

(V)


(VI)


(VII)


(VIII)


(IX)


(X)

† Denotes lower intensity than *.

carbocyclic formulæ were discussed. The most likely formulation for an aldol of diacetyl is (III), and this is supported by the infrared spectrum with carbonyl bands at 1770 (five-membered ring) and 1720 cm.$^{-1}$ (open chain) and a hydroxyl band at 3600 cm.$^{-1}$ (in $CCl_4$).

This aldol is evidently capable of reacting in aqueous solution as the hexanetrione (IV) and condenses with 4 : 5-diaminouracil to give a good yield of what seems to be the expected

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* Part IX, preceding paper.

[1] Birch and Donovan, *Austral. J. Chem.*, 1953, **6**, 360.

[2] Birch, Massy-Westropp, and Moye, *ibid.*, 1955, **8**, 539.

[3] Diels, Blanchard, and d'Heyden, *Ber.*, 1914, **47**, 2539.

derivative (V) or (VI), probably the former. The infrared spectrum shows a carbonyl band at 1710 cm.$^{-1}$ (open-chain ketone). Heating this substance on the steam-bath with N-sodium hydroxide led to precipitation of the sodium salt of lumichrome (VII), which was identified by analysis, by its characteristic ultraviolet absorption in 0·4N-sodium hydroxide, identical with that found by McNutt,[4] and by comparison of the infrared spectrum with that of an authentic specimen. A further peak we have defined at 220 mμ was also found in natural lumichrome and had not been reported by McNutt.

Condensation of diacetyl itself with 4 : 5-diaminouracil has been re-examined.[5] It gave a colourless product instead of the yellow compound previously described. It seems, however, to be the expected compound (VIII) and its ultraviolet spectrum in alkaline solution differs markedly from that of lumichrome, but is similar to that of the intermediate (V or VI).

The ready formation of the dimethylbenzene ring suggests that the reaction is worthy of further investigation from the biochemical side. The evidence already available fits the assumption that pyruvic acid is involved. It has been shown [6] that this stimulates riboflavin production in *Eremothecium ashbyii*. In the related *Ashbya gossypii*, labelled acetic acid was incorporated [7] in low yield, as well as [1-$^{14}$C]- and [6-$^{14}$C]-glucose in the manner shown in (IX) and (X). These distributions of label are compatible with the production of methyl-labelled pyruvic acid from the glucose, incorporation being twice as efficient from the 6-$^{14}$C as from the 1-$^{14}$C in line with the view that degradation of the latter may in part involve the Warburg–Dickens–Lipmann shunt.[8] They indicate also that incorporation of the acetic acid cannot be through any very direct pathway.

## EXPERIMENTAL

*5-Acetyltetrahydro-2-hydroxy-2 : 5-dimethyl-3-oxofuran.*—Diacetyl (100 g.) was caused to condense with itself under conditions given in the literature.[3] The product, after distillation, crystallised at 0° (25—30% yield) and, recrystallised from ether–light petroleum (b. p. 60—80°), had m. p. 51—54°, $\lambda_{max.}$ 293 mμ (ε 54·5). Further distillation of the residue from the original condensation gave a fraction, b. p. 110—120°/0·1 mm. (20 g.), which slowly deposited another crystalline *substance* (3 g.). Recrystallised from benzene containing a little ethyl acetate, this had m. p. 142·5—143·5° [Found : C, 55·85; H, 7·2. $(C_4H_6O_2)_n$ requires C, 55·8; H, 7·0%]. No absorption could be detected between 220 and 350 mμ; the infrared spectrum (in Nujol) demonstrated the presence of a hydroxyl group or groups (2910, 3330, 3420 cm.$^{-1}$) and a carbonyl group in an open chain or a six-membered ring (1715 cm.$^{-1}$).

*Condensation of Diacetyl with 4 : 5-Diaminouracil.*—4 : 5-Diaminouracil sulphate (1 mole), diacetyl (1 mole) and water (about 20 vols.) were heated on the steam-bath until all the sulphate had disappeared, then set aside to crystallise. The colourless 2 : 4-dihydroxy-6 : 7-dimethylpteridine was recrystallised several times from water with no change in properties [m. p. 340° (decomp.)] (Found : C, 49·7, 49·85; H, 4·3, 4·4. Calc. for $C_8H_8O_2N_4$ : C, 50·0; H, 4·2%). Repetition of the recorded preparation did not give the yellow compound reported but the above colourless substance.

The ultraviolet spectrum in 0·4N-sodium hydroxide showed the following peaks : $\lambda_{max.}$ 252 [ε 17,600 (sharp)] and 362—366 mμ [ε 7520 (broad)]; $\lambda_{min.}$ 231 [ε 9180 (sharp)] and 295 [ε 980 (sharp)]; $\lambda_{infl.}$ 276 mμ (ε 6040). The infrared spectrum (in Nujol) contained the following peaks (cm.$^{-1}$) : 3130—3150 s, 3040—3060 s, 2900 s, 2840 s, 1720 s, 1690 s, 1570 s, 1530 s, 1460 s, 1400 s, 1380 s, 1350 s, 1335 s, 1280 s, 1220 s, 1200 m, 1120 m, 1050 m, 1015 s, 870 m, 830 w, 815 s, 760 m, 750 m, 710 m, 690 s (s = strong, m = medium, w = weak).

*Condensation of 5-Acetyltetrahydro-2-hydroxy-2 : 5-dimethyl-3-oxofuran with 4 : 5-Diaminouracil.*—4 : 5-Diaminouracil sulphate (2·45 g.) was added to a solution of the aldol (1·85 g.) in water (40 c.c.). The suspension was heated on the steam-bath for several minutes until the sulphate had dissolved, then a little charcoal was added and the solution boiled and filtered as

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[4] McNutt, *J. Biol. Chem.*, 1954, **210**, 511.

[5] Cf. Kuhn and Cook, *Ber.*, 1937, **70**, 761.

[6] Brown, Goodwin, and Pendlington, *Biochem. J.*, 1955, **61**, 37.

[7] Plaut, *J. Biol. Chem.*, 1954, **211**, 111.

[8] West and Todd, "Textbook of Biochemistry," Macmillan Co., New York, 1955, p. 979.

rapidly as possible. Pale yellow plates of 2 : *4-dihydroxy-7-(2-hydroxy-2-methyl-3-oxobutyl)-6-methylpteridine* (2·5 g.) gradually separated and, crystallised from water, had m. p. 230—232° (Found, in material dried at 150°/10 mm.: C, 49·9; H, 6·0; N, 19·4. $C_{12}H_{14}O_4N_4,5H_2O$ requires C, 50·0; H, 5·2; N, 19·5%). The ultraviolet spectrum in 0·4N-sodium hydroxide showed $\lambda_{max.}$ 254 ($\varepsilon$ 19,500 (fairly broad)], 364—367 ($\varepsilon$ 7730 (broad)], $\lambda_{min.}$ 230 [$\varepsilon$ 9775 (fairly broad)], and 302—305 ($\varepsilon$ 2225 (broad)], and $\lambda_{infl.}$ 232—233 ($\varepsilon$ 9770) and 272·5 mμ ($\varepsilon$ 9040). The spectrum is generally similar to that of 2 : 4-dihydroxy-6 : 7-dimethylpteridine except that the peaks and troughs are less clearly defined.

*Formation of Lumichrome.*—The compound above, (V) or (VI) (500 mg.), was heated on the steam-bath with N-sodium hydroxide (50 c.c.) for 1 hr. On cooling, an almost quantitative yield of the sodium salt of lumichrome was precipitated. Acidification with acetic acid, followed by the addition of water, gave a gelatinous precipitate of lumichrome which was recrystallised twice from pyridine. Since this product contains pyridine of solvation, for analysis it was dissolved in N-aqueous sodium hydroxide and acidified with dilute hydrochloric acid and warmed, and the precipitate was washed free from chloride with distilled water and dried at 15°/10 mm. (Found: C, 57·1; H, 5·1; N, 22·0. Calc. for $C_{12}H_{10}O_2N_4,0{\cdot}5H_2O$: C, 57·0; H, 4·35; N, 22·0%). McNutt[4] also obtained an analysis for lumichrome containing $0{\cdot}5H_2O$. The ultraviolet spectrum in 0·4N-sodium hydroxide was identical with that of an authentic specimen of lumichrome: $\lambda_{max.}$ 222 ($\varepsilon$ 33,900), 265 ($\varepsilon$ 46,200 (sharp)], 340 [$\varepsilon$ 7420 (medium broad)], and 428—430 [$\varepsilon$ 7280 (broad)]; $\lambda_{min.}$ 237 ($\varepsilon$ 11,040), 300 [$\varepsilon$ 2630 (sharp)], and 370—374 mμ [$\varepsilon$ 4420 (broad)]. The infrared spectra of the pyridine solvate and of the similar compound from authentic lumichrome (in Nujol) were also identical apart from a few minor differences in intensities: $\nu_{max.}$ 3135 s, 3020—3050 s, 2890 s, 2825 s, 1715 s, 1695 s, 1620 m, 1575 s, 1560 s, 1485 s, 1460 s, 1425 s, 1380 s, 1360 s, 1345 s, 1290 s, 1220 m, 1025 m, 1000 m, 880 m, 830 m, 810 m, 795 m, 750 m, 730 m.

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