533. *Studies in Relation to Biosynthesis. Part XVI.* The Synthesis of Lumiflavin from Non-benzenoid Precursors.*

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

Condensation of 5-amino-4-methylaminouracil (II; R = Me) with the aldol (I) from diacetyl produces the pteridine (III) (or the isomer with the pteridine section inverted) which can be cyclised by polyphosphoric acid to lumiflavin (IV; R = Me). These experiments are considered to represent a valid model reaction sequence for the biosynthesis of riboflavin.

In Part XIII [1] the suggestion was made that the dimethylbenzene ring of riboflavin (IV; R = ribityl) arises biochemically from two molecules of diacetyl. This idea was supported by demonstrating that condensation of the aldol (I) derived from two molecules of diacetyl with 4 : 5-diaminouracil (II; R = H) gave a pteridine which was converted by alkali into lumichrome containing the dimethylbenzene ring. In order to provide a closer model we have now examined the condensation of the aldol (I) with 5-amino-4-methylaminouracil (II; R = Me) to give a pteridine (III) (or the isomer with the pteridine rings inverted). This pteridine could not be cyclised to the benzene derivative with alkali as in the previous work, possibly because of the greater sensitivity of lumiflavin to alkali. The action of polyphosphoric acid, however, readily produced lumiflavin (IV; R = Me) identified by comparison of ultraviolet and infrared spectra and by paper chromatography.

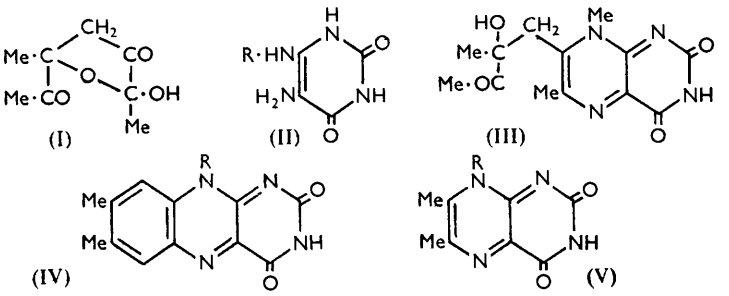
In the meantime further biochemical support for our hypothesis has been provided by Masuda [2] who identified acetoin in cultures of *Eremothecium ashbyii* and isolated compound (V; R = ribityl) from them. Goodwin and Treble [3] have also isolated 4 : 5-diaminouracil, as a derivative, from cultures of this organism. Masuda converted his compound (V; R = ribityl) into riboflavin by heating it with diacetyl. He prepared the analogue (V; R = Me) from the ribityl compound (V; R = ribityl) by illumination and synthesised it from the uracil derivative (II; R = Me) and diacetyl.[2] We have synthesised compound (II; R = Me) by a slightly different route and have converted it into compound (V; R = Me): the properties of the final product and of some intermediates are in reasonable, though not complete, agreement with those given by Masuda. We were unable to convert compound (V; R = Me) into lumiflavin by the action of diacetyl, possibly because

* Part XV, *J.*, 1958, 369.

[1] Birch and Moye, *J.*, 1957, 412.
[2] Masuda, *Pharm. Bull.* (*Japan*), 1957, **5**, 28, 136.
[3] Goodwin and Treble, *Biochem. J.*, 1957, **67** 10P.

of the very sparing solubility of the pteridine. A small amount of the pteridine (III) was detected in the product by paper chromatography.

The natural occurrences and the results above, as well as the tracer results already cited,[1] strongly suggest the truth of our hypothesis as a *structural* one without distinguishing



the exact sequence of events. We believe that intervention of the semiketal (I) is more likely in a biochemical route than is the condensation of diacetyl with compound (V; R = ribityl), but only biochemical experiments can decide this.

Experimental

5-Amino-2 : 4-dihydroxy-6-methylaminopyrimidine (II; R = Me).—Our preparation of this compound was similar to that of Masuda [2] except that 2 : 4-dihydroxy-6-methylaminopyrimidine was made by King and King's method.[4] Reduction of 2 : 4-dihydroxy-6-methylamino-5-nitrosopyrimidine with sodium hydrogen sulphite [2] gave after crystallisation from water, not the bisulphite salt, m. p. 350° reported by Masuda,[2] but the free base, *5-amino-2 : 4-dihydroxy-6-methylaminopyrimidine*, m. p. 250—260° (Found: C, 36·3; H, 5·6; N, 33·75. $C_5H_8O_2N_4,0{\cdot}5H_2O$ requires C, 36·4; H, 5·45; N, 33·9%).

6 : 7 : 8-Trimethyl-lumazine.—The above pyrimidine (250 mg.) was heated in water (4 c.c.) to 80° for 10 min. with excess of diacetyl. The product (110 mg.) slowly crystallised at 0°, to give yellow needles, with a blue-green fluorescence in aqueous solution, m. p. 320—322° (Found: C, 52·3; H, 4·6. Calc. for $C_9H_{10}O_2N_4$: C, 52·4; H, 4·85%). Masuda [2] gives m. p. 300—301°. After being refluxed with diacetyl (5 c.c.) for 10 hr. the substance was recovered almost entirely.

Lumiflavin.—The above aminodihydroxymethylaminopyromidine (400 mg.) in water (5 c.c.) was shaken with 5-acetyltetrahydro-2-hydroxy-2 : 5-dimethyl-3-oxofuran [1] (300 mg.) for 2 min. The golden-yellow solution was left at 0° for several days; golden cubes, m. p. 346—348° (240 mg.), separated which could not be recrystallised and were analysed after being washed and dried. They were either *7-(2-hydroxy-2-methyl-3-oxobutyl)-6 : 8-* or *6-(2-hydroxy-2-methyl-3-oxobutyl)-7 : 8-dimethyl-lumazine* (Found: C, 52·6; H, 5·35. $C_{13}H_{16}O_4N_4$ requires C, 53·4; H, 5·5%), and had $\lambda_{max.}$ (in H_2O) 257, 277, 405 mμ (ε 14,230, 10,150, 11,150), $\lambda_{min.}$ 225, 272, 336 mμ (ε 7425, 9800, 330), $\lambda_{inflex.}$ 302 mμ (ε 1245).

Attempted recrystallisation from hot water led to dark green crystals with a different absorption spectrum (in H_2O) [$\lambda_{max.}$ 235, 300, 445 mμ (ε 14,750, 12,649, 17,762), $\lambda_{min.}$ 270, 352 mμ (ε 4890, 680), $\lambda_{inflex.}$ 253 mμ (ε 10,450)] but a similar analysis (Found: C, 54·0; H, 5·3%). This may be the substance produced by dehydration of the aldol part of the molecule which then crystallises with a mole of water.

The pteridine, m. p. 346—348° (20 mg.), was added to polyphosphoric acid (4 c.c.) at 90°. Heating was continued for 30 min., water (50 c.c.) added, and the solution saturated with sodium acetate and extracted with chloroform. After evaporation the product was purified by chromatography on paper strips in butanol–ethanol–water (50 : 15 : 35) and had R_F 0·35. The product was eluted from the paper with methanol, the solvent evaporated, and the lumiflavin separated from some paper extractives by dissolution in water. Saturation of the aqueous solution with sodium acetate and extraction with chloroform gave lumiflavin, identical

[4] F. E. and T. J. King, *J.*, 1947, 726; Winkelman, *J. prakt. Chem.*, 1927, **115**, 292.

in ultraviolet spectrum ($\lambda_{max.}$ 225, 269, 372, 445 mμ; $\lambda_{min.}$ 242, 300, 400 mμ) and infrared spectrum with a specimen kindly provided by Dr. T. W. Goodwin (Liverpool). The R_F (0·35) on paper in the above system was identical with that of an authentic specimen and the fluorescence colour was the same.

THE UNIVERSITY, MANCHESTER, 13. [*Received, February 28th*, 1958.]