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ISOLATION AND STRUCTURE OF ANKAFLAVIN: A NEW PIGMENT FROM *MONASCUS ANKA*

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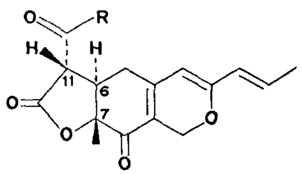
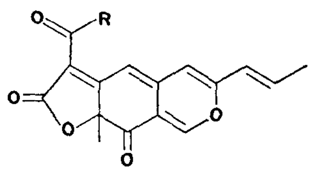
In certain regions of Asia the red rice ('Ang Khak') is often used as a colouring matter in the preparation of certain foods and alcoholic beverages, for example the Taiwan wine, 'Hong Ru'. The fungus *Monascus purpureus* has been shown to be responsible for this colour.[1]

A systematic and comprehensive study[1] of the secondary metabolites produced by the genus *Monascus* has resulted in the isolation and structural characterization of monascin (I) and rubropunctatin (III) from *M. rubropunctatus* Sato, monascin and monascorubrin (IV) from *M. purpureus* Wenti, and monascin from *M. rubriguosus* Sato. As a continuation of this study, and with the hope of isolating metabolites which may be of significance in the biogenesis of these interesting pigments, we initiated studies on the species *M. anka* Sato, the isolation of a new pigment, ankaflavin (II), from this organism is now reported.



(I) R = n-C_5H_{11}

(II) R = n-C_7H_{15}



(III) R = n-C_5H_{11}

(IV) R = n-C_7H_{15}

DISCUSSION AND RESULTS

Extraction of the mycelia of *M. anka* and chromatography of the extract on cellulose yielded a yellow and a red fraction. Further chromatography of the red fraction on celite[2] gave rubropunctatin and monascorubrin. Extensive TLC on silica, with 25% ether in benzene, gave ankaflavin and the slightly more polar monascin.

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[1] WHALLEY, W. B. (1963) *Pure Appl. Chem.* **7**, 565; and references cited therein.
[2] HADFIELD, J. R., HOLKER, J. S. E. and STANWAY, D. W. (1967) *J. Chem. Soc.* **C**, 751.

Ankaflavin has the molecular formula $C_{23}H_{30}O_5$ (M^+ 386·2055), m.p. 120–121° and $[\alpha]_D^{25}$ + 454·0° (*c* 1·01, $CHCl_3$). Its uv spectrum (λ_{max}^{dioxan} 212, 228 and 382 nm. ϵ = 14 300, 16 400 and 13 200 respectively) was virtually superimposable on that of monascin, suggesting a common chromophore. The IR spectrum of ankaflavin indicated the presence of a γ-lactone (1785 cm^{-1}), a saturated ketone (1720 cm^{-1}), an α,β-unsaturated ketone (1673 cm^{-1}), a vinyl ether (1648 cm^{-1}) and a *trans* double bond (958 cm^{-1}). The NMR spectrum revealed a close similarity to that of monascin,[3a,b] the only difference being the greater intensity of the 'methylene envelope' centred at δ 1·30, integration of this band indicates the presence of 10 alkyl methylene protons.

The above spectral evidence suggests that ankaflavin is structurally very similar to monascin. This conclusion is substantiated by a study of the MS of the two metabolites. Ankaflavin showed a molecular ion peak (*m/e* 386) 28 m.u. higher than that of monascin, but in both compounds (see Ref. 3c) the base peak occurs at *m/e* 162, this results from a *retro*-Diels–Alder cleavage of the cyclohexenone ring giving fragments *a* and *b*; fragmentation of *b* to give the oxepin *c* (m/e 134) is also common to both compounds. The only other prominent peak in the MS of ankaflavin (and monascin) occurred at *m/e* 69, this could arise from *a* as indicated.

The spectral evidence presented, taken in conjunction with the fact that ankaflavin and monascin are co-metabolites of *M. anka*, suggests structure (II) for ankaflavin. Ankaflavin thus bears the same structural relationship to monascin as rubropunctatin does to monascorubrin. The configuration at C_6, C_7 and C_{11} is still uncertain, but certain spectral features support the relative stereochemistry depicted in (II). Thus, the large coupling constant (*J* 12 Hz) associated with the protons on C_6 and C_{11} dictates that these protons must bear a *trans* relationship to each other. The high IR frequency (1785 cm^{-1}) at which the lactone group in ankaflavin absorbs suggests a *trans* fusion of this function to the cyclohexenone ring.

EXPERIMENTAL

Monascus anka Sato was grown at 31–33° for 1 month in a liquid medium consisting (for 1 l.) of 1·0 g peptone, 2·0 g KNO_3, 3·0 g tartaric acid, 100 g sucrose, 2·0 g $(HN_4)_2HPO_4$, 0·5 g $MgSO_4.7H_2O$, 0·5 g $ZuSO_4.7H_2O$ and 0·1 g $CaCl_2.6H_2O$. The mycelia were harvested, washed with H_2O, air dried at room temp. and powdered. This powder was then extracted with *n*-hexane. The extract was concentrated, stored at 0° for 2 days and filtered. The crude yellow pigment was collected and separated into a yellow and a red band by chromatography on cellulose (Whatman, standard grade) with *n*-hexane as eluent.

The yellow band was then subjected to repeated preparative-scale TLC on silica gel (1 mm thick, 20 × 20 cm plates) with 25% ether in benzene. Ankaflavin, which is slightly less polar than monascin was collected (by extracting into methylene-chloride and ethyl acetate) and crystallized from ethanol to give yellow prisms, m.p. 120–121°. One gram of crude yellow pigment yielded 74 mg of ankaflavin. Ankaflavin has the following physical properties: $[\alpha]_D^{25}$ + 454·0 (*C* 1·01, $CHCl_3$); λ_{max} (Dioxan): 212, 228 and 382 nm(ϵ = 14 300, 16 400 and 13 200 respectively); ν_{max} ($CHCl_3$): 1785, 1720, 1673, 1648 and 958 cm^{-1}; NMR ($CDCl_3$): δ 0·90 (3H,*t*,*J* 5 Hz), 1·30 (10H,*m*), 1·48 (3H,*s*), 1·86 (3H, *q*, *J* 7, 1 Hz), 2·62 (2H,*m*), 3·88 (1H,*q*,*J* 10, 5 Hz), 3·84 (1H,*d*,*J* 14 Hz), 4·7 (1H, *d*, *J* 14 Hz), 5·04 (2H,*d*,*J* 12·6), 5·32 (1H,*s*) 5·90 (1H,*q*,*J* 15, 1 Hz) and 6·50 (1H,*d* of *q*,*J* 15, 7 Hz); MS: *m/e* (rel. intensity) 386·2055 (M^+, 33) 188(33), 169(100), 134(33), 127(53), 69(83), 57(70), 55(66), 44(90), 43(90), 43(90).

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[3] (a) FIELDING, B. C., HOLKER, J. S. E., JONES, D. F., POWELL, A. D. G., RICHMOND, K. W., ROBERTSON, A. and WHALLEY, W. B. (1961) *J. Chem. Soc.* 4579; (b) KUMASAKI, S., NAKANISHI, K., NISHIKAWA, E. and OHASHI, M. (1962) *Tetrahedron* **18**, 1195; (c) CHEN, F. C., MANCHAND, P. S. and WHALLEY, W. B. (1971) *J. Chem. Soc.* **C**, 3577.