8. *Studies in Mycological Chemistry. Part VIII.* The Structure of Flavasperone ("Asperxanthone"), a Metabolite of* Aspergillus niger.

By B. W. Bycroft, T. A. Dobson, and John C. Roberts.

By degradative and other methods, the structure of flavasperone is established as (III). Demethylation of this compound is accompanied by a molecular rearrangement to give nor-rubrofusarin (I; R = R′ = R″ = H). The isomer of flavasperone, rubrofusarin monomethyl ether, has structure (I; R = R″ = Me, R′ = H).

Asperxanthone, a bright-yellow metabolite produced in small yield by various strains of *Aspergillus niger*, was first isolated by Lund, Robertson, and Whalley [1] who established its formula as $C_{13}H_5O_3(OMe)_2Me$, and considered it to be a 1-hydroxydimethoxymethylxanthone. This opinion was based on the observation that it possessed a number of properties (including a strong green ferric reaction and the ability to form a diacetoborate) characteristic of 1-hydroxyxanthones, and that didemethylasperxanthone appeared to be identical with demethylated rubrofusarin (nor-rubrofusarin) Rubrofusarin itself (a metabolite of certain species of *Fusarium*) was, at that time, also thought to be xanthonoid.[2,3]

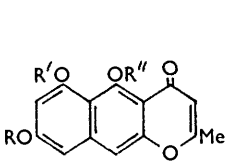
We repeated and confirmed most of the work of Lund *et al.*[1] and, in particular, established the identity of didemethylasperxanthone with nor-rubrofusarin. In addition, we found that didemethylasperxanthone, on potash fusion, gave a poor yield of orcinol. However, we discovered that asperxanthone could not be methylated by standard procedures, and that it had a carbonyl stretching frequency (1677 cm.$^{-1}$) which was abnormally high for a xanthone carbonyl group;[4] further, the metabolite gave no recognisable product when it was submitted to a general method [5] for degrading 1-hydroxyxanthones. Its xanthonoid structure was thus in doubt.

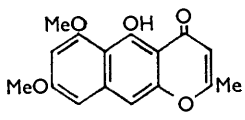
At this point we were informed [6] that the structure of rubrofusarin had been established as (I; R = Me, R′ = R″ = H). It should be noted that rubrofusarin is linear (*X*-ray crystallography [6]), and that nor-rubrofusarin must also be linear since either of these compounds, on methylation, gives rubrofusarin monomethyl ether.[2] Nor-rubrofusarin (didemethylasperxanthone) therefore has structure (I; R = R′ = R″ = H). It then appeared probable that asperxanthone possesses structure (II) or, in the event of a

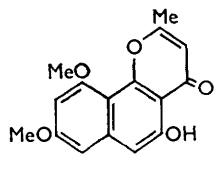
* Part VII, *J.*, 1960, 2169.

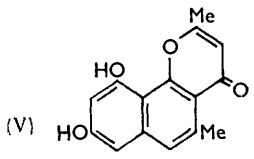
[1] Lund, Robertson, and Whalley, *J.*, 1953, 2434.
[2] Ashley, Hobbs, and Raistrick, *Biochem. J.*, 1937, **31**, 385.
[3] Mull and Nord, *Arch. Biochem. Biophys.*, 1944, **4**, 419.
[4] Roberts, *Chem. Rev.*, 1961, in the press.
[5] Roberts, *J.*, 1960, 785.
[6] Stout, Dreyer, and Jensen, personal communication and *Chem. and Ind.*, 1961, 289.

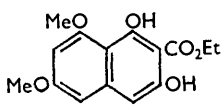
Wessely–Moser type rearrangement [7] during its demethylation, structure (III). We now report evidence for structure (III).

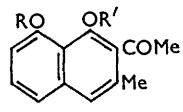

(I)


(II)

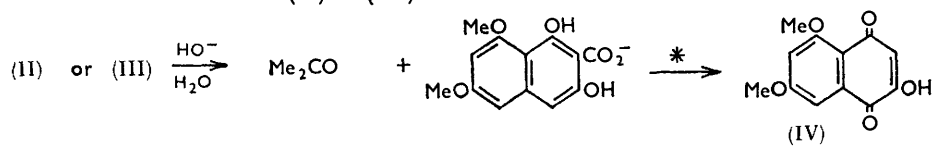

(III)


(V)


(VI)


(VII)

Alkaline hydrolysis of asperxanthone produced acetone and a non-volatile residue which, on vigorous oxidation, gave, in poor yield, 3,5-dimethoxyphthalic acid, and which, on gentle oxidation, appeared to contain 2-hydroxy-5,7-dimethoxy-1,4-naphthaquinone [5,7-*O*-dimethylflaviolin [8] (IV)] but this substance could not be obtained pure. The crude material was methylated, by means of methanolic hydrogen chloride, and the product was purified by chromatography and sublimation to give tri-*O*-methylflaviolin [8,9] (IV; OMe for OH). The precursor of the trimethyl ether was therefore indeed 5,7-*O*-dimethylflaviolin. Hence the degradation of asperxanthone follows the annexed route and asperxanthone has structure (II) or (III)



* Aerial oxidation (cf. ref. 10), acidification, and decarboxylation.

In the Gibbs test [11]—a method for detecting an unsubstituted position *para* to a phenolic group—both asperxanthone and β-naphthol gave green colours. In contrast, nor-rubrofusarin (didemethylasperxanthone), rubrofusarin, and rubrofusarin monomethyl ether gave intense blue colours (positive tests). Structure (III) was thus indicated for asperxanthone.

The ultraviolet absorption spectrum of asperxanthone is fundamentally different from those of nor-rubrofusarin, rubrofusarin, and rubrofusarin monomethyl ether (see annexed Table), but bears a close relation to that of eleutherinol (V), the structure of which has been unambiguously determined.[12,13]

We conclude that asperxanthone has structure (III) and that a rearrangement occurs when it is demethylated.

The metabolite is thus a naphthopyrone and not a xanthone. Although the name asperxanthone has priority,[1] we feel that it should be changed in order to avoid confusion. We suggest the trivial name flavasperone. Dr. Whalley has kindly informed us (personal communication) that he agrees with this suggestion.

[7] Wessely and Moser, *Monatsh.*, 1930, **56**, 97; Philbin, Swirski, and Wheeler, *J.*, 1956, 4455.
[8] Bycroft and Roberts, unpublished work.
[9] Davies, King, and Roberts, *J.*, 1955, 2782.
[10] Birch and Donovan, *Austral. J. Chem.*, 1955, **8**, 529.
[11] King, King, and Manning, *J.*, 1957, 563.
[12] Ebnöther, Meijer, and Schmid, *Helv. Chim. Acta*, 1952, **35**, 910; Frei and Schmid, *Annalen*, 1957, **603**, 169.
[13] Birch and Donovan, *Austral. J. Chem.*, 1953, **6**, 373.

Ultraviolet absorption spectra (in ethanol).

Compound	($\lambda_{max.}$ in mμ; $10^{-3}\varepsilon$ in parentheses)			
Nor-rubrofusarin [a]	225 (28·0)	280 (43·4)	330 (3·01)	414 (5·52)
Rubrofusarin	225 (28·2)	278 (47·6)	326 (3·21)	406 (5·48)
Rubrofusarin monomethyl ether [b]	226 (29·7)	275 (53·9)	329 (2·85)	387 (4·72)
" Asperxanthone "	241 (38·4)	282 (22·4)	—	370 (4·64)
Eleutherinol [c]	240 (41·7)	273 (30·2)	—	362 (10·0)

[a] Inflexion at 250 (19·5). [b] Another max. at 345 (2·87). Values taken from a curve.[12]

The production of orcinol, noted above, may be rationalised by assuming a series of reactions involving hydrolysis, reversed Claisen condensations, and decarboxylations. We have found that orcinol can also be obtained by dealkylation and potash fusion of synthetic ethyl 1,3-dihydroxy-6,8-dimethoxynaphthalene-2-carboxylate [10] (VI).

Rubrofusarin (I; R = Me, R′ = R″ = H) yields a monomethyl ether [2] which is an isomer of flavasperone. The monomethyl ether has a linear structure (see above), is readily soluble in aqueous alkali, and has no ferric reaction. It therefore has structure (I; R = R″ = Me, R′ = H). This unexpected course of monomethylation of rubrofusarin closely parallels the monomethylation of musizin [14] (VII; R = R′ = H) which yields a mixture of two products, the isomer (VII; R = H, R′ = Me) preponderating.

From a biogenetic standpoint it is noteworthy that the skeletal structures of both flavasperone and rubrofusarin [6] probably originate by different operations on a common precursor—an unbranched " polyacetic acid " chain [15] containing seven acetate residues.

Experimental

Infrared absorption spectra, unless otherwise stated, were measured for compounds in potassium bromide discs. Ultraviolet absorption spectra were determined for compounds in ethanolic solution.

Flavasperone.—This was obtained from the dried mycelium of *A. niger* van Tieghem (C.B.S., Baarn, No. 141,260) or of *A. fonsecaeus* (N.R.R.L., No. 67) by the method of Lund *et al.*,[1] but we found that a more efficient purification of the crude material could be accomplished by (i) chromatography in chloroform solution on a column of " heavy " magnesium carbonate (Hopkin and Williams), and (ii) sublimation of the eluted material at 180°/0·1 mm. Flavasperone formed bright-yellow needles, m. p. 203—204° (Found: C, 67·2; H, 5·2. Calc. for $C_{16}H_{14}O_5$: C, 67·1; H, 4·9%), $\nu_{max.}$ 3060w, 2925w, 2855w, 2351w, 2331w, 1677s, 1622s, 1588s, 1530, 1465s, 1442s, 1397s, 1383s, 1357 cm.$^{-1}$, and numerous peaks (medium to strong) at lower frequencies. No significant absorption due to an O–H stretching vibration could be detected in the infrared absorption spectrum. In carbon tetrachloride solution, the compound showed a carbonyl stretching frequency at 1670 cm.$^{-1}$.

Flavasperone was recovered unchanged after its solution in acetic acid had been shaken with 5% palladised charcoal in hydrogen. Treatment of the metabolite with diazomethane led to recovery of original material. An attempt to methylate flavasperone by heating its solution in acetone with anhydrous potassium carbonate and methyl sulphate gave an intractable red resin.

Rubrofusarin Monomethyl Ether.—A sample was sublimed at 180°/0·1 mm. to give orange needles, m. p. 205—206° (lit.,[2] m. p. 203—204°), $\nu_{max.}$ 3300w, 3090w, 2990w, 2950w, 2844w, 2373w, 1650s, 1639s, 1627s, 1607, 1569, 1557, 1485, 1463, 1428, 1395, 1373s cm.$^{-1}$, etc. This compound gave (in both aqueous and ethanolic solution) a negative ferric reaction. It was readily soluble in 2N-sodium hydroxide to give an orange solution and was recovered unchanged after treatment with ethereal diazomethane.

Rubrofusarin.—This was obtained by the recorded procedure,[2] in poor yield, from the

[14] Covell, King, and Morgan, *J.*, 1961, 702.
[15] Birch, *Fortschr. Chem. org. Naturstoffe*, 1957, **14**, 186.

mycelium of *Fusarium culmorum* (C.M.I., No. 35,099) and was sublimed at 190°/0·1 mm. to give red cubes, m. p. 210—211° (lit.,[2] m. p. 210—211°), $\nu_{max.}$ 3389 (wide band), 1662s, 1627s, 1590s, 1517, 1483s, 1459, 1406s, 1376s cm.$^{-1}$, etc.

Nor-rubrofusarin.—(i) A sample prepared [2] from rubrofusarin was sublimed at 220°/10^{-5} mm. to give the pure substance (A) as orange needles, m. p. 280—285° (decomp.). (ii) A suspension of flavasperone (140 mg.) in hydriodic acid (5 ml.; *d* 1·7) and acetic anhydride (2 ml.) was heated under reflux for 5 hr. The resulting solution was poured into a concentrated solution of sodium hydrogen sulphite (10 ml.), and the product was collected and repeatedly sublimed at 230°/10^{-5} mm. to give the pure substance (B) as orange needles (78 mg.), m. p. 278—285° (decomp.) unaltered on admixture with A. Its colour reactions [1] and its ultraviolet absorption spectrum were identical with those of material A. The infrared absorption spectrum of B [$\nu_{max.}$ 3395, 3185 (wide band), 1658s, 1615s, 1482s, 1415s, 1380s, 1349s cm.$^{-1}$, etc.] was virtually identical with that of A. Methylation of product B with diazomethane (cf. ref. 2) yielded rubrofusarin monomethyl ether as the main product. The yellow triacetate of B had m. p. 209—211° (lit.,[1] m. p. 210°) and $\lambda_{max.}$ 221, 252, 311, 325, and 360 mμ ($10^{-3}\varepsilon$ 19·3, 67·8, 4·39, 4·49, and 5·40 respectively).

Alkali-fusion of Nor-rubrofusarin (" *Didemethylasperxanthone* ") —Substance B (20 mg.), potassium hydroxide (0·5 g.), and sodium hydroxide (0·5 g.) were fused at 290—300° for 20 min. (cf. ref. 16). A solution of the cooled melt in water was acidified with 4N-hydrochloric acid, and the product was isolated by ether-extraction. A very small quantity of material (M) was obtained and was identified as orcinol by positive colour reactions (with ferric chloride and bleaching-powder solutions) and by paper chromatography (see annexed table). In all cases it gave only one " spot."

Solvent system	Whatman paper No.	$100R_F$ M	Orcinol	Resorcinol	2-Methyl-resorcinol	4-Methyl-resorcinol	Phloro-glucinol
A(a.s.)	4	87	86	—	—	—	—
B(a.s.)	1	25	25	51	43	33	—
B(d.s.)	1	49	51	90	80	69	—
C(a.s.)	1	80	80	—	—	—	63

A = Upper layer from butan-1-ol, acetic acid, and water (4 : 1 : 5 by vol.). B = Upper layer from butan-1-ol, benzene, and water (1 : 9 : 10 by vol.). C = Upper layer from pentan-1-ol and water (1 : 1 by vol.). (a.s.) = Ascending solvent; (d.s.) = descending solvent. Detector spray: Pauly's reagent (see ref. 17).

By a similar process of dealkylation and alkali fusion, a synthetic specimen of ethyl 1,3-dihydroxy-6,8-dimethoxy-2-naphthoate [10] (VI) gave a small yield of product which was also identified (paper chromatography) as orcinol.

Degradation of Flavasperone (III) *to Acetone and* 5,7-O-*Dimethylflaviolin* (IV).—A solution of flavasperone (50 mg.) in 25% aqueous tetraethylammonium hydroxide (10 ml.) was added dropwise to refluxing 3% aqueous potassium hydroxide (20 ml.). The solution was heated under reflux for further ½ hr. and then distilled. The distillate (5 ml.) was added to 15 ml. of a solution of 2,4-dinitrophenylhydrazine hydrochloride (made from 0·2 g. of the base and 100 ml. of 2N-hydrochloric acid). The precipitate was purified by chromatography (in chloroform) on acid-washed alumina. The fast-moving yellow band was eluted and the solvent was removed. Crystallisation of the residue from ethanol gave yellow rods (9 mg.; m. p. 119—124°) which at 110°/0·1 mm. gave a sublimate of acetone 2,4-dinitrophenylhydrazone, m. p. and mixed m. p. 125—126° (Kofler block).

The solution which remained (after the hydrolysis and distillation) was kept overnight with free access of air. The deep-red solution was acidified and was extracted with ether. The ethereal solution was then extracted with successive portions of 6% aqueous sodium hydrogen carbonate solution until the aqueous extracts were no longer coloured. The combined aqueous extracts were acidified to give (by ether-extraction) a yellow amorphous product (15 mg.). Paper chromatography of this material in two different solvent systems and use of either 2% aqueous sodium hydroxide solution or Pauly's reagent [17] as detector spray, gave results virtually identical with those given by a synthetic specimen of 5,7-*O*-dimethylflaviolin.[8] Attempts to purify the crude material were unsuccessful, so the remainder (10 mg.) was heated under reflux

[16] Kulkarni and Merchant, *J. Sci. Ind. Res., India*, 1955, **14**,*B*, 153; *Chem. Abs.*, 1956, **50**, 7012.
[17] Cramer, " Papierchromatographie," Verlag Chemie, Weinheim, 4th edn., 1958, pp. 94, 149.

for 15 min. with *ca.* 3% methanolic hydrogen chloride (5 ml.). The solvent was removed and the residue was dissolved in benzene, washed with aqueous sodium hydrogen carbonate and dried (Na_2SO_4). The material in this benzene solution was then chromatographed as previously described.[9] The yellow band was eluted and the residue, obtained by evaporation of the solvents, was sublimed at 170°/0·1 mm. to give golden prisms (3 mg.), m. p. 189—191° (Kofler block), unaltered on admixture with synthetic tri-*O*-methylflaviolin [8,9] of m. p. 191—192°. Ultraviolet light absorption: synthetic, $\lambda_{max.}$ 215, 262, 298, and 414 mμ ($10^{-3}\varepsilon$ 31·6, 15·1, 11·0, and 2·69, respectively); degradation product, $\lambda_{max.}$ 215, 262, 296, and 419 mμ ($10^{-3}\varepsilon$ 34·7, 16·2, 11·5, and 3·16, respectively).

Degradation of Flavasperone (III) *to* 3,5-*Dimethoxyphthalic Acid.*—Flavasperone (45 mg.) was hydrolysed, as described above, and the acetone was removed by distillation. The residual solution was acidified and the product was collected in ether. To a solution of the yellow product (36 mg.) in 2N-sodium hydroxide (5 ml.) was added a 15% solution of hydrogen peroxide (2·5 ml.). After the initial reaction the solution was heated on the steam bath for ½ hr. Ether-extraction of the acidified solution gave a small amount of a yellow syrup. This material was heated with acetic anhydride (2 ml.) on the steam bath for ½ hr. and the solvents were removed *in vacuo*. The residue was sublimed at 120°/0·1 mm. and the sublimate was washed with light petroleum (b. p. 40—60°) to eliminate an oil. Resublimation gave colourless needles (*ca.* 2 mg.), m. p. 146—148° (Kofler block), altered to 147—148° after admixture with authentic 3,5-dimethoxyphthalic anhydride, m. p. 147—149°, which had been prepared from 3,5-dimethoxyphthalic acid [18] by the action of acetic anhydride.

We thank Professor J. H. Birkinshaw for gifts of nor-rubrofusarin and of rubrofusarin monomethyl ether; Drs. G. H. Stout and D. L. Dreyer of the University of Washington for useful discussions and for release of information before publication; and the Department of Scientific and Industrial Research for maintenance grants (to B. W. B. and T. A. D.).

THE UNIVERSITY, NOTTINGHAM. [*Received, July 18th,* 1961.]

[18] Fritsch, *Annalen*, 1897, **296**, 344.