929. *Studies in Mycological Chemistry. Part XIV.* Synthesis of Flavasperone.*

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Flavasperone (I; R = H) has been synthesised in a ten-stage process from 3,5-dimethoxybenzoic acid. Since flavasperone may be degraded to nor-rubrofusarin (V; R = R′ = R″ = H), this synthesis may also be regarded as a formal synthesis of the parent compound of the mould metabolites, rubrofusarin and fonsecin.

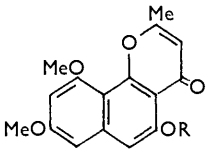
Flavasperone (" asperxanthone ") is a yellow metabolite [1,2] produced in small yield by various strains of *Aspergillus niger*. Degradative and spectroscopic studies [2] have led to the assignment of the angular naphthopyrone structure (I; R = H) to this metabolite. We now describe an unambiguous synthesis of a compound of this structure and a proof of its identity with flavasperone.

We first tried to convert an ester or an acetyl group in an appropriately substituted naphthalene (*e.g.*, II; R = CO_2Et or Ac, R′ = H) into an acetoacetyl group by standard Claisen-condensation procedures. It was expected that the product would then undergo ring-closure to give a separable mixture of flavasperone (I; R = H) and of its linear

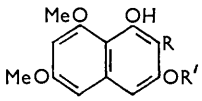
* Part XIII, *J.*, 1963, 3542.

[1] Lund, Robertson, and Whalley, *J.*, 1953, 2434.
[2] Bycroft, Dobson, and Roberts, *J.*, 1962, 40.

isomer, *O*-methylfonsecin [3] (V; R = R′ = Me, R″ = H). However, the ester [4] (II; R = CO_2Et, R′ = H) failed, under a variety of conditions, to undergo the required



(I)



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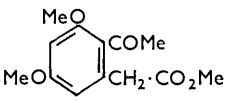

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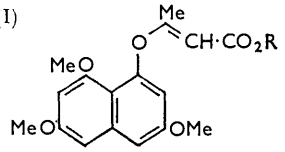

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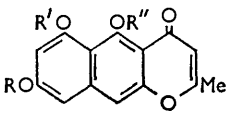

(V)

Claisen condensation. We thus turned to the alternative route involving the 2-acetylnaphthalene derivative (II; R = Ac, R′ = H) which we had hoped to prepare by acetoacetylation of ethyl 3,5-dimethoxyphenylacetate (in the 2-position by use of diketen [5]), followed by base-induced ring-closure of the product. The ester, however, failed to react with the diketen.

An obvious precursor for projected syntheses of the Simonis [6] or Dann [7] type (see below) appeared to be the trimethoxynaphthol (II; R = H, R′ = Me). This was prepared [8] by acetylation of methyl 3,5-dimethoxyphenylacetate to give the ester-ketone (III) which readily cyclised in the presence of base to give the naphthalene derivative (II; R = R′ = H). Monomethylation of the last compound by diazomethane led to the desired trimethoxynaphthol. There can be no doubt that it is the hydroxyl group in the 3-position that is methylated in this reaction since (*a*) the 1-hydroxyl group is hydrogen-bonded to the 8-methoxyl group and (*b*) the product (II; R = H, R′ = Me) gave a positive (blue) Gibbs test [9] (β-naphthols yield a green colour in this reaction [2]). When this naphthol was heated with ethyl acetoacetate (Simonis synthesis [6] of chromones), a small quantity of *O*-methylflavasperone (I; R = Me) was obtained.

A more successful synthesis of *O*-methylflavasperone was achieved by an application of the Dann chromone synthesis.[7] The trimethoxynaphthol (II; R = H, R′ = Me) was caused to react with methyl β-chlorocrotonate to give the naphthyloxy-derivative (IV; R = Me). Careful hydrolysis of this compound gave the naphthyloxycrotonic acid (IV; R = H), ring-closure of which, by the specialised Dann method, gave an acceptable yield of *O*-methylflavasperone (I; R = Me). (Various methods for bringing about ring-closure of this acid were investigated but only two were successful, namely, that mentioned and one using trifluoroacetic anhydride.[10] The reaction may proceed through a mixed-anhydride intermediate.)

We found it extremely difficult to prepare *O*-methylflavasperone from the metabolite itself. This difficulty is undoubtedly due to (*a*) strong hydrogen-bonding between the hydroxyl group and the carbonyl group and (*b*) susceptibility of the γ-pyrone grouping to alkali. Diazomethane was ineffective and methylation procedures involving alkaline reagents led to coloured, intractable products. On one occasion (by the use of dry silver oxide and an excess of methyl iodide) we obtained a very small quantity of material (see below) which was almost certainly the desired, but slightly impure, methyl ether.

[3] Galmarini, Stodola, Raper, and Fennell, *Nature*, 1962, **195**, 502.
[4] Birch and Donovan, *Austral. J. Chem.*, 1955, **8**, 529.
[5] Cf. Boese, *Ind. Eng. Chem.*, 1940, **32**, 16.
[6] Petschek and Simonis, *Ber.*, 1913, **46**, 2014; Mentzer, Molho, and Vercier, *Compt. rend.*, 1951, **232**, 1488; cf. Frei and Schmid, *Annalen*, 1957, **603**, 169.
[7] Dann and Illing, *Annalen*, 1957, **605**, 158; and other papers by Dann *et al.*
[8] Cf. Bycroft and Roberts, *J.*, 1962, 2063.
[9] King, King, and Manning, *J.*, 1957, 563.
[10] Cf. Ferrier and Tedder, *J.*, 1957, 1435.

We then sought for an efficient, selective-demethylation procedure for the conversion of the naphthopyrone (I; R = Me) into flavasperone (I; R = H). A number of attempts with aluminium chloride under various conditions gave indifferent results. We found later that " magnesium iodide etherate " [11] gave a reasonable yield of the desired product (I; R = H) which proved to be identical with natural flavasperone.

Rubrofusarin,[2,12,13] a metabolite of some *Fusarium* species, has structure (V; R = Me, R′ = R″ = H) and, on demethylation, yields nor-rubrofusarin (V; R = R′ = R″ = H). Another metabolite, fonsecin (V; R = R″ = H, R′ = Me), which is obtainable [3] from a mutant strain of *Aspergillus fonsecaeus*, also yields nor-rubrofusarin on demethylation. Since we have previously established [2] that the demethylation of flavasperone is accompanied by a Wessely–Moser rearrangement to give nor-rubrofusarin, it follows that our synthesis of flavasperone may, in addition, be formally regarded as a synthesis of the parent compound of rubrofusarin and fonsecin.

Added in proof (August 7th, 1963).—It is now known that nor-rubrofusarin itself is a natural product. It occurs, together with rubrofusarin, in the seeds of *Cassia tora* (Rangaswami, *Proc Indian Acad. Sci.*, 1963, **57**, *A*, 88).

Experimental

M. p.s were determined on the Kofler block. Ultraviolet absorption spectra were measured for ethanolic solutions with a Perkin-Elmer spectrophotometer (model 137 UV). Infrared absorption spectra, unless otherwise stated, were measured for compounds in potassium bromide discs with a Unicam spectrophotometer (S.P. 200).

Attempted Preparation of 2-Acetoacetyl-6,8-dimethoxynaphthalene-1,3-diol (II; R = $CO{\cdot}CH_2Ac$, R′ = H).—Ethyl 1,3-dihydroxy-6,8-dimethoxynaphthalene-2-carboxylate [4] and acetone failed to react (in an atmosphere of nitrogen) in the presence of sodium ethoxide, sodium, or sodium hydride.

Attempted Acetoacetylation of Ethyl 3,5-Dimethoxyphenylacetate.—The ester and diketen, in methylene dichloride or in nitrobenzene solution, failed to react in presence of anhydrous aluminium chloride.

Methyl 3,5-Dimethoxyphenylacetate.—A solution of 3,5-dimethoxybenzoyl chloride [8] (20 g., 0·1 mole) in dry ether (50 ml.) was added, with stirring, to an ethereal solution of diazomethane (0·15 mole) and triethylamine (10·5 g., 0·1 mole).[14a] The solution was kept at room temperature overnight. Removal of the precipitated triethylamine hydrochloride, and evaporation of the solvent from the filtrate, left the diazoketone as yellow crystals. To a solution of this material in methanol (400 ml.) was added, with stirring, a solution of dry silver benzoate [14b] (1·7 g.) in triethylamine (15 ml.). The solution was kept at room temperature for 1 hr., then further additions of silver benzoate in triethylamine were made until nitrogen was no longer evolved. The mixture was boiled (charcoal) and filtered. The solvents were removed and an ethereal solution of the residue was extracted with aqueous sodium hydrogen carbonate solution and then dried. Removal of the ether and distillation of the residue gave the ester as a pale yellow oil (12·5 g.), b. p. 104—105°/0·1 mm.

Methyl 2-Acetyl-3,5-dimethoxyphenylacetate (III).—This was prepared from the foregoing ester as previously reported.[8]

6,8-Dimethoxynaphthalene-1,3-diol (II; R = R′ = H).—A solution of the foregoing ester-ketone (2·5 g.) in methanol (10 ml.) was added slowly to a refluxing solution of sodium methoxide (from 0·5 g. of sodium) in methanol (10 ml.) under nitrogen. The solution was heated for 20 min., cooled, acidified with 2N-sulphuric acid (15 ml.), and poured into water (60 ml.). The aqueous solution was extracted several times with chloroform, and the combined extracts were washed with water and dried. Removal of the chloroform and crystallisation of the residue from benzene gave the *naphthalenediol* as colourless prisms (1·8 g., 82%), m. p. 126—127° [Found: C, 65·4; H, 6·1; OMe, 28·1. $C_{10}H_6O_2(OMe)_2$ requires C, 65·4; H, 5·5; OMe, 28·2%],

[11] Arkley, Attenburrow, Gregory, and Walker, *J.*, 1962, 1260.
[12] Ashley, Hobbs, and Raistrick, *Biochem. J.*, 1937, **31**, 385.
[13] Stout, Dreyer, and Jensen, *Chem. and Ind.*, 1961, 289.
[14] Newman and Beal, *J. Amer. Chem. Soc.*, (*a*) 1949, **71**, 1506; (*b*) 1950, **72**, 5163.

$\lambda_{max.}$ 243, 294 (inf.), 302, 312 (inf.), and 328 mμ (log ε 4·84, 3·61, 3·62, 3·58, and 3·46, respectively), $\nu_{max.}$ (in $CHCl_3$) 3610, 3410, and 1637 cm.$^{-1}$.

3,6,8-*Trimethoxy*-1-*naphthol* (II; R = H, R′ = Me).—To a solution of 6,8-dimethoxynaphthalene-1,3-diol (1·5 g.) in a minimum of methanol was added an excess of ethereal diazomethane, and the combined solutions were kept overnight. Removal of the solvents and filtration of an ethereal solution of the residue through a short column of alumina gave an eluate which was evaporated. The residue crystallised from benzene–light petroleum (b. p. 60—80°) to give the *naphthol* as colourless prisms (1·4 g., 87%), m. p. 143—144°, or, after sublimation at 130°/0·1 mm., m. p. 145—146° [Found: C, 66·3; H, 6·0; OMe, 39·6. $C_{10}H_5O(OMe)_3$ requires C, 66·6; H, 6·0; OMe, 39·8%], $\lambda_{max.}$ 244, 290, 298, 312, and 326 mμ (log ε 4·86, 3·66, 3·61, 3·52, and 3·40, respectively), $\nu_{max.}$ (in CCl_4) 3430, 1637, and 1620 cm.$^{-1}$. This compound was insoluble in aqueous sodium hydroxide, gave a positive (blue) Gibbs test,[9] and formed a deep-red *picrate* (needles from benzene), m. p. 193—195° (decomp.) [Found: C, 49·4; H, 4·0; OMe, 19·7. $C_{16}H_8O_8N_3(OMe)_3$ requires C, 49·2; H, 3·7; OMe, 20·1%].

Methyl β-(3,6,8-*trimethoxy*-1-*naphthyloxy*)*crotonate* (IV; R = Me).—Methyl β-chlorocrotonate (2 g.) in dry ethyl methyl ketone (10 ml.) was added, during 3 hr., to a refluxing solution (nitrogen atmosphere) of 3,6,8-trimethoxy-1-naphthol (1·2 g.) in ethyl methyl ketone (40 ml.) containing potassium carbonate (*ca.* 5 g.). The mixture was heated under reflux for a further 24 hr. Filtration, removal of the solvent, and chromatography of an ethereal solution of the residue on alumina gave a pale yellow oil which crystallised from methanol–water to give the *ester* (1·4 g., 77%) as needles, m. p. 102—103° (Found: C, 65·3; H, 6·4. $C_{18}H_{20}O_6$ requires C, 65·1; H, 6·1%), $\nu_{max.}$ 1710 and 1637 cm.$^{-1}$.

β-(3,6,8-*Trimethoxy*-1-*naphthyloxy*)*crotonic Acid* (IV; R = H).—A solution of the foregoing ester (1 g.) in methanol (10 ml.) was heated on the steam-bath, in an atmosphere of nitrogen, with 2N-sodium hydroxide (15 ml.) for 1½ hr. The cooled solution was diluted with water (50 ml.) and was extracted with ether. Acidification of the aqueous layer with 5N-hydrochloric acid (*ca.* 6 ml.) precipitated the *acid*, which was collected, washed with water, and crystallised from methanol–water as needles (0·75 g., 81%), m. p. 185—187° (decomp.) [Found: C, 63·6; H, 5·5; OMe, 28·8%; Equiv., by titration (as a monobasic acid), 333. $C_{14}H_9O_3(OMe)_3$ requires C, 64·1; H, 5·7; OMe, 29·2%; Equiv., 318].

6-*Methyl*-3′,6′,8′-*trimethoxynaphtho*-(1′,2′:2,3)-4-*pyrone* (I; R = Me), O-*Methylflavasperone*.—(i) The foregoing acid (700 mg.) was dissolved in acetyl chloride (10 ml.). 66% Perchloric acid (8 drops) was added and the solution was kept at room temperature for 4 days. The deep-red solution was poured on ice (20 g.), and the mixture was extracted several times with chloroform. The organic layer was washed with aqueous sodium hydrogen carbonate and then with water. Removal of the solvent and crystallisation of the residue from acetone gave the *pyrone* (450 mg., 63%) as colourless prisms, m. p. 219—221° [Found: C, 68·2; H, 5·6; OMe, 30·2. $C_{14}H_7O_2(OMe)_3$ requires C, 68·0; H, 5·4; OMe, 31·0%], $\lambda_{max.}$ 239, 274, and 358 mμ (log ε 4·59, 4·54, and 3·79, respectively), $\nu_{max.}$ 1657, 1621, and 1585 cm.$^{-1}$.

(ii) The foregoing acid (50 mg.) was dissolved in trifluoroacetic anhydride (1 ml.). The solution was kept, with stirring, for 12 hr. at room temperature and was then poured into aqueous sodium hydrogen carbonate. The mixture was extracted with chloroform, and the organic layer was washed with water and then dried. The solvent was removed and the residue was fractionally sublimed at 190°/0·05 mm., to give prisms (15 mg.), m. p. 219—221°, identical with the foregoing pyrone.

(iii) 3,6,8-Trimethoxy-1-naphthol (500 mg.) and ethyl acetoacetate (500 mg.) were heated under reflux, in a stream of nitrogen, for 4½ hr. The excess of ethyl acetoacetate was removed under reduced pressure, and the residue was dissolved in ether and washed repeatedly with aqueous sodium hydrogen carbonate (to remove dehydroacetic acid) and finally with water. Removal of the ether from the dried solution, and distillation of the residue at 0·1 mm., gave two fractions, (A) b. p. 160—190°, and (B) b. p. 200—240°. Crystallisation of fraction (A) from benzene–light petroleum (b. p. 60—80°) gave the original naphthol (320 mg.). Chromatography of fraction (B) in benzene, on acid-washed alumina (Spence's type H) gave a pale yellow solid which on fractional sublimation at 190°/0·05 mm. gave colourless prisms (10 mg.), m. p. 219—221°, identical with the above pyrone.

(iv) To a solution of flavasperone (30 mg.) in methyl iodide (5 ml.) was added freshly prepared, dry silver oxide (50 mg.). The mixture was heated under reflux for 6 hr. in an atmosphere of nitrogen. Removal of the solids and evaporation of the filtrate gave a red gum.

Fractional sublimation (180°/0·05 mm.) of this material gave *ca.* 2 mg. of needles, m. p. 217—221°, $\lambda_{max.}$ 240, 276, and 360 mμ, $\nu_{max.}$ 1660, 1620, and 1590 cm.$^{-1}$.

Flavasperone (I; R = H).—(i) Aluminium chloride (5 mg.) was added to a solution of the above pyrone (5 mg.) in dry ether (10 ml.), and the mixture was heated under reflux for 12 hr. The ether was removed and the residue was heated for 5 min. with acetic acid (1 ml.) and concentrated hydrochloric acid (1 drop). The solution was diluted with water (15 ml.) and extracted with chloroform. The organic layer was washed repeatedly with water, then dried, and the solvent was removed. The residue gave a dark green ferric reaction, and paper chromatography indicated that this material contained some flavasperone together with another (unidentified) product.

(ii) A solution of *O*-methylflavasperone (70 mg.) in dry benzene (10 ml.) was mixed with 0·4 ml. of a solution of " magnesium iodide etherate " [made [11] from magnesium (0·4 g.), iodine (2 g.), dry ether (2·5 ml.), and dry benzene (5 ml.)], and the combined solutions were heated under reflux, in an atmosphere of nitrogen, for 3 hr. The cooled solution was mixed with an excess of 0·5N-hydrochloric acid and extracted with chloroform. The organic layer was washed with (*a*) a dilute aqueous solution of sodium hydrogen sulphite and (*b*) water, and was then dried ($MgSO_4$). Removal of the solvents and repeated fractional sublimation of the residue, at 180°/0·1 mm., gave flavasperone (40 mg., 63%) as pale yellow needles, m. p. 201—202°, unaltered on admixture with an authentic sample, m. p. 200—201°. Ultraviolet light absorption: (synthetic) $\lambda_{max.}$ 241, 283, and 371 mμ (log ε 4,56, 4·35, and 3·63, respectively); (natural) $\lambda_{max.}$ 241, 281, and 372 mμ (log ε 4·57, 4·37, and 3·64, respectively). The synthetic and the natural substance had virtually identical infrared absorption spectra.[2]

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