437. *Studies in Mycological Chemistry. Part VII.* Sterigmatocystin, a Metabolite of* Aspergillus versicolor *(Vuillemin) Tiraboschi.*

By J. E. Davies, D. Kirkaldy, and John C. Roberts.

Isolation of, and degradative studies on, the metabolite, sterigmatocystin, are described. The structure (VII) is suggested for it.

Moulds of the *Aspergillus versicolor* group are of ubiquitous distribution and have been found on a wide variety of decaying animal and vegetable products.[1] Some strains of *A. versicolor* (Vuill.) Tiraboschi produce a crystalline metabolite named sterigmatocystin.[2a]

Three independent isolations of this metabolite have been recorded.[2a,3-6] It soon became evident [4,6] that the structure (I) proposed by Japanese workers [2b] was untenable and, through the courtesy of Professor Birkinshaw of the London School of Hygiene and Tropical Medicine, it was mutually agreed that we should continue the structural investigations in Nottingham. This paper records the progress.[7]

Pure sterigmatocystin may be isolated from the dried and powdered mycelium, in a yield of *ca.* 1·3%, by successive solvent extraction, chromatography, crystallisation, and sublimation. It forms pale yellow needles of m. p. 246° but, after crystallisation from ethanol or acetone, its m. p. is always lower (*ca.* 242°). The molecular weight was found [6] (by the Rast method, by quantitative hydrogenation, and by *X*-ray crystallography) to be 321 ± 9. Analysis of the sublimed material established the molecular formula, $C_{18}H_{12}O_6$ (*M*, 324),† rendering formula (I), $C_{15}H_{12}O_5$, unacceptable.

Sterigmatocystin is strongly lævorotatory. Hot ethanolic potassium hydroxide slowly converts it into an optically inactive isomer, isosterigmatocystin. Sterigmatocystin yields a green ferric reaction in ethanol and gives a deep-yellow colour with, but is insoluble in, aqueous sodium hydroxide. The molecule contains one methoxyl group (Zeisel) and one free hydroxyl group (*O*-monomethyl and *O*-monobenzoyl derivative). It does not contain a methylenedioxy-group or a *C*-methyl group (Kuhn–Roth). Its behaviour on acetylation is anomalous (see below).

Some of these properties indicated that sterigmatocystin might be a derivative of 1-hydroxyxanthone, and this view was confirmed by a number of lines of evidence: (i) the ultraviolet light absorption (Fig. 1) agrees with those recorded for many hydroxylated and/or methoxylated xanthones; [9] (ii) the infrared absorption spectrum has a strong band at 1650 cm.$^{-1}$ which is assigned to the stretching vibration of a xanthone hydrogen-bonded carbonyl group (cf. 1-hydroxyxanthone, which has the corresponding band at 1652 cm.$^{-1}$); (iii) *O*-methylsterigmatocystin, when reduced with lithium aluminium hydride,[10] yielded a

* Part VI, *J.*, 1960, 785.

† Compare ref. 6 which records the formula, $C_{18}H_{14}O_6$, as deduced from analyses on a crystallised sample. Strong retention of traces of solvents appears to be characteristic of certain xanthones (cf. draconol [8]).

[1] Thom and Raper, " A Manual of the Aspergilli," Williams and Wilkins Coy., Baltimore, 1945, p. 183.

[2] (*a*) Hatsuda and Kuyama, *J. Agric. Chem. Soc. Japan*, 1954, **28**, 989; (*b*) Hatsuda, Kuyama, and Terashima, *ibid.*, pp. 992, 998; (*c*) *Chem. Abs.*, 1956, **50**, 15,522.

[3] Abou-Zeid, Ph.D. Thesis, London, 1953.

[4] Birkinshaw and Hammady, *Biochem. J.*, 1957, **65**, 162.

[5] Davies, Ph.D. Thesis, Nottingham, 1956.

[6] Davies, Roberts, and Wallwork, *Chem. and Ind.*, 1956, 178.

[7] Cf. Roberts, Davies, and Kirkaldy, Communication Abs., 4th Internat. Congr. Biochemistry, Vienna, 1958, p. 9.

[8] (*a*) Brockmann, Haase, and Freiensehner, *Ber.*, 1944, **77**, 279; (*b*) Robertson, Whalley, and Yates, *J.*, 1950, 3117.

[9] Yates and Stout, *J. Amer. Chem. Soc.*, 1958, **80**, 1691.

[10] Mustafa and Hilmy, *J.*, 1952, 1343; Shah, Kulkarni, and Joshi, *J. Sci. Ind. Res., India*, 1954, **13**, *B*, 186; *Chem. Abs.*, 1955, **49**, 6929.

compound which, from its analysis and ultraviolet absorption spectrum (Fig. 2), was considered to be a xanthen derivative; (iv) degradation of sterigmatocystin with aluminium chloride in chlorobenzene yielded (as had been shown by Hatsuda *et al.*[2b]) 1,3,8-trihydroxyxanthone; (v) the ultraviolet absorption spectrum of *O*-methylsterigmatocystin was very similar to that of 1,3,8-trimethoxyxanthone (Fig. 3). There was therefore little doubt that sterigmatocystin was a 3,8-substituted 1-hydroxyxanthone.

FIG. 1.

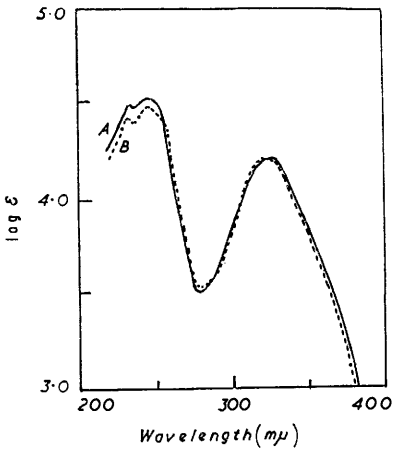


FIG. 2.

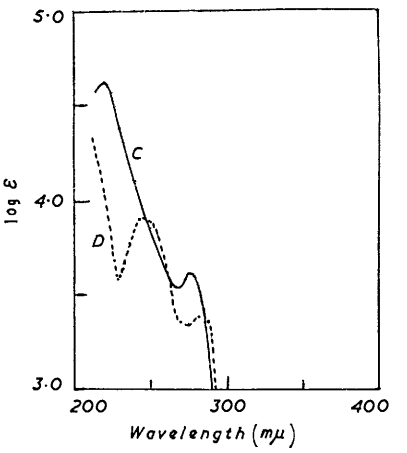


FIG. 3.

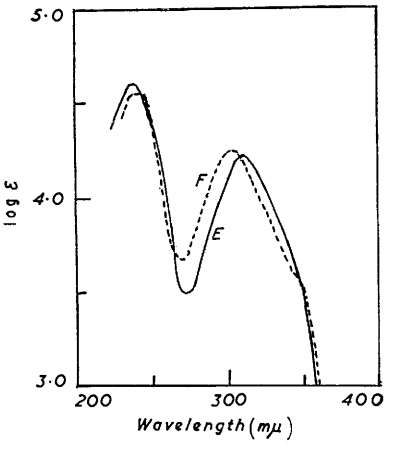
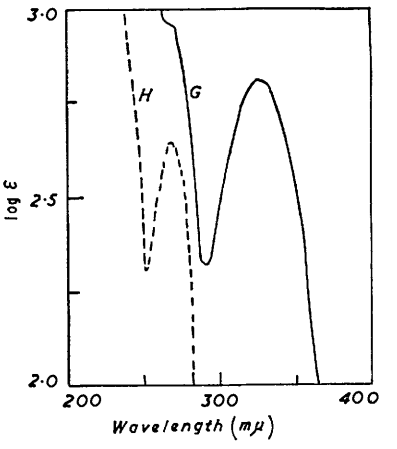


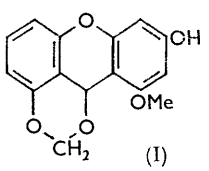
FIG. 4.



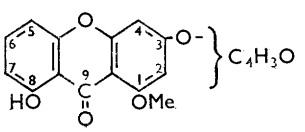
FIGS. 1—4. *Ultraviolet absorption spectra of*: *A*, *sterigmatocystin* (VII); *B*, *dihydrosterigmatocystin*; *C*, *xanthen derivative of* O-*methylsterigmatocystin*; *D*, *xanthen*; *E*, O-*methylsterigmatocystin*; *F*, 1,3,8-*trimethoxyxanthone*; *G*, *oxidation product* (VIII) *of* 5-*hydroxydihydrosterigmatocystin*; *H*, O-*dimethylphloroglucinol*.

Oxidation of *O*-methylsterigmatocystin, with potassium permanganate, gave, among other products, 2-hydroxy-6-methoxybenzoic acid which was identified by paper chromatography. Similar oxidation of sterigmatocystin gave γ-resorcylic acid, proving the unprotected hydroxyl group in this compound to be in position 8. With a smaller proportion of oxidant, sterigmatocystin gave a result in agreement with that previously obtained by Hatsuda *et al.*;[2b] in this case, the product was a crystalline acid, $C_{15}H_{10}O_7$,

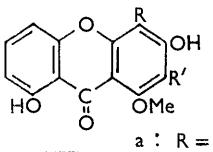
which, when pyrolysed and sublimed, gave 3,8-dihydroxy-1-methoxyxanthone ($C_{14}H_{10}O_5$), identical with a specimen synthesised by the Tanase method [2b,11] from γ-resorcylaldehyde and *O*-monomethylphloroglucinol. The methoxyl group in sterigmatocystin therefore occupies position 1 and we may now formulate the metabolite as (II) and the acidic oxidation product mentioned above as (IIIa or b).



(I)



(II)



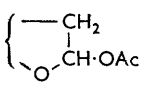
(III)

a : R = H, R′ = CO_2H
b : R = CO_2H, R′ = H

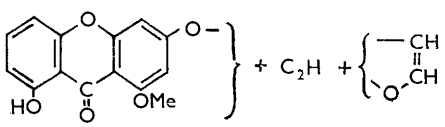
Quantitative hydrogenation of sterigmatocystin, under mild conditions, established the presence of one ethylenic bond in the molecule. Infrared absorption bands for sterigmatocystin [3099(w), 1610(s), 1067, and 722 cm.$^{-1}$, which were absent from the spectrum of dihydrosterigmatocystin] indicated the presence of a vinyl ether grouping and the bands corresponded closely with those recorded for 2,3-dihydrofuran.[12] The presence of a vinyl ether group was confirmed by ozonolysis of *O*-methylsterigmatocystin, hydrolysis of the product, and isolation of formic acid (0·85 mol.).

Sterigmatocystin, with pyridine and acetic anhydride at 90° (1—2 hr.), gave crystalline but heterogeneous material. Birkinshaw *et al.*[4] (using more rigorous conditions) reported the preparation of a " diacetate " $C_{22}H_{18}O_9$ which they formulated as $C_{18}H_{10}O_6(CO{\cdot}CH_3)_2,H_2O$. We have prepared this " diacetate," m. p. 228—229°, and found that it cannot be hydrogenated. We conclude that a molecule of acetic acid has added to the vinyl ether grouping, to give acetoxymono-*O*-acetyldihydrosterigmatocystin (IV). The infrared absorption spectrum of the compound had no bands at 710 and 1610 cm.$^{-1}$ (absence of an isolated double bond) but possessed an unsymmetrical band at 1767 cm.$^{-1}$ with a shoulder at about 1750 cm.$^{-1}$, corresponding to the carbonyl absorption of phenyl acetate [13] (1766 cm.$^{-1}$) and an alkyl acetate [14] (1735—1750 cm.$^{-1}$). Addition of a molecule of acetic acid to a vinyl ether grouping has been noted previously.[15] Dihydrosterigmatocystin behaves normally on acetylation, yielding a mono-*O*-acetyl derivative.

Structure (II) for sterigmatocystin may now be extended to partial structure (V). Since the ultraviolet absorption spectrum of dihydrosterigmatocystin is essentially similar



(IV)

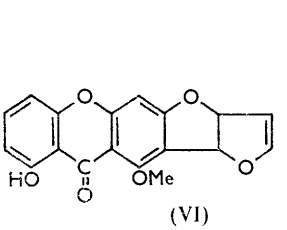


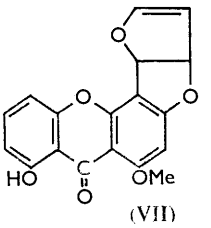
(V)

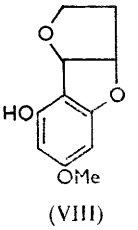
to that of sterigmatocystin (Fig. 1), the ethylenic link of the vinyl ether system cannot be in conjugation with the main chromophore. Valency analysis of dihydrosterigmatocystin indicates the presence of two rings in addition to those in the xanthone nucleus. Three further facts are pertinent: sterigmatocystin is optically active; dihydrosterigmatocystin is stable towards moderately concentrated mineral acid (absence of an acetal group); and one carbon atom must be attached to position 2 or 4 in the xanthone nucleus [cf. (III)

[11] Tanase, *J. Pharm. Soc. Japan*, 1941, **61**, 341.
[12] (*a*) Meakins, *J.*, 1953, 4170; (*b*) Barr and Rose, *J.*, 1954, 3766.
[13] Hartwell, Richards, and Thompson, *J.*, 1948, 1436.
[14] Thompson and Torkington, *J.*, 1945, 640.
[15] Normant, *Compt. rend.*, 1949, **228**, 102.

above]. Hence sterigmatocystin has structure (VI) or (VII): a decision between them was arrived at in the following way.


(VI)

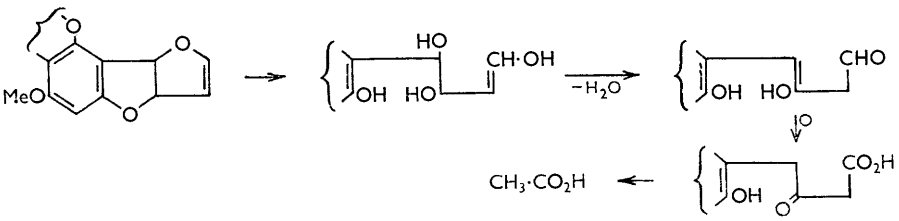

(VII)


(VIII)

It has been shown that some 1(or 8)-hydroxyxanthones, having a free 4(or 5)-position, can be hydroxylated and oxidised to give a salicylic acid.[16] By this method dihydrosterigmatocystin yielded dihydro-5-hydroxysterigmatocystin but oxidation of this with alkaline hydrogen peroxide gave, by spontaneous decarboxylation, a phenol, $C_{10}H_9O_3$·OMe, whose ultraviolet absorption spectrum resembles that of *O*-dimethylphloroglucinol (Fig. 4); the bathochromic shift (59 mμ), due to nuclear substitution and ring fusion, is, however, unexpectedly large (cf. the bathochromic shift of 13 mμ in the spectra [17] of anisole and of 2,3-dihydrobenzofuran). The infrared absorption spectrum of this compound showed bands at 908 and 1078 cm.$^{-1}$(s) which are attributed to C–O–C stretching in the fully reduced furan ring.[12b] The position *para* to the phenolic group is free (Gibbs test [18]) and we therefore formulate this product as (VIII) and the carboxylic acid, produced by oxidation of sterigmatocystin with potassium permanganate, as (IIIb). Attempts to synthesise this acid have so far been abortive.

We therefore suggest structure (VII) for sterigmatocystin. Discussion of the structure of isosterigmatocystin is reserved until we have obtained more information.

One further observation requires comment. Birkinshaw *et al.*[4] found that sterigmatocystin, when fused with potassium hydroxide, gave resorcinol and acetic acid (*ca.* 0·7 mol.). The production of acetic acid, which is remarkable since sterigmatocystin contains no *C*-methyl group, may be rationalised as in the annexed scheme.



Sterigmatocystin has no significant tuberculostatic or amœbicidal activity. We thank Dr. D. A. Peak of Boots Pure Drug Co. Ltd. for this information.

Experimental

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

Isolation of Sterigmatocystin.—*Aspergillus versicolor* (Vuillemin) Tiraboschi (Commonwealth Mycological Institute, No. 49,124) was kept in sub-culture on Czapek–Dox agar slopes. For production of sterigmatocystin, the mould was grown in surface culture on a solution of sucrose

[16] Roberts, *J.*, 1960, 785.
[17] Burawoy and Chamberlain, *J.*, 1952, 2310; Entel, Ruof, and Howard, *J. Amer. Chem. Soc.*, 1951, **73**, 4152.
[18] King, King, and Manning, *J.*, 1957, 563.

(3%) and inorganic salts (Czapek–Dox formula [4]) in de-ionised water. Flat, round culture flasks,[19] each containing 500 ml. of the medium, were sterilised and, after having been inoculated with a heavy aqueous spore suspension, were kept at 30° ± 1° in the dark for 21 days. The mycelium was collected, washed, and dried *in vacuo* at 45°. The finely ground mycelium (*ca.* 380 g. from 100 flasks) was extracted (Soxhlet) with acetone for 48 hr. The extract (1 l. from 100 g. of mycelium) was kept at 0° overnight, filtered, concentrated to *ca.* 40 ml., and chilled. The crude sterigmatocystin (m. p. 220—225°) which was deposted was collected and dissolved in chloroform. The filtered chloroform solution was poured on to a column (30 × 5 cm.) of magnesium oxide (Hopkin and Williams Ltd., heavy quality; previously heated to 250° for 2 hr.). Development of the column with chloroform produced (in order from the top) purple-brown, orange-brown, and yellow bands. Continued percolation of chloroform through the column eluted the yellow band. The yellow eluate was evaporated to dryness, and the residue was crystallised from acetone to give sterigmatocystin, m. p. 241—242° (decomp.) (*ca.* 1·3% calc. on the dry mycelium). Sublimation of this material at 180°/0·5 mm. gave pure sterigmatocystin, m. p. 246° (decomp.).

Subsequent extraction of the acetone-extracted mycelium with ethanol yielded mannitol, identified as its hexa-acetate which formed needles, m. p. and mixed m. p. 123—124°.

Sterigmatocystin was also isolated in a yield of *ca.* 1·2% from another strain of *A. versicolor* (L.S.H.T.M. Cat. No. Ac. 59) which had been kindly supplied by Professor Birkinshaw. Attempts to obtain the metabolite from *A. versicolor* (C.M.I. 16,139) were abortive.

General Properties of Sterigmatocystin.—The sublimed material forms pale-yellow needles, m. p. 246° (decomp.), $[\alpha]_D^{20\cdot5}$ −387° (*c* 0·424 in $CHCl_3$) (Found: C, 66·9; H, 3·7; OMe, 9·5; *C*-Me, 0. Calc. for $C_{17}H_9O_5$·OMe: C, 66·7; H, 3·7; OMe, 9·6%), $\lambda_{max.}$ 205, 233, 246, and 325 mμ (log ε 4·40, 4·49, 4·53, and 4·21 respectively), $\nu_{max.}$ 3450w, 3099w, 2995w, 2975w, 2920w, 1650s, 1627s, 1610s, 1590s, 1496, 1482s, 1447, 1415, 1400, 1362s, 1347, 1322, 1300, 1272s, 1239s, 1197, 1179, 1122, 1098, 1067, 1044, 1019w, 993, 978, 952, 932w, 904w, 895w, 846, 823, 774, 756, 735, 722, 702w, and 668w cm.$^{-1}$. Sterigmatocystin is insoluble in water, aqueous sodium carbonate, and aqueous sodium hydroxide but gives a deep-yellow colour with the last-mentioned reagent. It is sparingly soluble in most organic solvents but quite readily soluble in chloroform and pyridine. With concentrated sulphuric acid it gives a dark, green-brown colour. It gives a green colour with ethanolic ferric chloride and a yellow-brown colour with aqueous ferric chloride. It gives a positive Gibbs reaction.[18] Sterigmatocystin (0·1 g.) was recovered unchanged after having been shaken with ethanol (50 ml.) and concentrated hydrochloric acid (10 ml.) for 7 days. When a similar mixture was kept, with continuous stirring, at 40—50° for three days a *product* was obtained which separated from ethanol in yellow needles, m. p. 225—227° (decomp.) (Found: C, 64·1; H, 5·7%). This compound has not been identified.

Since sterigmatocystin, when treated with concentrated sulphuric acid, produces a deep colour, the normal methods [20] of testing for a methylenedioxy-group are vitiated. The following method was devised.[21] About 2 mg. of the substance were heated in a sealed glass tube with 1 ml. of 90% phosphoric acid at 80—90° for 20 min. The tube was cooled thoroughly and crushed under 5 ml. of iced water. The solution was distilled, the first 0·6 ml. of distillate being collected. 0·3 Ml. of distillate was used in a chromotropic acid test for formaldehyde.[22] Sterigmatocystin yielded a negative test (cf. Hatsuda *et al.*[2b]).

1-*Hydroxyxanthone.*—This compound, m. p. 146—147°, was prepared by Michael's method.[23] Infrared absorption bands were at 1652s, 1616s, 1580, 1555, 1483s, 1468s, 1382, 1355w, 1337w, 1290s, 1240s, 1221, 1183w, 1166, 1121w, 1067, 1029w, 938w, 868w, 821, 780, 730, and 677 cm.$^{-1}$.

Dihydrosterigmatocystin.—Sterigmatocystin (31·1 mg.), glacial acetic acid (10 ml.), and 5% palladised charcoal (30 mg.) were shaken in hydrogen. Up-take (2·08 ml., 0·97 mol.) ceased after 40 min. The catalyst was filtered off and the solvent removed *in vacuo*. The residue (30 mg.) was crystallised twice from ethanol to yield *dihydrosterigmatocystin* as yellow plates, m. p. 229—230° [Found (on a sublimed specimen): C, 66·7; H, 4·4. $C_{18}H_{14}O_6$ requires C, 66·3;

[19] See *Biochem. J.*, 1944, **38**, 456.

[20] Houben-Weyl, "Methoden der Organischen Chemie," 4th edn., Thieme, Stuttgart, 1953, Vol. II, p. 420.

[21] Cf. (*a*) Feigl, "Spot Tests in Organic Analysis," 5th English edn., Elsevier, Amsterdam, 1956, p. 190; (*b*) Pavolini and Malatesta, *Ann. Chim. appl.*, 1947, **37**, 495; *Chem. Abs.*, 1951, **45**, 8407.

[22] Feigl, *op. cit.*, p. 331.

[23] Michael, *Amer. Chem. J.*, 1883—1884, **5**, 81.

H, 4·3%], $\lambda_{max.}$ 208, 232, 247, and 325 mμ (log ε 4·31, 4·42, 4·49, and 4·21, respectively), $\nu_{max.}$ 3450w, 2995w, 2975w, 2920w, 1648s, 1622s, 1582s, 1495, 1482, 1450, 1415, 1398, 1346w, 1312, 1297w, 1275, 1238, 1202, 1181w, 1127, 1093, 1054, 1028w, 988w, 958, 926w, 912w, 893, 777, 751w, 735w, 705w, and 667 cm.$^{-1}$.

Dihydrosterigmatocystin was recovered unchanged after it had been heated under reflux with constant-boiling hydrochloric acid for 24 hr.

Isosterigmatocystin.—Sterigmatocystin (0·3 g.) was heated under reflux for 9 hr. with 15% ethanolic potassium hydroxide (100 ml.). The ethanol was removed by evaporation *in vacuo*, and the brown residue was mixed with water (100 ml.). The mixture was filtered, the filtrate was acidified (concentrated hydrochloric acid) and the mixture warmed to coagulate the precipitate, which was collected and crystallised from ethanol to yield isosterigmatocystin as light-brown rods (0·12 g.), m. p. 233—234° (Found: C, 66·6; H, 3·8; OMe, 12·7; active H, 0·54. Calc. for $C_{17}H_9O_5$·OMe: C, 66·7; H, 3·7; OMe, 9·6, two active H, 0·62%), $\lambda_{max.}$ 252 and 336 mμ (log ε 4·54 and 4·15, respectively), $\nu_{max.}$ 3484w, 3226, 2990w, 2922w, 2849w, 2748w, 1652s, 1601s, 1571s, 1514, 1483s, 1464, 1446, 1408s, 1348, 1325, 1297s, 1269, 1228s, 1192w, 1171, 1161, 1120, 1100, 1065w, 1041, 1016, 980w, 916w, 874, 817s, 799w, 787w, 774, 758, 727, 722, 683w, and 654 cm.$^{-1}$. The compound was optically inactive (in chloroform solution) and could not be hydrogenated under mild conditions. It was soluble in aqueous 2N-sodium hydroxide and 2N-sodium carbonate but was insoluble in sodium hydrogen carbonate solution. It gave a green colour with ethanolic ferric chloride and a purple colour with aqueous ferric chloride.

O-*Methylsterigmatocystin.*—Sterigmatocystin (0·35 g.), methyl sulphate (4 ml.), anhydrous potassium carbonate (4 g.), and dry acetone (100 ml.) were heated under reflux for 12 hr. Removal of the solids and evaporation of the filtrate yielded an oil which, when treated with a solution of ammonia (*d* 0·880; 2 ml.) and then with water (100 ml.), yielded a flocculent, white precipitate which was collected, washed and dried (0·32 g.). Crystallisation of this material from methanol gave *O*-methylsterigmatocystin as faintly yellow, slender rods, m. p. 265—267° [Found (on a sublimed sample): C, 67·4; H, 4·4; OMe, 18·6. Calc. for $C_{17}H_8O_4(OMe_2)$: C, 67·5; H, 4·2; OMe, 18·3%], $\lambda_{max.}$ 236, 309 mμ (log ε, 4·61 and 4·23 respectively), $\nu_{max.}$ (in $CHBr_3$): 3124w, 1662s, 1643s, 1603s, 1473s, 1438, 1418, 1382w, 1347, 1267s, 1250, 1229, 1204, 1075, 1040w, 1016, 970, 890w, 840w, 811, 770, and 752w cm.$^{-1}$. This compound gave no ferric reaction. In concentrated hydrochloric acid solution it yielded a yellow-orange precipitate of a ferrichloride on the addition of a solution of ferric chloride in concentrated hydrochloric acid.

O-Methylsterigmatocystin was also obtained by using methyl iodide (3·5 ml.), in place of methyl sulphate, in the method described above. Sterigmatocystin was recovered unchanged after treatment with diazomethane in ether–methanol.

Dihydro-O-*methylsterigmatocystin.*—(i) Dihydrosterigmatocystin (0·15 g.) with methyl iodide (2 ml.), anhydrous potassium carbonate (1 g.), and acetone (50 ml.) gave a *product* (0·13 g.), which, repeatedly crystallised from methanol, formed colourless rods, m. p. 282—283° [Found: C, 67·0; H, 4·7; OMe, 19·4. $C_{17}H_{10}O_4(OMe)_2$ requires C, 67·1; H, 4·8; OMe, 18·2%], $\lambda_{max.}$ 203, 237, and 311 mμ (log ε 4·42, 4·59, and 4·24, respectively).

(ii) *O*-Methylsterigmatocystin (0·25 g.), dissolved in ethyl acetate (125 ml.), was hydrogenated in the presence of 5% palladised charcoal (0·25 g.). The product was isolated in the usual way and crystallised from methanol as rods, m. p. 280°, unaltered on admixture with a sample prepared by method (i). Dihydro-*O*-methylsterigmatocystin (0·1 g.) was recovered unchanged after it had been vigorously shaken for 3 days with ethanol (20 ml.) and concentrated hydrochloric acid (5 ml.).

O-*Benzoylsterigmatocystin.*—To a solution of sterigmatocystin (0·25 g.) in pyridine (6 ml.) was added gradually benzoyl chloride (3 ml.). After the solution had been left overnight at room temperature, more benzoyl chloride (1 ml.) was added. The solution was heated under reflux for 15 min., poured into ice-water (50 g.), and left overnight. Extraction with chloroform (2 × 50 ml.), washing the extract with 2N-hydrochloric acid (2 × 50 ml.) and then with water (50 ml.), and evaporation of the chloroform yielded a brown oil which, on the addition of ethanol (5 ml.), gave a crude product (0·25 g.). Two crystallisations from ethanol yielded O-*benzoylsterigmatocystin* as colourless needles, m. p. 258—260° (Found: C, 69·6; H, 4·1. $C_{25}H_{16}O_7$ requires C, 70·1; H, 3·8%).

O-*Benzoyldihydrosterigmatocystin.*—(i) By the foregoing procedure dihydrosterigmatocystin yielded the required *derivative* which crystallised from ethanol as colourless needles, m. p. 256—258° (Found: C, 69·8; H, 4·3. $C_{25}H_{18}O_7$ requires C, 69·8; H, 4·2%).

(ii) Hydrogenation of *O*-benzoylsterigmatocystin, in glacial acetic acid and in the presence of 5% palladised charcoal, yielded an identical product (m. p. and mixed m. p.).

Acetoxymono-O-*acetyldihydrosterigmatocystin.*—Sterigmatocystin (0·3 g.), anhydrous sodium acetate (0·6 g.), and acetic anhydride (3 ml.) were heated under reflux for 6 hr. The *product*, isolated in the usual way, was crystallised twice from methanol to give colourless rods (50 mg.), m. p. 227—228° [Found: C, 62·1; H, 4·1; Ac, 22·5. $C_{18}H_{12}O_7(CO{\cdot}CH_3)_2$ requires C, 62·0; H, 4·3; Ac, 20·2%], $\nu_{max.}$ 1767s, 1662s, 1641s, 1601s, 1490, 1470s, 1421w, 1370, 1350w, 1315w, 1288, 1239s, 1211s, 1136, 1108, 1087, 1062, 1013, 973, 926, 900w, 816w, and 791w cm.$^{-1}$. No hydrogen was absorbed (during 50 min.) under the conditions described above for the hydrogenation of sterigmatocystin; the starting material was recovered unchanged.

O-*Acetyldihydrosterigmatocystin.*—By the foregoing acetylation technique, dihydrosterigmatocystin yielded O-*acetyldihydrosterigmatocystin*, needles (from methanol), m. p. 215—216° (Found: C, 65·0; H, 4·7; Ac, 11·8. $C_{18}H_{13}O_6{\cdot}CO{\cdot}CH_3$ requires C, 65·2; H, 4·4; Ac, 11·7%).

Reduction of O-*Methylsterigmatocystin by Lithium Aluminium Hydride.*—Lithium aluminium hydride (1·75 g.), *O*-methylsterigmatocystin (0·325 g.), and dry ether (175 ml.) were heated under reflux for 18 hr. After the mixture had been cooled, the excess of hydride was decomposed by the cautious addition of 2N-sulphuric acid. The organic layer was separated, washed with water, and dried (Na_2SO_4). Evaporation of the solvent yielded a residue (0·3 g.) which was repeatedly crystallised from methanol to give the *xanthen derivative* as small, colourless rods, m. p. 220—222° (Found: C, 70·1; H, 5·3. $C_{19}H_{16}O_5$ requires C, 70·4; H, 5·0%), $\lambda_{max.}$ 221, 275 mμ (log ε 4·62 and 3·62 respectively). Hydrogenation of this material in ethyl acetate in the presence of 5% palladised charcoal led to the *dihydro-derivative* which, after three crystallisations from ethanol, formed colourless needles, m. p. 233—236° (Found: C, 70·4; H, 5·8. $C_{19}H_{18}O_5$ requires C, 69·9; H, 5·6%).

Degradation of Sterigmatocystin with Aluminium Chloride.—A solution of sterigmatocystin (0·5 g.) in chlorobenzene (50 ml.) was heated under reflux with powdered aluminium chloride (3 g.) for 3 hr. The solvent was removed *in vacuo* at 100° and the residue, after addition of ice and 2N-hydrochloric acid, was kept overnight. The mixture was exhaustively extracted with ether, and the combined extracts were shaken with successive quantities of 10% aqueous potassium hydroxide until the aqueous layer was no longer coloured. The combined aqueous layers were acidified with concentrated hydrochloric acid, and the product was extracted into ether. The deep-red, fluorescent, ethereal solution was washed with water, dried (Na_2SO_4), and evaporated. The residue (0·4 g.) was sublimed at 140°/0·1 mm. to give yellow needles (0·3 g.), m. p. *ca.* 253°. Repeated crystallisation of this material from benzene (or from benzene containing a trace of ethyl acetate) gave 1,3,8-trihydroxyxanthone as pale-yellow needles, m. p. and mixed m. p. 258—259° (Found: C, 64·1; H, 3·2. Calc. for $C_{13}H_8O_5$: C, 63·9; H, 3·3%). The acetylated degradation product crystallised from methanol as colourless rods, m. p. and mixed m. p. 192—193°.

1,3,8-*Trihydroxyxanthone.*—This compound had previously been prepared by Hatsuda *et al.*[2b] using a Tanase-type [11] synthesis. We found the following synthesis [24] to be more convenient.

A mixture of 2,6-dihydroxybenzoic acid [25] (2 g.), dry phloroglucinol (2 g.), freshly fused zinc chloride (8 g.), and phosphorus oxychloride (24 ml.) was heated at 80° for 1½ hr. The cooled mixture was stirred into iced water (300 g.), and the resulting mixture was neutralised with sodium hydrogen carbonate solution. The solid product was collected, washed, dried, and exhaustively extracted (Soxhlet) with acetone. Evaporation of the acetone gave a deep-red residue which sublimed at 140—150°/0·05 mm. to give a yellow product (*ca.* 0·5 g.). This material was crystallised from aqueous methanol and then sublimed to give pure 1,3,8-trihydroxyxanthone, m. p. 259° (Found: C, 64·0; H, 3·2%) (Hatsuda *et al.*[2b] record m. p. 265°), $\lambda_{max.}$ 208, 228, 247, and 329 mμ (log ε 4·31, 4·39, 4·46, and 4·28, respectively), giving a green-brown colour with aqueous-ethanolic ferric chloride.

The acetyl derivative, prepared by pyridine–acetic anhydride, separated from ethanol in colourless rods, m. p. 193—194° [Found: C, 61·6; H, 4·1; Ac, 34·2. Calc. for $C_{13}H_5O_5(CO{\cdot}CH_3)_3$: C, 61·6; H, 3·8; Ac, 34·8%].

The tri-*O*-methyl derivative was prepared by methyl sulphate, anhydrous potassium carbonate, and acetone and isolated in the usual way. The crude material was sublimed

[24] Cf. Grover, Shah, and Shah, *J.*, 1955, 3982.
[25] Cartwright, Jones, and Marmion, *J.*, 1952, 3499.

and the sublimate crystallised from aqueous acetone. The product was sublimed at 170°/0·1 mm., to give 1,3,8-trimethoxyxanthone as colourless rods, m. p. 188—190° (Found: C, 66·7; H, 4·8. Calc. for $C_{16}H_{14}O_5$: C, 67·1; H, 4·9%), $\lambda_{max.}$ 204, 242, and 303 mμ (log ε 4·46, 4·56, and 4·26 respectively).

Oxidations involving a Large Proportion of Oxidant.—(i) *Sterigmatocystin.* Powdered potassium permanganate (0·51 g.) was added during 3 hr. to sterigmatocystin (67 mg.) in refluxing acetone (23 ml.). Water (75 ml.) was added and the mixture warmed (50°) and then filtered. The filtrate was adjusted to pH 10 with aqueous sodium hydroxide and reduced by evaporation *in vacuo* to 30 ml. Acidification with 2N-hydrochloric acid gave a yellow precipitate which was filtered off and washed with ether (20 ml.). The filtrate was extracted with ether (20 ml.), and the combined ethereal solutions were extracted with saturated sodium hydrogen carbonate solution (7 ml.). The aqueous layer was acidified and the liberated acids were extracted with ether. The acids were investigated by chromatography [26] on Whatman No. 1 paper, with (as descending solvent) the upper layer formed by equilibrating butan-1-ol (40 ml.), acetic acid (10 ml.), and water (50 ml.). The chromatograms were allowed to run for *ca.* 16 hr. and, after having been dried, were sprayed with a 1% solution of ferric chloride. Under these conditions, the residue was found to contain a substance (R_F 0·62, blue colour with ferric chloride) which was indistinguishable from γ-resorcylic acid. None of the other substances present was identifiable.

(ii) O-*Methylsterigmatocystin.* This substance, when degraded as described above, yielded a residue which on chromatography, with three solvent systems and a ferric chloride spray, gave a purple " spot " with R_F values as follows: (i) solvent as above, 0·83; (ii) solvent composed of ethanol (80 ml.), pyridine (12 ml.), and water (8 ml.), 0·45; (iii) solvent composed of the lower layer from *m*-cresol (50 ml.), acetic acid (2 ml.), and water (48 ml.), 0·90. Under the same conditions, 2-hydroxy-6-methoxybenzoic acid gave identical results.

Oxidations involving a Smaller Proportion of Oxidant.—(i) *Sterigmatocystin.* Powdered potassium permanganate (3 g.) was added in 2 hr. to sterigmatocystin (1 g.) in refluxing acetone (250 ml.). Water (400 ml.) was added, the mixture was warmed (50°) and filtered, and the residue washed with warm water (200 ml.). The combined filtrate and washings were adjusted to pH 10 with N-sodium hydroxide and concentrated, by distillation *in vacuo*, to 500 ml. After acidification with 2N-hydrochloric acid the solution was extracted with ether (3 × 500 + 200 ml.). The combined ethereal solutions were shaken with saturated sodium hydrogen carbonate solution (2 × 100 ml.). The aqueous layers were acidified and the precipitate, separated by centrifugation, was washed and dried (0·45 g.). This material, after two crystallisations from glacial acetic acid, followed by crystallisation from ethanol (charcoal), yielded 3,8-*dihydroxy*-1-*methoxyxanthone*-4-*carboxylic acid* as yellow needles, decomp. 190—210° (Found: C, 59·3; H, 3·2; OMe, 11·6. $C_{14}H_7O_6$·OMe requires C, 59·6; H, 3·3; OMe, 10·3%), $\lambda_{max.}$ 231, 250 (infl.), 260, 310, and 350 (infl.) mμ (log ε 4·44, 4·50, 4·56, 4·01, and 3·73, respectively), giving a brown colour with ethanolic ferric chloride. Fractional sublimation of this acid yielded a main fraction which was resublimed at 290°/0·5 mm. to give 3,8-dihydroxy-1-methoxyxanthone as yellow needles, m. p. and mixed m. p. 331—332° (decomp.) (Found: C, 64·8; H, 4·0; OMe, 12·0. Calc. for $C_{13}H_7O_4$·OMe: C, 65·1; H, 3·9; OMe, 12·0%), $\lambda_{max.}$ 206, 232, 247, and 319 mμ (log ε 4·36, 4·43, 4·50, and 4·23, respectively).

(ii) O-*Methylsterigmatocystin.* Oxidation of *O*-methylsterigmatocystin by the foregoing method yielded an acid (0·5 g.). This could not be crystallised, but gave after treatment (12 hr.) with an excess of diazomethane in ether, a substance which crystallised from methanol in pale-yellow prisms, m. p. 201—203°, and gave analytical figures corresponding to a hydrated form of *methyl* 1,3,8-*trimethoxyxanthone*-4-*carboxylate* [Found: C, 61·3; H, 4·5; OMe, 35·7. $C_{14}H_4O_3(OMe)_4$,$\frac{1}{2}H_2O$ requires C, 61·2; H, 4·9; OMe, 35·1%], $\lambda_{max.}$ 204, 236, and 306 mμ (log ε 4·28, 4·50, and 4·19 respectively).

3,8-*Dihydroxy*-1-*methoxyxanthone.*—The following synthesis is a modification of that described by Hatsuda *et al.*[2b] A solution of γ-resorcylaldehyde [27] (1 g.) and *O*-methylphloroglucinol [28] (1 g.) in acetic acid (7·5 ml.) and concentrated hydrochloric acid (3 ml.) was heated under reflux for 20 min. and then left overnight. The dark red crystalline 3,8-dihydroxy-1-methoxyxanthylium chloride was collected, washed with glacial acetic acid (2 ml.), and dried

[26] Bate-Smith and Westall, *Biochim. Biophys. Acta*, 1950, **4**, 427.
[27] Shah and Laiwalla, *J.*, 1938, 1828.
[28] Weidel and Pollak, *Monatsh.*, 1900, **21**, 15.

(0·57 g.). A suspension of this salt in ethanol (30 ml.) was hydrogenated at atmospheric pressure by using Adams catalyst (10 mg.). Absorption of hydrogen (44 ml.) was complete in 50 min. The mixture was filtered and the ethanol evaporated *in vacuo*, to give crude 3,8-dihydroxy-1-methoxyxanthen as a red resin. A solution of this substance in acetic anhydride (25 ml.) was heated under reflux for ½ hr. and poured into iced water (100 g.). The mixture was extracted with ether (3 × 30 ml.), and the ethereal solution, after having been extracted with a solution of sodium hydrogen carbonate (2 × 50 ml.) and washed with water (50 ml.), was dried ($MgSO_4$). Removal of the ether and crystallisation of the residue from ethanol gave 3,8-diacetoxy-1-methoxyxanthen (0·3 g.) as colourless rods, m. p. 145—146°. To a warm (45—50°) solution of this material (100 mg.) in acetic anhydride (2 ml.) and acetic acid (1 ml.), was added during 3 hr. a solution of chromium trioxide (100 mg.) in water (0·1 ml.) and glacial acetic acid (2 ml.), diphenylamine being used as external indicator. The solution was mixed with water (45 ml.), and the product extracted into ether (2 × 100 ml.). The ethereal solution was washed with sodium hydrogen carbonate solution (2 × 50 ml.) and then with water (25 ml.). Removal of the ether gave 3,8-diacetoxy-1-methoxyxanthone (60 mg.), m. p. 152°. A solution of this product in ethanol (10 ml.) and concentrated hydrochloric acid (1 ml.) was heated under reflux for 2 hr. and then cooled. The precipitated material was collected, washed, dried (48 mg.), and sublimed at 250—260°/0·05 mm. to give 3,8-dihydroxy-1-methoxyxanthone, m. p. 331—334° (decomp.) (Found: C, 65·0; H, 4·4; OMe, 11·6%). Its ultraviolet absorption spectrum was identical with that of the specimen which had been obtained in the degradation described above.

Ozonolysis of O-*Methylsterigmatocystin.*—A stream of ozonised oxygen was bubbled through a solution of *O*-methylsterigmatocystin (0·325 g.) in chloroform (120 ml.) which was cooled in acetone and solid carbon dioxide. When absorption was complete the solution was allowed to regain room temperature and the chloroform was removed *in vacuo*. The residue was treated with water (50 ml.) and left overnight. The mixture was heated on a steam-bath for 1 hr. and then filtered (to give a residue A; see below). The filtrate was distilled to minimum volume. Water (20 ml.) was added to the residue and distillation was again carried out. This operation was repeated several times. The combined distillates required 15·9 ml. of 0·5N-sodium hydroxide (≡ 0·85 mol. of a monobasic acid) for neutralisation to phenolphthalein (external indicator). The volume of the neutralised solution was reduced by distillation to 10 ml. and this concentrate was shaken with " Zeocarb-225 " and then filtered. The filtrate gave positive tests for formic acid (i) with Tollens's reagent, (ii) with mercuric chloride (in neutral solution), and (iii) in the chromotropic acid reaction [ref. 21(*a*), p. 340]. No acid, other than formic acid, could be detected in the filtrate by paper chromatography.[29]

The residue, A (*ca.* 0·25 g.), was a green-yellow, acidic substance which could not be obtained crystalline.

Elbs Persulphate Oxidation of Dihydrosterigmatocystin.—Dihydrosterigmatocystin (2 g.) was shaken with 25% w/v aqueous tetraethylammonium hydroxide (50 ml.) for 1 hr. To the resulting solution was added, during 8 hr. and with continuous shaking, a solution of potassium persulphate (1·8 g.) in water (50 ml.). The mixture, which had become gelatinous, was shaken for a further 12 hr. and was then acidified to Congo Red with 2N-hydrochloric acid. The precipitate was removed. Concentrated hydrochloric acid (20 ml.) was added to the filtrate and the mixture was heated on the steam-bath for 1 hr. and then kept at room temperature overnight. The brown precipitate was collected, washed, dried (0·8 g.), and crystallised first from ethanol (charcoal) and then from ethanol–benzene to give *dihydro-5-hydroxysterigmatocystin* (0·2 g.) as yellow rods, m. p. 260—262° (decomp.) (Found: C, 61·5, 61·4; H, 4·3, 4·5; OMe, 10·2, 9·0. $C_{17}H_{11}O_6 \cdot OMe, \frac{1}{2}H_2O$ requires C, 61·7; H, 4·3; OMe, 8·8%), $\lambda_{max.}$ 248, 280, and 329 mμ (log ε 4·41, 4·03, and 4·01 respectively). Two sublimations of this compound at 200°/0·02 mm. gave the anhydrous *compound*, m. p. 264—265° (Found: C, 63·0; H, 4·1. $C_{18}H_{14}O_7$ requires C, 63·2; H, 4·1%).

Oxidation of Dihydro-5-hydroxysterigmatocystin.—To a solution of the hydrated form of this substance (0·8 g.) in 1% aqueous sodium hydroxide (200 ml.) was added 3% hydrogen peroxide solution (160 ml.). The solution was kept at room temperature for 2½ days and then filtered from a small amount of deposited solid. The filtrate was acidified with 2N-hydrochloric acid and extracted with ether (3 × 150 ml.). The combined ethereal extracts were washed with a saturated solution of sodium hydrogen carbonate and then with water until the pH of the washings did not exceed 7. Evaporation of the *wet* ether left a mixture of water and an oil which

[29] Reid and Lederer, *Biochem. J.*, 1951, **50**, 60.

subsequently crystallised. The crystals were collected, washed with ether (3 ml.), and dried at 60° *in vacuo*. The *product* (21 mg.) appeared as clusters of colourless rods, m. p. 152—154° (decomp.) (Found: C, 63·6; H, 5·9; OMe, 15·0. $C_{10}H_9O_3$·OMe requires C, 63·5; H, 5·8; OMe, 14·9%), $\lambda_{max.}$ 326 mμ (log ε 2·81), $\nu_{max.}$ 3350, 3008w, 2983w, 2960w, 2890w, 2855w, 1635s, 1570w, 1518s, 1477, 1451s, 1380, 1348, 1326, 1300w, 1254s, 1215, 1200s, 1150s, 1078s, 1062s, 1040, 969s, 932s, 908, 864, 834, 823, 789, 731w, and 699w cm.$^{-1}$. This material was almost insoluble in water but dissolved readily in 2N-sodium hydroxide and 2N-sodium carbonate. Its ethanolic solution gave no colour with aqueous ferric chloride but gave a brown colour with ethanolic ferric chloride. It gave a strong, positive Gibbs test,[18] the blue solution having maximum light absorption at 635—640 mμ.

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