808. *Studies in Mycological Chemistry. Part XI.* The Structure of Isosterigmatocystin and an Amended Structure for Sterigmatocystin.*

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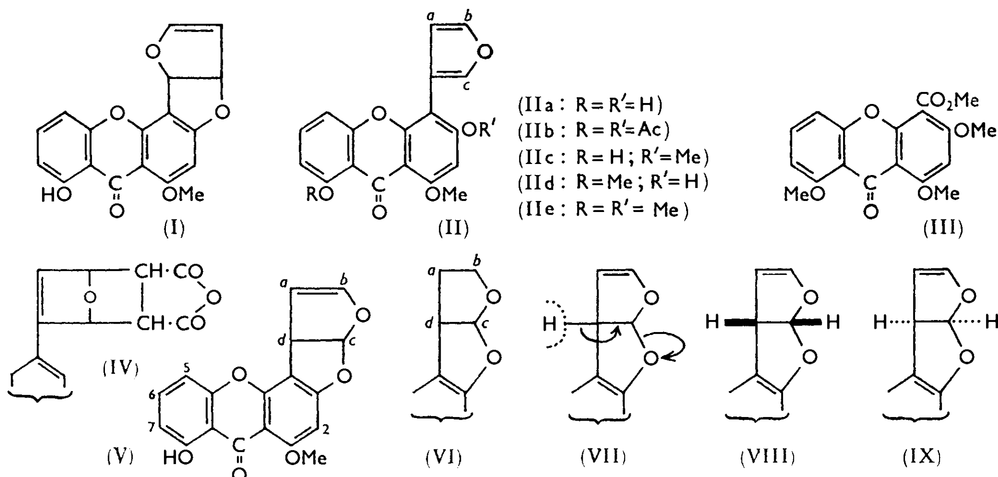
Isosterigmatocystin, produced by alkali treatment of sterigmatocystin, is shown to be 4-3′-furyl-3,8-dihydroxy-1-methoxyxanthone (IIa). The previously assigned structure (I) for sterigmatocystin is amended to (V).

We have previously suggested [1,2] that sterigmatocystin, an optically active metabolite of *Aspergillus versicolor* (Vuill.) Tiraboschi, has structure (I). The presence of a vinyl ether system [1] in sterigmatocystin has been further confirmed by titration with per-acid, and by the addition (in the presence of mineral acid) of methanol, ethanol, and acetic acid to the double bond. Hot ethanolic potassium hydroxide converts sterigmatocystin into an optically inactive compound, isosterigmatocystin,[1,3] for which we now record the establishment of structure (IIa).

Isosterigmatocystin, $C_{18}H_{12}O_6$, is a pale yellow crystalline compound which gives a purple ferric reaction in aqueous ethanol. It is xanthonoid in nature (see below) and, in contrast to sterigmatocystin, is soluble in aqueous sodium carbonate solution, thus indicating the liberation, during the isomerisation, of a hydroxyl group in the 3-position of the xanthone nucleus. The molecule contains one methoxyl group (Zeisel) and, since it forms a di-*O*-acetyl derivative (IIb), two free hydroxyl groups.



Isosterigmatocystin yields, with diazomethane, a mono-*O*-methyl derivative (IIc) which is different from the monomethyl ether (IId) prepared by isomerisation of *O*-methylsterigmatocystin (I; OMe for OH). Both of these monomethyl ethers give the same dimethyl ether (IIe) on further methylation. (This fully methylated compound appears to be the same as that previously obtained [3] but incorrectly designated as a mono-*O*-methyl derivative.) Ozonolysis of di-*O*-methylisosterigmatocystin (IIe) yields formic acid (2 mol.) and, in good yield, a xanthonoid acid whose methyl ester is identical with a synthetic specimen [2] of methyl 1,3,8-trimethoxyxanthone-4-carboxylate (III). The latter observation, together with the proved stability of dihydrosterigmatocystin to the isomerisation conditions, is important in establishing the retention of the intact xanthone

* Part X, *J.*, 1962, 2063.

[1] Davies, Kirkaldy, and Roberts, *J.*, 1960, 2169.
[2] Roberts and Underwood, *J.*, 1962, 2060.
[3] Birkinshaw and Hammady, *Biochem. J.*, 1957, **65**, 162.

nucleus during the isomerisation. (Reaction conditions similar to those used are known [4] to convert some xanthones into the corresponding benzophenones.) Isosterigmatocystin is thus a derivative of 3,8-dihydroxy-1-methoxyxanthone with the remainder of the molecule (C_4H_3O) attached to the 4-position.

That isomerisation is accompanied by an extension of the chromophore is evident from a comparison of the ultraviolet absorption spectrum of sterigmatocystin with that of 3-*O*-methylisosterigmatocystin (IIc). The bathochromic and hyperchromic effects observed are of the order to be expected for the additional conjugation of one ethylenic link with the xanthone nucleus (cf. the values for ethylbenzene and styrene [5] and for dihydro-osajin and osajin [6]). A comparison of the infrared absorption spectrum of di-*O*-methylisosterigmatocystin (IIe) with that of 1,3,8-trimethoxyxanthone indicates the presence, in the former compound, of a furan ring. Confirmation of the presence of this feature in isosterigmatocystin is found in the ready formation of a Diels–Alder adduct (IV) of di-*O*-methylisosterigmatocystin (IIe) with maleic anhydride. Since ozonolysis of di-*O*-methylisosterigmatocystin (IIe) yields 2 mol. of formic acid, the furan ring is attached through its β-position to the xanthone nucleus. (An α-substituted furan would yield 1 mol. of formic acid.)

Isosterigmatocystin thus has structure (IIa). This conclusion necessitates an amendment to the structure (I) previously suggested for sterigmatocystin which we now formulate as (V). The stability to acid of the acetal group [1] in dihydrosterigmatocystin (VI), which made us formerly favour structure (I) for sterigmatocystin, is apparently attributable to its peculiar molecular environment. The mechanism of the conversion of sterigmatocystin into its isomer can be represented as shown (VII).

The structural assignments described above have been confirmed by investigation of the proton magnetic resonance spectra of sterigmatocystin and of some of its derivatives. The resonances due to the protons of the xanthone nucleus are identified by comparison of the spectra of sterigmatocystin (V), dihydrosterigmatocystin (VI), and di-*O*-methylisosterigmatocystin (IIe). The proton H_2 gives a singlet at relatively high field whilst H_6, H_5, and H_7 form an AXY system with $J_{AX} = J_{AY}$. Thus H_6 appears as a triplet (J = 8·05 c.p.s.) and the same coupling constant, together with another of about 2 c.p.s., is found in a group (intensity 2) which corresponds, therefore, to H_5 and H_7 (see annexed Table).

Proton Magnetic Resonance Absorptions of Xanthone Protons (τ Scale).

	H_2	H_6	H_5 and H_7
Sterigmatocystin (V)	3·55	2·47	*ca.* 3·2
Di-*O*-methylisosterigmatocystin (IIe)	3·62	2·63	*ca.* 3·25
Dihydrosterigmatocystin (VI)	3·76	2·50	*ca.* 3·25

The spectrum of di-*O*-methylisosterigmatocystin (IIe) shows, in addition to the xanthone proton absorptions, three methyl (*O*-methyl) absorptions (at 5·91, 5·96, and 5·97τ) and three single-proton absorptions. The latter (which show typical furan coupling constants [7]) are (i) a quartet (J ~0·75 and 1·31 c.p.s.) at 2·27 τ (H_c), (ii) a triplet (J ~1·31 c.p.s.) at 2·57 τ (H_b), and (iii) a complex signal, in which J values are not clear, at 3·13 τ (H_a). These results confirm the presence of a β-substituted furan ring in isosterigmatocystin (IIa).

Sterigmatocystin (V) shows, in addition to the xanthone proton absorptions, a singlet at −3·26 τ (*O*-H), a peak of intensity 3 at 5·98 τ (*O*-methyl), and signals equivalent to four other protons. The simplest signal of these is a doublet (J = 6·9 c.p.s.) at 3·16 τ coupled only to a proton at 5·19 τ (consisting of two groups of triplets, which are not,

[4] Meisenheimer, Hanssen, and Wächterowitz, *J. prakt. Chem.*, 1928, **119**, 315.
[5] Gillam and Stern, "An Introduction to Electronic Absorption Spectroscopy in Organic Chemistry," 2nd edn., Arnold, London, 1957, pp. 134 and 141.
[6] Wolfrom, Harris, Johnson, Mahan, Moffett, and Wildi, *J. Amer. Chem. Soc.*, 1946, **68**, 406.
[7] Abraham and Bernstein, *Canad. J. Chem.*, 1961, **39**, 905.

however, simple triplets) and these must represent protons H_c and H_d respectively (see V). From known values for dihydrofuran,[8] it follows that signals at 3·48 τ and 4·53 τ (coupled together, $J = 2{\cdot}51$ c.p.s.) arise from H_b and H_a. Allylic couplings, which increase the complexity of the last-mentioned three signals, confirm the assignments.

The spectrum of dihydrosterigmatocystin (VI) is in accord with the above structural assignments, since all signals ascribed to protons in the bicyclic side-chain in sterigmatocystin (V) are altered by removal of the vinyl ether system. The proton H_c now appears as a doublet ($J = 5{\cdot}8$ c.p.s.) at 3·58 τ and this coupling also appears in a signal at 6·35 τ which is therefore due to H_d. The remaining protons appear as complex signals at 5·80 τ and 6·35 τ (probably *O*-methylene), 7·32 τ, and 8·76 τ. The wide separation within the latter groups is a reflection of the rigid stereochemistry of the bicyclic side-chain in which protons in an apparently similar environment are held in different positions relative to the ring-currents of the xanthone nucleus.

We find that it is virtually impossible to construct a scale-model of sterigmatocystin in which the two dihydrofuran rings are *trans*-fused. (In absence of other information, proton magnetic resonance spectroscopy does not give an absolute indication of the *cis*- or *trans*-coupling. The J_{cd} value indicates a dihedral angle of either ~23° or 134° across the link.[9]) It is thus probable that sterigmatocystin, which is lævorotatory, is to be represented by (VIII) or (IX).

Experimental

M. p.s were determined on the Kofler block. Ultraviolet spectra (of ethanolic solutions) were recorded on a Unicam S.P. 700, and infrared spectra (of substances in potassium bromide discs) on a Unicam S.P. 100 spectrophotometer. Proton magnetic resonance spectra (on substances in chloroform, or methylene dichloride) were recorded on an A.E.I. (RS 2) spectrometer; these spectra were calibrated by the side-band technique, tetramethylsilane being used as internal reference.

Sterigmatocystin.—This was obtained [1] from the dried mycelium of *A. versicolor* (Vuill.) Tiraboschi (C.M.I., No. 49,124) but we found that a more efficient separation of the metabolite could be effected by extraction (Soxhlet) with (i) acetone and (ii) chloroform. The solvents were removed *in vacuo*. The combined residues were dissolved in chloroform and chromatographed on heavy magnesium oxide as before [1] to give sterigmatocystin, m. p. (of a sublimed sample) 246°, $\lambda_{max.}$ 208, 235, 249, and 329 mμ (log ε 4·28, 4·39, 4·44, and 4·12, respectively).

Per-acid Titration of O-*Methylsterigmatocystin.*—A solution of *O*-methylsterigmatocystin (0·0805 g.) in chloroform (5 ml.) was mixed with an ethereal solution of *o*-monoperphthalic acid (0·3485 g.). During ½ hr. at 0°, 1·1 atomic equivalents of oxygen were absorbed. No pure oxidation product could be isolated.

Addition Reactions of the Vinyl Ether System.—(i) A solution of sterigmatocystin (50 mg.) in methanol (50 ml.) and concentrated hydrochloric acid (5 ml.) was kept at 50° for 3 days. Distillation *in vacuo* of the volatile materials yielded a residue which, after two crystallisations from methanol, gave yellow prisms (40 mg.), m. p. 296—297°. Sublimation of this material at 275°/0·05 mm. gave *dihydromethoxysterigmatocystin*, m. p. 298—299° [Found: C, 63·9; H, 4·5; OMe, 16·1. $C_{17}H_{10}O_5(OMe)_2$ requires C, 64·0; H, 4·5; OMe, 17·4%]. (ii) By a similar procedure, sterigmatocystin and ethanol gave a product which crystallised from ethanol in yellow needles. Sublimation of this material (235°/0·05 mm.) yielded *ethoxydihydrosterigmatocystin*, m. p. 252—254° (Found: C, 64·6; H, 5·0. $C_{20}H_{18}O_7$ requires C, 64·8; H, 4·9%). (iii) Similarly, *O*-methylsterigmatocystin and acetic acid gave material which crystallised from ethanol as needles. These, when sublimed at 155°/0·05 mm., yielded *acetoxydihydro-*O*-methylsterigmatocystin*, m. p. 176—177° (Found: C, 63·2; H, 4·7; Ac, 10·9. $C_{19}H_{15}O_7Ac$ requires C, 63·3; H, 4·6; Ac, 10·8%).

None of these compounds could be hydrogenated under the conditions used [1] for the conversion of sterigmatocystin into dihydrosterigmatocystin. The ultraviolet absorption spectra of these three compounds were virtually identical with those of the corresponding starting

[8] Jackman, "Nuclear Magnetic Resonance Spectroscopy," Pergamon Press, London, 1959, pp. 62 and 87.
[9] Conroy, *Adv. Org. Chem.*, 1960, 2, 311.

materials. The infrared absorption bands in the spectrum of sterigmatocystin which are characteristic of the vinyl ether group [3099(w), 1610(s), 1067, and 722 cm.$^{-1}$] and are absent from the spectrum of dihydrosterigmatocystin were also absent from the spectra of the above three compounds.

Isosterigmatocystin (IIa).—The following method of preparation gives better results than that previously described.[1] Sterigmatocystin (1 g.) and 15% ethanolic potassium hydroxide (250 ml.) were heated under reflux, in an atmosphere of nitrogen, for 12 hr. The solvent was removed by evaporation *in vacuo*, and water (250 ml.) was added. The mixture was filtered and the filtrate was acidified (acetic acid). The product was collected and crystallised from ethanol, giving isosterigmatocystin as pale yellow slender rods (0·75 g.), m. p. 233—234°, $\lambda_{max.}$ 212, 238, 253, and 340 mμ (log ε 4·48, 4·52, 4·59, and 4·24, respectively).

Isosterigmatocystin remained unchanged when its solution in ethyl acetate was shaken with 10% palladised charcoal in an atmosphere of hydrogen. More rigorous conditions (Adams catalyst/ethyl acetate) led to uptake of hydrogen but the amorphous product could not be purified.

Di-O-acetylisosterigmatocystin (IIb).—Isosterigmatocystin (100 mg.), pyridine (5 ml.), and acetic anhydride (0·2 ml.) were kept at room temperature for 4 days. The solvents were removed *in vacuo*, and the residue was crystallised twice from ethanol to give the *product* (cf. ref. 3) as needles (85 mg.), m. p. 206—208° [Found: C, 64·6; H, 4·0; Ac, 20·8. $C_{18}H_{10}O_6Ac_2$ requires C, 64·7; H, 4·0; Ac, 21·0%], $\lambda_{max.}$ 209, 242, 301, and 350 mμ (log ε 4·34, 4·53, 3·86, and 3·51, respectively), $\nu_{max.}$ 1762 (aryl acetate) and 1665 (xanthone carbonyl group) cm.$^{-1}$.

3-O-*Methylisosterigmatocystin* (IIc).—To a solution of isosterigmatocystin (100 mg.) in methanol (25 ml.) was added a solution of diazomethane (from 0·25 g. of methylnitrosourea) in ether (25 ml.). The mixture was kept at room temperature for 12 hr., and the solvents were then removed *in vacuo*. The residue, having been washed with 2N-aqueous sodium hydroxide and then with water, was crystallised twice from methanol, giving the *methyl ether* as pale yellow rods (85 mg.), m. p. 248° [Found: C, 67·5; H, 4·3; OMe, 11·8. $C_{17}H_8O_4(OMe)_2$ requires C, 67·5; H, 4·2; OMe, 18·4%], $\lambda_{max.}$ 205, 213, 236, 254, and 336 mμ (log ε 4·39, 4·39, 4·46, 4·60, and 4·16, respectively). It was insoluble in aqueous sodium hydroxide and gave a green-brown ferric reaction in ethanol. Treatment of this ether with pyridine–acetic anhydride (as above) gave the *acetate* as rhombs (from ethanol), m. p. 268° (Found: C, 65·9; H, 4·2; Ac, 11·3. $C_{19}H_{13}O_6Ac$ requires C, 66·3; H, 4·2; Ac, 11·3%).

8-O-*Methylisosterigmatocystin* (IId).—*O*-Methylsterigmatocystin (0·5 g.) was caused to isomerise by the method described above for the preparation of isosterigmatocystin. The *product* crystallised from methanol as pale yellow needles (0·4 g.), m. p. 230° (Found: C, 67·7; H, 4·2; OMe, 18·3%), $\lambda_{max.}$ 211, 241, 260, and 321 mμ (log ε 4·41, 4·56, 4·37, and 4·17, respectively). It was soluble in sodium hydroxide and in sodium carbonate solutions but insoluble in aqueous sodium hydrogen carbonate, and gave a negative ferric reaction (in ethanol). The *acetate* (prepared as above) crystallised from ethanol in rods, m. p. 189—190° (Found: C, 66·2; H, 4·4; Ac, 11·0%).

3,8-*Di-O-methylisosterigmatocystin* (IIe).—(i) Isosterigmatocystin (0·4 g.), methyl iodide (10 ml.), anhydrous potassium carbonate (5 g.), and dry acetone (250 ml.) were heated under reflux for 18 hr. The product, isolated in the usual way, was crystallised twice from chloroform–light petroleum (b. p. 60—80°) to yield the *dimethyl ether* as needles (0·35 g.), m. p. 173—174° [Found: C, 67·8; H, 4·3; OMe, 22·2. $C_{17}H_7O_3(OMe)_3$ requires C, 68·2; H, 4·6; OMe, 26·4%], $\lambda_{max.}$ 216, 243, and 320 mμ (log ε 4·40, 4·55, and 4·16, respectively), $\nu_{max.}$ 3153, 1603, 1509, and 878 (furan),[10] and 1653 (carbonyl group) cm.$^{-1}$. It gave a negative ferric test and was insoluble in aqueous sodium hydroxide.

(ii) Both of the mono-*O*-methyl ethers (above), on further methylation by the appropriate method, yielded the same product, which was identical (m. p. and mixed m. p.) with the di-*O*-methyl ether.

1,3,8-*Trimethoxyxanthone*.—This was prepared as previously described.[1] Infrared absorption bands were present at 3001, 2930, 2865, 1651, 1606, 1579, 1471, 1440, 1425, 1390, 1339, 1327, 1311, 1290, 1268, 1229, 1208, 1170, 1153, 1117, 1097, 1085, 950, 889, 823, 771, and 722 cm.$^{-1}$.

Attempted Isomerisation of Dihydrosterigmatocystin.—This substance was treated with

[10] Cf. Barton, Pradhan, Sternhell, and Templeton, *J.*, 1961, 266; Bailey, Hakki, and Bost, *J. Org. Chem.*, 1955, **20**, 1035.

ethanolic potassium hydroxide for 36 hr. as described above for the isomerisation of sterigmatocystin. Only unchanged material was recovered.

*Ozonolysis of Di-*O*-methylisosterigmatocystin.*—Ozonised oxygen was bubbled through a solution of the dimethyl ether (0·5 g.) in methylene dichloride (300 ml.) at −10° until the solution turned deep yellow-green. The solvent was evaporated *in vacuo* and the residue was treated with water (250 ml.) and hydrogen peroxide (10 ml.; 100 vol.) overnight at room temperature. The mixture was filtered to give a residue A (see below). The filtrate was distilled to minimum volume. Water (20 ml.) was added and the process was repeated several times. The combined distillates required 27·8 ml. of 0·1045N-sodium hydroxide (≡2·05 mol. of a monobasic acid) for neutralisation to phenolphthalein as external indicator. The volume of the neutralised solution was reduced to 10 ml. by evaporation and the concentrate was shaken with Zeocarb-225 and filtered. The filtrate gave positive tests for formic acid with Tollens's reagent and with chromotropic acid.[11] No acid other than formic acid could be detected in the filtrate by paper chromatography.[12]

The residue A (*ca.* 0·3 g.) was methylated by diazomethane (from 3 g. of methylnitrosourea) in methanol–ether (1 : 1) for 12 hr. at room temperature. The product was crystallised from methanol and was then sublimed (180°/0·05 mm.) to give methyl 1,3,8-trimethoxyxanthone-4-carboxylate (250 mg.) as needles (Found: C, 62·4; H, 4·8. $C_{18}H_{16}O_7$ requires C, 62·8; H, 4·7%), m. p. (and mixed m. p. with a synthetic sample [2]) 203°. The degradation product and the synthetic material had identical infrared absorption spectra.

*Adduct of Di-*O*-methylisosterigmatocystin and Maleic Anhydride* (IV).—A solution of the dimethyl ether (100 mg.) and maleic anhydride (30 mg.) in benzene (20 ml.) was filtered and kept at room temperature for 12 hr. The precipitate was collected, washed with dry ether, and dried *in vacuo* at room temperature to give fine needles (30 mg.), m. p. 168° (Found: C, 64·4; H, 4·1. $C_{24}H_{18}O_9$ requires C, 64·0; H, 4·0%), $\lambda_{max.}$ 204, 245, 263, 305, and 320 mμ (log ε 4·20, 4·44, 4·31, 4·03, and 4·07, respectively), $\nu_{max.}$ 1852 and 1777 (anhydride) and 1657 (xanthone carbonyl group) cm.$^{-1}$. Its infrared absorption spectrum was different from that of a 1 : 1 molar mixture of the two components. Under similar experimental conditions no adduct was formed by 1,3,8-trimethoxyxanthone or by sterigmatocystin.

We thank the University of Nottingham for the award of the F. Stanley Kipping Memorial Research Scholarship (to J. G. U.).

The University, Nottingham. [*Received, April* 18*th*, 1962.]

[11] Feigl, "Spot Tests in Organic Analysis," 5th English edn., Elsevier, Amsterdam, 1956, p. 340.
[12] Bergmann and Segal, *Biochem. J.*, 1956, **62**, 542.