The Chemistry of Fungi. Part XXV. Oosporein, a Metabolite of* Chaetomium aureum *Chivers.*

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The polyhydroxy-diphenyldiquinone, oosporein (I; R = H) (Kögl and van Wessem, *Rec. Trav. chim.*, **1944**, **63**, 5), has been isolated from a strain of *Chaetomium aureum* Chivers and derivatives have been prepared.

During a comprehensive investigation of the metabolites from the *Chaetomium* genus, a copper-coloured pigment, $C_{14}H_{10}O_8$, was isolated from the mycelium and the metabolic liquors of a strain of *Chaetomium aureum* Chivers, grown on a modified William Saunders medium. This pigment exhibited properties typical of a polyhydroxyquinone, being readily converted into a tetra-acetate, $C_{14}H_6O_4(OAc)_4$ and reductively acetylated to an octa-acetate, $C_{14}H_6(OAc)_8$. The close correspondence between the properties of the metabolite and of the two acetates with oosporein (Kögl and van Wessem, *Rec. Trav. chim.*, **1944**, **63**, 5) (I; R = H) and its appropriate derivatives indicated identity. A direct comparison

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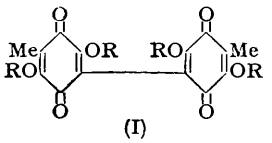
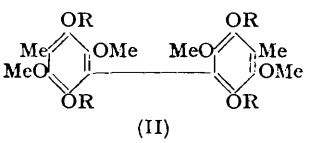
between the three substances kindly supplied by Professor F. Kögl and the products obtained by us, including a comparison of their infrared absorption spectra, confirmed this.

Methylation of oosporein with a large excess of diazomethane gave the tetramethyl ether (I; R = Me) which was readily reduced to tetrahydrotetra-*O*-methyloosporein (II; R = H). Reductive acetylation of tetra-*O*-methyloosporein (I; R = Me) furnished


(I)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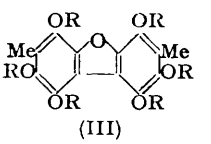
(III)

tetra-*O*-acetyltetrahydrotetra-*O*-methyloosporein (II; R = Ac), identical with the acetylation product of tetrahydrotetra-*O*-methyloosporein. Methylation of the tetrahydric phenol (II; R = H), obtained by the catalytic hydrogenation of tetra-*O*-methyloosporein, by either the methyl sulphate or the methyl iodide–potassium carbonate method furnished a mixture of tetrahydro-octa-*O*-methyloosporein (II; R = Me) and, unexpectedly, the dibenzofuran (III; R = Me), which was readily demethylated. Efforts to convert tetrahydrotetra-*O*-methyloosporein into a dibenzofuran of type (III) by dehydration with the usual reagents were uniformly unsuccessful (cf. Part XXIV, *loc. cit.*).

Experimental

(With D. H. Johnson) *Isolation of Oosporein.*—The mould *Chaetomium aureum* Chivers was grown on a modified William Saunders medium, containing glucose (50 g.), glycine (2 g.), potassium dihydrogen phosphate (2 g.), calcium chloride (0·5 g.), malt extract (1·4 g.), ammonium sulphate (0·3 g.), magnesium sulphate (0·25 g.), boric acid (0·5 g.), ferric chloride (0·25 g.), thallous chloride (0·5 g.), potassium iodide (0·05 g.), copper sulphate (0·05 g.), manganous chloride (0·5 g.), and zinc sulphate (0·5 g.; all quantities per l.) at 25° for 21 days. The mycelial felts were collected, dried, powdered, and exhaustively extracted (Soxhlet) with light petroleum (b. p. 40—60°) for 4 days and then with ether; a part of the pigment separated in a state of high purity in the Soxhlet boiler. The metabolic liquid was concentrated in a vacuum to about one-tenth of its volume. Several days later the brown precipitate (*ca.* 10 g., from 180 l. of metabolic fluid) was collected, dried, and repeatedly extracted with boiling acetone (1 l.), and the extract reduced by distillation (to about 30 ml.), oosporein then separating as bronze plates. The combined yield of pigment from mycelium and liquor varied from 0·02 to 0·11 g. per l. of culture fluid, and the relative distribution between metabolic liquid and mycelium also varied widely. Growth of the mould at 30° did not appear to alter the yield of metabolite materially but at room temperature (*ca.* 15—16°) the yield was considerably lower; when glucose was replaced by glycerol the organism did not produce the pigment. Thus obtained, oosporein separated from acetone–light petroleum (b. p. 60—80°) in bronze-coloured plates, m. p. 260—275° (decomp.) (Found: C, 55·0; H, 3·7. Calc. for $C_{14}H_{10}O_8$: C, 54·9; H, 3·3%).

Oosporein tetra-acetate crystallised from ethyl acetate–light petroleum (b. p. 60—80°) in yellow needles, m. p. 191° undepressed on admixture with a specimen supplied by Professor F. Kögl; the infrared absorption spectra of the two derivatives were identical [Found: C, 55·9 55·8; H, 3·7, 3·9; OAc, 35·5. Calc. for $C_{14}H_6O_4(OAc)_4$: C, 55·7; H, 3·8; OAc, 36·3%]; Kögl and van Wessem (*loc. cit.*) record m. p. 184°.

Octa-O-acetyltetrahydro-oosporein separated from alcohol in prisms, m. p. 250° [Found: C, 55·8, 55·7; H, 4·7, 4·9; OAc, 46·7. Calc. for $C_{14}H_6(OAc)_8$: C, 55·7; H, 4·7; OAc, 53·3%]. The infrared absorption spectrum of this compound was identical with that of a specimen supplied by Professor Kögl; Kögl and van Wessem (*loc. cit.*) give m. p. 231°.

Methylation of Oosporein.—Ethereal diazomethane (from 12 g. of methylnitrosourea) was rapidly added to oosporein (1 g.) in cold methanol (175 ml.) and 5 min. later the mixture was acidified with 2N-sulphuric acid (2 drops) and concentrated to *ca.* 20 ml. in a vacuum. When precipitated from the concentrate with water (50 ml.), *tetra-O-methyloosporein* was purified from light petroleum (b. p. 60—80°) and then by chromatography from benzene on neutralised alumina, forming orange needles, m. p. 125° [Found: C, 59·7; H, 5·3; OMe, 34·0%; *M*, 360. $C_{14}H_6O_4(OMe)_4$ requires C, 59·7; H, 5·0; OMe, 34·2%; *M*, 362]. This ether is soluble in the

usual organic solvents, insoluble in aqueous 2N-sodium carbonate, and has a negative ferric reaction.

With less diazomethane (*ca.* 4 mol.) methylation furnished two isomeric *trimethyl ethers* of oosporein, *viz.*, (A) which separated from aqueous methanol in golden plates, m. p. 154° [Found : C, 58·2, 58·5; H, 4·9, 4·5; OMe, 26·3, 25·9. $C_{14}H_7O_5(OMe)_3$ requires C, 58·6; H, 4·7; OMe, 26·7%], and (B) forming yellow plates, m. p. 192°, from aqueous methanol (Found : C, 58·1; H, 4·4; OMe, 22·5%), a mixture of the two melting at 130—142°. The compounds, which gave blood-red ferric reactions in alcohol, formed deep purple solutions in aqueous 2N-sodium hydrogen carbonate from which they were recovered unchanged on acidification. Treatment of ether (A) or (B) with an excess of diazomethane gave an almost quantitative yield of tetra-*O*-methyloosporein.

Tetrahydro-octa-O-*methyloosporein.*—Tetra-*O*-methyloosporein (3 g.), dissolved in methanol (150 ml.), was hydrogenated with a palladium–charcoal catalyst (from 2 g. of charcoal and 0·2 g. of palladium chloride) for 30 min. (absorption, 304 ml.; calc. for 2 mol., 370 ml.). Removal of the solvent in a vacuum left *tetrahydrotetra*-O-*methyloosporein* which separated from aqueous methanol in needles (2·2 g.), m. p. 197°, readily soluble in aqueous 2N-sodium hydroxide and having a negative ferric reaction in alcohol [Found : C, 58·9; H, 5·9; OMe, 33·5. $C_{14}H_{10}O_4(OMe)_4$ requires C, 59·0; H, 6·0; OMe, 33·8%]. The same product was obtained by the reduction of tetra-*O*-methyloosporein in methanol with sulphur dioxide.

Acetylation of tetrahydrotetra-*O*-methyloosporein (0·2 g.) with acetic anhydride (5 ml.) and concentrated sulphuric acid (2 drops) on the steam-bath for 15 min. furnished *tetra*-O-*acetyltetrahydrotetra*-O-*methyloosporein* which crystallised from aqueous acetic acid in needles (0·18 g.), m. p. 146°, identical with the product formed by the reductive acetylation of tetra-*O*-methyloosporein. This compound was insoluble in aqueous 2N-sodium hydroxide and had a negative ferric reaction in alcohol (Found : C, 58·3; H, 5·5. $C_{26}H_{30}O_{12}$ requires C, 58·4; H, 5·6%).

When tetrahydrotetra-*O*-methyloosporein (1 g.) was methylated by either methyl sulphate or methyl iodide–potassium carbonate–acetone for 6 hr., the product was almost invariably a mixture (0·8 g.), m. p. 130—140°, which was separated by fractional crystallisation from methanol into 1 : 3 : 4 : 5 : 6 : 8-*hexamethoxy*-2 : 7-*dimethyldibenzofuran*, plates, m. p. 141—142° [Found : C, 64·0; H, 6·5; OMe, 49·2%; *M*, 374. $C_{14}H_6O(OMe)_6$ requires C, 63·9; H, 6·4; OMe, 49·0%; *M*, 376], and *tetrahydro-octa*-O-*methyloosporein*, octahedra, m. p. 179° [Found : C, 63·0; H, 7·1; OMe, 57·4. $C_{14}H_6(OMe)_8$ requires C, 62·6; H, 7·1; OMe, 58·7%]. These products had negative ferric reactions and were insoluble in aqueous 2N-sodium hydroxide.

Demethylation of 1 : 3 : 4 : 5 : 6 : 8-hexamethoxy-2 : 7-dimethyldibenzofuran (0·5 g.) with a boiling mixture of hydriodic acid (10 ml.; *d* 1·7) and acetic acid (from 5 ml. of anhydride) for 10 min. and dilution with water furnished 1 : 3 : 4 : 5 : 6 : 8-hexahydroxy-2 : 7-dimethyldibenzofuran which separated from methanol in needles, m. p. 300° (decomp.), and on exposure to air rapidly oxidised. On acetylation with acetic anhydride–sulphuric acid this unstable phenol gave 1 : 3 : 4 : 5 : 6 : 8-*hexa-acetoxy*-2 : 7-*dimethyldibenzofuran*, forming prisms, m. p. 274°, from methanol [Found : C, 57·6; H, 4·6; OAc, 43·1. $C_{14}H_6O(OAc)_6$ requires C, 57·4: H, 4·5; OAc, 47·4%]. Methylation of the phenol by methyl sulphate–potassium carbonate regenerated the parent ether, m. p. and mixed m. p. 141—142°.

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