Studies on the Metabolites of *Zygosporium masonii*. Part II.[1] Structures of Zygosporins D, E, F, and G

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Zygosporins D, E, F, and G were isolated as minor metabolites of the fungus *Zygosporium masonii* and their cyclo-undeca[*d*']isoindole-1,11-dione structures were elucidated as (II), (IV), (III), and (IX), respectively. These compounds showed cytotoxicity against HeLa cells.

We have previously reported the isolation [2] of a new cytotoxic antibiotic cytochalasin D [3] (zygosporin A) as a metabolite of the fungus *Zygosporium masonii*. Its structure was elucidated as (I) by chemical degradation [1] and *X*-ray analysis.[4]

Many minor metabolites were also isolated from the culture filtrate and these showed the same characteristic cytotoxicity as cytochalasin D. We name them zygosporins D, E, F, and G † and report their structural elucidation.

The culture filtrate was extracted with ethyl acetate

† Zygosporin B, $C_{15}H_{28}O_4$, m.p. 143—144°, and zygosporin C, m.p. 260—263°, were reported in a Japanese patent Matsushima, Jap. P. Appl. 65,294/1966.

[1] Part I, preceding paper.

[2] S. Hayakawa, T. Matsushima, T. Kimura, H. Minato, and K. Katagiri, *J. Antibiotics*, 1968, **21**, 523.

[3] D. C. Aldridge and W. B. Turner, *J. Chem. Soc.* (*C*), 1969, 923; *J. Antibiotics*, 1969, **22**, 170.

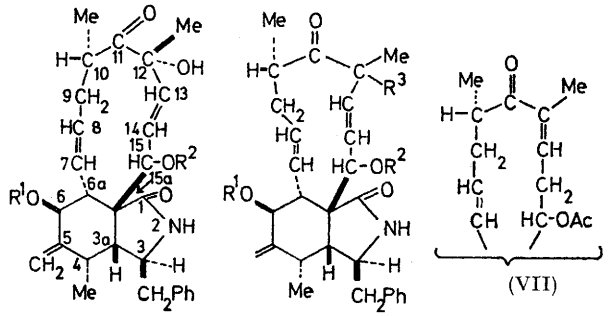
[4] Y. Tsukuda, M. Matsumoto, H. Minato, and H. Koyama, *Chem. Comm.*, 1969, 41.

and the extract was crystallised from acetone to give crude cytochalasin D. The mother liquor and the recrystallisation liquor were combined and chromatographed on silica gel and alumina. Fractions were separated by preparative t.l.c. to give zygosporins D, E, F, and G.

Zygosporin D, $C_{28}H_{35}NO_5$, m.p. 180—190°, $[\alpha]_D$ −14·9°, showed a lower t.l.c. R_F value than cytochalasin D and $\nu_{max.}$ 3400, 1700, 968, and 910 cm.$^{-1}$. These data suggest that this compound is a deacetyl-derivative of cytochalasin D. It was in fact shown to be identical with desacetylcytochalasin D[1] (II), a hydrolysis product of cytochalasin D, by comparison of i.r. and mass spectra, and of $[\alpha]_D$ and t.l.c. R_F values.

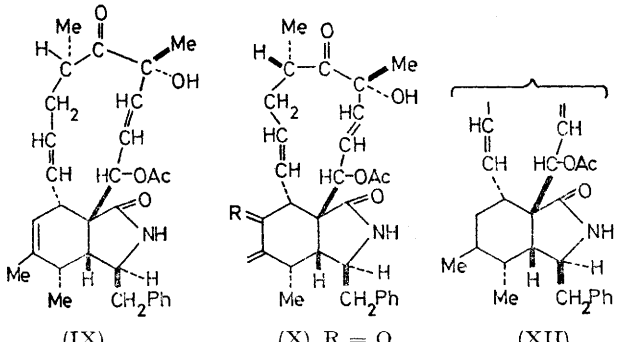
Zygosporin F, $C_{32}H_{39}NO_7$, m.p. 126—129°, $[\alpha]_D$ −12·0°, showed a higher t.l.c. R_F value than cytochalasin D and $\nu_{max.}$ 3420, 1740, 1704, 965, and 910 cm.$^{-1}$. These data agreed with those of cytochalasin D monoacetate[1] (III). I.r. and mass spectra and $[\alpha]_D$ values were also in agreement.

Zygosporin E, $C_{30}H_{37}NO_5$, m.p. 218—223·5°, $[\alpha]_D$ +6·2°, $\nu_{max.}$ 3525, 3420, 1743, 1703, 973, and 912 cm.$^{-1}$, showed three doublet methyl signals [τ 9·09 (*J* 6·8 c./sec.), 8·85 (*J* 6·4), and 8·75 (*J* 6·8)] and an acetoxy-signal [τ 7·77(s)] in the n.m.r. spectrum. The pattern of the spectrum is very similar to that of cytochalasin D (I)

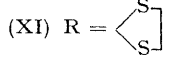


(I) R^1 = H, R^2 = Ac
(II) R^1 = R^2 = H
(III) R^1 = R^2 = Ac

(IV) R^1 = R^3 = H, R^2 = Ac
(V) R^1 = R^2 = Ac, R^3 = H
(VI) R^1 = R^2 = Ac, R^3 = Cl



(IX) (X) R = O (XII)

(XI) R = 

except that a singlet methyl at τ 8·51 (CH_3·C·OH) has been replaced by the doublet methyl at τ 8·75. On acetylation with acetic anhydride in pyridine at room temperature, zygosporin E readily gave an acetate (V), an amorphous powder, m.p. 86—98°, $[\alpha]_D$ +13·7°. We therefore assume that zygosporin E is a derivative obtained by the replacement of the tertiary hydroxy-group of cytochalasin D with a hydrogen atom.

In order to confirm this structure, acetylcytochalasin D (III) was treated with thionyl chloride in pyridine to give a chloro-derivative (VI), m.p. 133—136°, together with a dehydrated product. When compound (VI) was reduced with zinc powder in acetic acid at room temperature, it gave a dechloro-derivative (V), m.p. 88—98°, $[\alpha]_D$ +14·7°, a resinous αβ-unsaturated ketone [possibly (VII)], $\lambda_{max.}$ 252 mμ (ε 8570), and an unidentified compound, m.p. 112—116° (VIII). The product (V) was identical with zygosporin E acetate (i.r. and mass spectra, and $[\alpha]_D$ and t.l.c. R_F values). Moreover, zygosporin E showed an n.m.r. signal for C*H*·OH at τ 6·22 (d, *J* 10·3 c./sec.), very similar to that for the C-6 proton of cytochalasin D (I) [τ 6·23 (d, *J* 10·4 c./sec.)]. We therefore assign the hydroxy-group to C-6 and the acetoxy-group to C-15 in zygosporin E, and the structure is confirmed as (IV).

Zygosporin G, $C_{30}H_{37}NO_5$, m.p. 115—125°, $[\alpha]_D$ −82·0°, $\nu_{max.}$ 3410, 1742, 1701, 1008, and 965 cm.$^{-1}$, showed n.m.r. signals for two doublet methyls [τ 8·92 (*J* 7·0 c./sec.) and 8·82 (*J* 6·4)], a singlet methyl (CH_3·C·OH) at τ 8·51, a methyl on a double bond at 8·31, and an acetoxy-group at 7·76. It also showed a higher R_F value than cytochalasin D (I) on t.l.c., and no n.m.r. signals corresponding to those of the C-6 proton [τ 6·23 (C*H*·OH)] and the exocyclic methylene group (τ 4·95 and 4·75) in the spectrum of the latter compound. In the light of these data the formula (IX) is proposed.

In order to confirm this structure, cytochalasin D (I) was oxidised with chromium trioxide to give a ketone[1] (X). When this was treated with ethanedithiol and boron trifluoride, it gave an ethylene thioacetal (XI), an amorphous powder, which was converted into a compound (IX), m.p. 113—123°, $[\alpha]_D$ −75·5°, and its dihydro-derivative (XII) on treatment with Raney nickel in acetone. Compound (IX) was identical with zygosporin G (i.r. and mass spectra, and $[\alpha]_D$ and t.l.c. R_F values).

The cytotoxicities ($E.D._{50}$) against HeLa cells (monolayer culture) of zygosporins D, E, F, and G were 0·79, 2·65, >10·0, and 4·9 μg./ml., respectively.

EXPERIMENTAL

N.m.r. spectra were taken for solutions in deuteriochloroform with a Varian A60 spectrometer. Unless otherwise stated, i.r. spectra were taken for solutions in chloroform. M.p.s were measured with a Kofler hot-stage apparatus and are corrected.

Isolation of Zygosporins D (II), *E* (IV), *F* (III), *and G* (IX).—*Zygosporium masonii* was cultivated under submerged culture conditions for 3 days at 28° in a medium containing 3% glucose, 2% peptone, and 0·5% sodium chloride by use of four tanks (each 100 l.). The culture filtrate (260 l.) was extracted with ethyl acetate (2 × 100 l.).

The ethyl acetate solution (*ca.* 200 l.) was washed with 2N-sodium carbonate and water, and evaporated at 50—60° *in vacuo* to *ca.* one-third of its volume, during which time the separated product was filtered off. The filtrate was evaporated *in vacuo* to give crude cytochalasin D (16 g.) and a paste (A) (82 g.).

Recrystallisation of the crude cytochalasin D (16 g.) from acetone gave pure (I), m.p. 268—270° (14·8 g.), and a residue (B) (1·0 g.).

Residues (A) and (B) were combined and chromatographed on silica gel (830 g.) (Table).

Chromatography of residues (A) and (B)

Fraction	Solvent	Product
1	Benzene, benzene–$CHCl_3$ (9 : 1, 7 : 3, and 5 : 5), and $CHCl_3$	Oil (42 g.)
2	$CHCl_3$	Paste (25 g.)
3	$CHCl_3$–MeOH (9 : 1)	Paste (16 g.)

Fraction 2 was crystallised from ethyl acetate to give a crystalline product (C) (270 mg.) and a paste (D) (24 g.). The latter was chromatographed on alumina (690 g.) to give an oil (22 g.), eluted with benzene and then chloroform, and a paste (E) (1·2 g.), eluted with chloroform–methanol (20 : 1, 9 : 1, and 8 : 2).

Fraction 3 was dissolved in light petroleum and the precipitate (F) (340 mg.) was collected.

The crystalline product (C) (270 mg.) was separated into cytochalasin D (R_F 0·40) (150 mg.) and *zygosporin E* (R_F 0·48) (51 mg.) by preparative t.l.c. (ethyl acetate).

The paste (E) (1·2 g.) was rechromatographed on silica gel to give an amorphous powder (660 mg.), which was separated into *zygosporin G* (R_F 0·35) (450 mg.) and *zygosporin F* (R_F 0·28) (90 mg.) by preparative t.l.c. [toluene–methanol (10 : 1)].

The precipitate (F) (340 mg.) was separated into cytochalasin D (R_F 0·50) (135 mg.) and *zygosporin D* (R_F 0·40) (32 mg.) by preparative t.l.c. [chloroform–methanol (10 : 1)]. Zygosporin D (II) gave colourless plates, m.p. 180—190° (from methanol), *M* 465 (mass spectrum), $[\alpha]_D^{23}$ −14·9° (±0·7°) (*c* 0·759 in dioxan), $\nu_{max.}$ 3400, 1700, 1127, 1107, 1075, 1005, 968, and 910 cm.$^{-1}$ (Found: C, 71·9; H, 7·45; N, 2·85. $C_{28}H_{35}NO_5$ requires C, 72·25; H, 7·6; N, 3·0%).

Zygosporin E (IV) gave colourless needles, m.p. 218—223·5° (from acetone and n-hexane), *M* 491 (mass spectrum), $[\alpha]_D^{24}$ +6·2° (±0·5°) (*c* 0·971 in dioxan), $\nu_{max.}$ 3525, 3420, 1743, 1703, 1113, 1010, 973, and 912 cm.$^{-1}$ (Found: C, 72·9; H, 7·6; N, 2·8; O, 16·65. $C_{30}H_{37}NO_5$ requires C, 73·3; H, 7·6; N, 2·85; O, 16·25%).

Zygosporin E acetate (V) was an amorphous powder, m.p. 86—98° (from cyclohexane), $[\alpha]_D^{23}$ +13·7° (±3·5°) (*c* 0·153 in dioxan), $\nu_{max.}$ 3420, 1739, 1703, 1018, 972, and 910 cm.$^{-1}$ (Found: C, 71·8; H, 7·45; N, 2·35; O, 17·8. $C_{32}H_{39}NO_6$ requires C, 72·0; H, 7·35; N, 2·6; O, 18·0%).

Zygosporin F (III) gave colourless prisms, m.p. 126—129° (from di-isopropyl ether), *M* 549 (mass spectrum), $[\alpha]_D^{24}$ −12·0° (±0·7°) (*c* 0·775 in dioxan), $\nu_{max.}$ 3420, 1740, 1704, 1113, 1043, 1009, 965, and 910 cm.$^{-1}$ (Found: C, 69·55; H, 7·15; N, 2·25; O, 20·75. $C_{32}H_{39}NO_7$ requires C, 69·9; H, 7·15; N, 2·55; O, 20·4%).

Zygosporin G (IX) gave colourless prisms, m.p. 115—125° (from di-isopropyl ether), *M* 491 (mass spectrum), $[\alpha]_D^{24}$ −82·0° (±1·4°) (*c* 0·870 in dioxan), $\nu_{max.}$ 3410, 1743, 1701, 1122, 1045, 1008, and 965, $\nu_{max.}$ (Nujol) 3430, 3285, 1742, 1693, 1228, 1121, 1044, 1007, 965, 745, and 703 cm.$^{-1}$ (Found: C, 72·95; H, 7·55; N, 2·7; O, 16·4. $C_{30}H_{37}NO_5$ requires C, 73·3; H, 7·6; N, 2·85; O, 16·25%).

Conversion of (III) *into the Chloro-derivative* (VI).—Thionyl chloride (1·14 ml.) was added dropwise to a solution of cytochalasin D monoacetate (III, 465 mg.) in pyridine (4 ml.) in an ice-bath, and the solution was left for 30 min. at room temperature. It was then poured into ice–water and extracted with chloroform. The extract was washed with 5% hydrochloric acid, water, 5% potassium carbonate, and water, dried (Na_2SO_4), and evaporated, leaving an amorphous residue (465 mg.). This was separated into a chloride (244 mg.), R_F 0·23, and a dehydrated product (44 mg.), R_F 0·26 [t.l.c. in cyclohexane–ethyl acetate (4 : 3)]. The chloride was crystallised from ether to give colourless *prisms* (VI), m.p. 133—136° (from acetone and n-hexane), $[\alpha]_D^{23}$ +189·5° (±9·3°) (*c* 0·248 in dioxan), *M* 567 (mass spectrum), $\nu_{max.}$ 3420, 1737, 1705, 1028, 1018, 983, and 905 cm.$^{-1}$, τ 9·21 (d, *J* 6·5 c./sec.), 8·88 (d, *J* 6·5 c./sec.), 8·10(s), 8·06(s), and 7·82(s) (Found: C, 67·4; H, 6·55; Cl, 5·85; N, 2·45; O, 16·95. $C_{32}H_{38}ClNO_6$ requires C, 67·65; H, 6·75; Cl, 6·25; N, 2·45; O, 16·9%).

Treatment of (VI) *with Zinc in Acetic Acid.*—Zinc powder (30 mesh, 5 g.) was added to a solution of (VI) (200 mg.) in acetic acid and the mixture was stirred for 1 hr. at room temperature, then filtered. The filtrate was extracted with chloroform. The extract was washed with 5% potassium carbonate and water, dried (Na_2SO_4), and evaporated, leaving a residue (179 mg.). This was separated into a *dechloro-product* (V) (23 mg.), R_F 0·40, a resinous *αβ-unsaturated ketone* [possibly (VII) (36 mg.)], R_F 0·48, $\lambda_{max.}$ (EtOH) 252 mμ (ε 8600), $\nu_{max.}$ 3420, 1735, 1703, 1655, 1605, 1020, 980, and 910 cm.$^{-1}$, τ 9·10 (d, *J* 6·5 c./sec.), 8·85 (d, *J* 6·5 c./sec.), 8·21(s), 8·08(s), and 7·78(s), starting material (VI) (17 mg.), R_F 0·43, and an unidentified product (VIII), R_F 0·32, m.p. 112—116° [preparative t.l.c. in cyclohexane–ethyl acetate (1 : 1)]. The dechloro-product (V) was treated with cyclohexane to give an amorphous powder, m.p. 88—98°, $[\alpha]_D^{23}$ +14·7° (±1·2°) (*c* 0·455 in dioxan) (Found: C, 71·95; H, 7·55; N, 2·5%), identical with zygosporin E acetate (i.r. and mass spectra and t.l.c. R_F value).

Conversion of Ketone (X) *into Zygosporin G* (IX).—A solution of the ketone [1] (X) (100 mg.) in ethanedithiol (1·5 ml.) and boron trifluoride–ether complex (0·1 ml.) was left for 3 hr. at room temperature. The mixture was poured into ice–water and extracted with chloroform. The extract was washed with 5% sodium hydroxide and water, dried (Na_2SO_4), and evaporated, leaving a residue (130 mg.). The residue was subjected to preparative t.l.c. to give an *amorphous powder* (XI) (60 mg.), R_F 0·25 [benzene–methanol (10 : 1)]. Deactivated Raney nickel (400 mg.) (W-2 Raney nickel heated under reflux for 30 min. in acetone) was added to a solution of (XI) (42 mg.) in ethanol (2 ml.) and the mixture was heated under reflux for 6·5 hr. The catalyst was filtered off and the filtrate was evaporated to leave a residue (34 mg.). This was separated into a *product* (IX) (8·5 mg.), R_F 0·41, colourless prisms, m.p. 113—123° (from di-isopropyl ether), $[\alpha]_D^{23}$ −75·5° (±18·1°) (*c* 0·045 in dioxan) (Found: C, 73·1; H, 7·5; N, 2·65%), identical with zygosporin G (i.r. and mass spectra and t.l.c. R_F values), and a resinous dihydro-derivative (XII), τ 9·13 (d, *J* 6·5 c./sec.), 8·95 (d, *J* 7·0 c./sec.), 8·85 (d, *J* 6·5 c./sec.), 8·55(s), and 7·80(s), by preparative t.l.c. [ether–ethyl acetate (10 : 1)].

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