A Steroid Hydroxylase Inhibitor, Diplodialide-A, and Related Metabolites from *Diplodia pinea*

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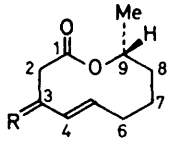
The isolation and structural elucidation of four ten-membered lactones, diplodialide-A (a steroid hydroxylase inhibitor), -B, -C, and -D are reported.

In the course of searching for steroid hydroxylase inhibitors of microbial origin, we isolated four new metabolites from the culture filtrate of the plant pathogenic fungus, *Diplodia pinea* (IFO 6472), namely diplodialide-A (1),† -B (2), -C (3), and -D (4).[1,2] These are the first members of ten-membered lactones belonging to pentaketides. We now describe in full the structural elucidation of these metabolites.

RESULTS AND DISCUSSION

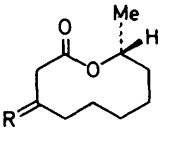
Diplodialide-A is a colourless oil, $[\alpha]_D$ +142° ($CHCl_3$), with the molecular formula $C_{10}H_{14}O_3$, and shows $\lambda_{max.}$ 232 nm (ε 6 560) in the u.v. spectrum and $\nu_{max.}$ 1 740, 1 700, and 1 645 cm^{-1} in the i.r. spectrum. These spectra indicate the presence of an αβ-unsaturated ketone and an ester or lactone. The n.m.r. spectrum of (1) at high field shows a methyl doublet at δ 1.28 (*J* 7 Hz), which collapses to a singlet on irradiation of the signal at δ 5.16 (1 H, m), indicating the presence of the Me–CH–O–CO unit. An AB quartet centred at δ 3.56 (*J* 14 Hz) was assigned to a methylene flanked by two carbonyl groups, and the signals in the vinyl region [d 5.88 (1 H, d, *J* 16 Hz) and 6.72 (1 H, m)] to the protons of a *trans*-disubstituted olefinic moiety conjugated with a carbonyl group. On this evidence diplodialide-A must contain the partial structure Me–CH–O–CO–CH_2–CO–CH=CH–. The n.m.r. spectrum of (1) has ill-defined signals at δ 1.4—2.6 corresponding to the remaining six protons. From these spectral data, it may thus be deduced that diplodialide-A is a ten-membered lactone with an αβ-unsaturated ketone function. Hydrogenation of (1) with 10% palladium–charcoal in ethyl acetate gave the dihydro-derivative (5);



(1) R = O

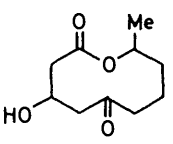
(2) R = OH, H

(9) R = OAc, H



(3) R = OH, H

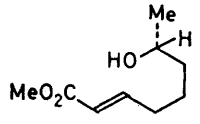
(5) R = O



(4)

† Diplodialide-A inhibited 11α-hydroxylation of progesterone in the cells of *Rhizopus stolonifer* (IFO 5781) at a concentration of 25 p.p.m. The biological study will be reported in detail elsewhere.

this, in accord with the structure shown, showed $\nu_{max.}$ at 1 745 (lactone) and 1 715 (saturated ketone) cm^{-1} and had no absorptions at 1 700 or 1 645 cm^{-1}, and had no olefinic proton signals in the n.m.r. spectrum. Confirmation of the presence of a β-keto-lactone group in (5) was provided by the u.v. spectrum, showing a bathochromic shift from weak end-absorption to 277 nm (ε 12 500) in an alkaline solution, which is reversed by acids. Compound (1) was hydrolyzed and methylated with diazomethane to give an αβ-unsaturated ester (6) [λ 210 (sh) nm (ε 11 200) and $\nu_{max.}$ 1 730 and 1 660 cm^{-1}]. The formation of (6) by alkaline hydrolysis shows that hydroxyl anion attacks the ketone group in (1) to cleave the bond between C-2 and C-3. This evidence defined the structure of diplodialide-A as shown (1).

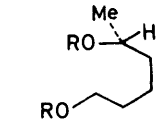


(6)

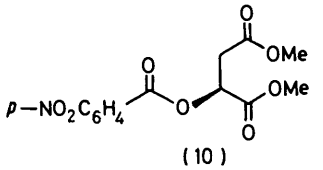
Diplodialide-B is a colourless oil, $[\alpha]_D$ −37.3° ($CHCl_3$) with the molecular formula $C_{10}H_{16}O_3$, and shows $\nu_{max.}$ 3 550 and 1 715 cm^{-1} in the i.r. spectrum. Diplodialide-B was oxidized with activated manganese dioxide in dichloromethane to give a compound identical in all respects, including optical rotation, with (1), and (1) was reduced with sodium borohydride in tetrahydrofuran to give diplodialide-B as the main product. Thus, the structure of diplodialide-B was defined as (2), the C-9 configuration of which is the same as that of (1).

Diplodialide-C is a colourless oil, $[\alpha]_D$ −41.0° ($CHCl_3$), with the molecular formula $C_{10}H_{18}O_3$, and shows $\nu_{max.}$ 3 650, 3 450, and 1 730 cm^{-1} in the i.r. spectrum, and the absence of olefinic proton signals in the n.m.r. spectrum. Diplodialide-C (3) was identical (i.r. and n.m.r. spectra and optical rotation) with the dihydro-derivative of diplodialide-B (2), prepared by hydrogenation of (2) in the presence of 10% palladium–charcoal. The ^{13}C n.m.r. spectra of diplodialide-A, -B, and -C are also compatible with the structures (1), (2), and (3), respectively. The above chemical conversions of diplodialide-A (1) to (2) and (3) established the absolute stereochemistry at C-9 in these compounds to be the same. Hence diplodialide-B was used for the determination of the absolute configuration at C-9. Ozonolysis of (2) followed by lithium aluminium hydride reduction gave (−)-hexane-1,5-diol (7), $[\alpha]_D$ −11° (MeOH), which

was converted to the di-*p*-nitrobenzoate (8), m.p. 110.0—110.5 °C, $[\alpha]_D$ −39.7° ($CHCl_3$). These optical



(7) R = H
(8) R = $COC_6H_4NO_2$-*p*



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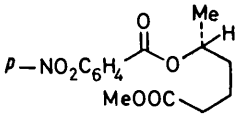


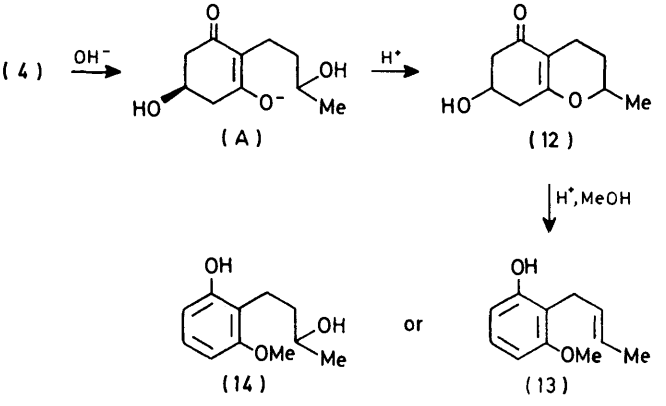
(11)

rotations have the opposite signs to the values reported for (*S*)-(+)-hexane-1,5-diol, $[\alpha]_D$ +8° (MeOH), and its di-*p*-nitrobenzoate, $[\alpha]_D$ +43° ($CHCl_3$).[3] The formation of (*R*)-(−)-hexane-1,5-diol indicates the (9*R*)-configuration for diplodialide-A, -B, and -C. The same stereochemistry of the lactone ring in polyketides has been reported for ochratoxins,[4] 5-methylmellein,[5] and pyrenophorin.[6]

The absolute configuration at C-3 of (2) was determined by ozonolysis of its acetate (9), followed by oxidation (H_2O_2–HCO_2H), and hydrolysis (KOH–MeOH–H_2O). The products were treated with excess of diazomethane and isolated as their *p*-nitrobenzoates. As expected, optically active dimethyl (−)-*p*-nitrobenzoylmalate (10), $[\alpha]_D$ −5.1° (MeOH), and the 5-hydroxyhexanoic acid derivative (11), $[\alpha]_D$ −30.5° ($CHCl_3$), were obtained. The former has the same sign of the optical rotation as the authentic (*S*)-(−)-compound, $[\alpha]_D$ −4.2° (MeOH), prepared from (*S*)-(−)-malic acid. These results indicate the (3*S*)-configuration shown in (2) for diplodialide-B and the (3*R*)-configuration shown in (3) for diplodialide-C.

Diplodialide-D is a colourless oil, $[\alpha]_D$ +0.8° ($CHCl_3$), has the molecular formula $C_{10}H_{16}O_4$, and shows $\nu_{max.}$ 3 450, 1 730, and 1 700 cm^{-1} in the i.r. spectrum. As shown in other diplodialides, the n.m.r. spectrum shows the presence of a methyl group (δ 1.23, d, *J* 6 Hz) attached to a methine group (δ 4.56, 1 H, m) bearing the oxygen atom of the lactone. The latter changes to a doublet of doublets (*J* 6 and 2 Hz) by irradiation at the former, showing the presence of the grouping Me–$CH(CH_2–)$–O–CO. Signals due to two methylene groups were observed at δ 2.57 (2 H, d, *J* 5 Hz), 2.60 (1 H, dd, *J* 14 and 4 Hz), and 2.90 (1 H, dd, *J* 14 and 3 Hz). Irradiation at δ 4.37 (1 H, m) simplified these signals to a singlet and an AB quartet (*J* 14 Hz), suggesting that diplodialide-D has the unit CO–CH_2–CH(OH)–CH_2–CO. From these spectral data it may thus be deduced that the structure of diplodialide-D is as shown in (4). The u.v. spectrum of (4) showed weak end-absorption, but an absorption maximum [$\lambda_{max.}$ 290 nm (ε 9 000)] appeared after the addition of 0.1N sodium hydroxide, which shifted irreversibly to 263 nm upon acidification. Treatment of (4) with potassium hydroxide in methanol–water (1 : 2) at room temperature followed by acidification gave an αβ-unsaturated ketone (12) as a single product ($C_{10}H_{14}O_3$). Ketone (12) shows $\nu_{max.}$ 3 400, 1 650, and 1 615 cm^{-1} in the i.r. spectrum and $\lambda_{max.}$ 263 nm (ε 11 800) in the u.v. spectrum, indicating the formation of an αββ′-trisubstituted-αβ-unsaturated ketone group,[7] and the disappearance of the lactone function of (4). The n.m.r. spectrum of (12) also confirmed the assigned structure. Accordingly, the changes in the u.v. spectrum of (4) on addition of base and acid are presumed to be due to the formation of a 1,3-dicarbonyl intermediate (A) by transannular cyclization of (4) and ring closure of the enolate ion (A) to (12) with acid.

Furthermore, (12), when heated under reflux in 1.75% hydrochloric acid in methanol for 1 h, gave a phenolic compound which has the structure (13) or (14). The similar transannular cyclization and aromatization was



reported for curvularin.[8] These evidence defined the structure of diplodiaolide-D as (4), but insufficient material precluded an examination of the configurations at C-3 and C-9 in (4). The presence of (4) in the culture filtrate of *Diplodia pinea* suggests that diplodialides may be derived from a partially reduced pentaketide.[9]

EXPERIMENTAL

M.p.s were determined with a Kofler hot-stage apparatus. I.r. spectra were recorded with a JASCO IR-G spectrometer, u.v. spectra with a Hitachi EPS-3T spectrophotometer, ^1H n.m.r. spectra with a JEOL JNM-MH-100 (100 MHz) spectrometer(tetramethylsilane as internal standard), and ^{13}C n.m.r. spectra with a JEOL JNM-FX 60 (15 MHz) Fourier-transform spectrometer (tetramethylsilane as internal standard). Mass spectra were measured with a JEOL JMS-D100 spectrometer and high-resolution mass spectra with a JEOL JMS-01SG-2 spectrometer. C.d. spectra were recorded with a JEOL J-40 spectrometer. Optical rotations were measured with JEOL DIP-4 polarimeter. Analytical g.l.c. was performed with a JEOL JGC-1100 gas chromatograph. Kieselgel 60PF_{254} (Merck) was used for analytical and preparative t.l.c. Mallinckrodt silicic acid was used for column chromatography.

Isolation of Diplodialide-A, -B, and -C.—Diplodia pinea (IFO 6472) was grown as a shaking culture for 7 d at 30 °C

in 500-ml Sakaguchi flasks, each containing 100 ml of malt–glucose medium [malt extract (Difco brand) (2%), glucose (2%), and polypeptone (Daigo brand) (0.1%)]. The fermentation broth from 170 flasks (17 l) was filtered, and the filtrate was extracted with ethyl acetate (2 × 20 l). The organic layer was dried (Na_2SO_4) and evaporated *in vacuo* to give a brown oil (5.24 g), which was dissolved in ethyl acetate (500 ml), washed with 2% aqueous sodium hydrogencarbonate, and evaporated *in vacuo* to give a brown oil (neutral fraction, 2.43 g). The oily material was chromatographed on a column of silica gel (60 g) in n-hexane containing increasing amounts of ethyl acetate. Elution with ethyl acetate–n-hexane (5 : 95) gave diplodialide-A (274 mg). Elution with ethyl acetate–n-hexane (1 : 9) gave two compounds, diplodialide-B (less polar, 839 mg) and diplodialide-C (more polar, 236 mg).

Isolation of Diplodialide-D.—*D. pinea* was pre-cultured in 500-ml Sakaguchi flasks containing 100 ml of the medium described above for 2 d at 30 °C.

The seed culture (400 ml) was transferred into 20-l jar fermenters each containing 10 l of the same medium, and the fermentation was continued for 6 d at 30 °C. The fermentation broth was filtered, and the filtrate was extracted with ethyl acetate (2 × 20 l). The organic layer was treated as described above to give the neutral fraction (2.07 g), which was chromatographed on a column of silica gel (90 g) in n-hexane containing increasing amounts of ethyl acetate. Elution with ethyl acetate gave a polar fraction (519 mg), which was dissolved in ethyl acetate and extracted with 2% aqueous sodium hydroxide to remove phenolic compounds. The organic layer was further chromatographed on preparative t.l.c. [solvents acetone–dichloromethane (30 : 70) and ethyl acetate–n-hexane (1 : 1)] to give diplodialide-D (10 mg).

Diplodialide-A (1).—This is a colourless oil (Found: M^+, 182.093 9. $C_{10}H_{14}O_3$ requires M, 182.094 3); $[\alpha]_D^{26}$ +142° (*c* 1.023, $CHCl_3$); $\nu_{max.}(CCl_4)$ 1 740, 1 700, 1 645, 1 265, 1 200, 1 070, and 965 cm^{-1}; $\lambda_{max.}$(MeOH) 232 nm (ε 6 560); $\delta_H(CDCl_3)$ 1.28 (3 H, d, *J* 7 Hz, CH–*Me*), 3.34 (1 H, d, *J* 14 Hz, CO–C$H_A$$H_B$–$CO_2$), 3.78 (1 H, d, *J* 14 Hz, CO–CH$_A$$H_B$–$CO_2$), 5.16 (1 H, m, O–C*H*–Me), 5.88 (1 H, d, *J* 16 Hz, CO–C*H*=CH), and 6.72 (1 H, m, CO–CH=C*H*–CH_2); $\delta_C(CDCl_3)$ 19.479 (q), 23.635 (t), 32.205 (t), 33.115 (t), 51.555 (t), 72.852 (d), 127.525 (d), 151.419 (d), 168.171 (s), and 195.163 (s); *m/e* 182 (6%, M^+), 164 (7), 154 (9), 139 (22), 128 (43), 122 (87), 110 (36), 95 (52), 81 (80), 71 (48), 68 (100), 55 (40), and 43 (46); c.d. $[\theta]_{224}$ +3.51 × 10^4, $[\theta]_{320}$ −7.55 × 10^3 (MeOH).

Catalytic reduction of diplodialide-A (1). Compound (1) (11 mg) in ethyl acetate (2 ml) was hydrogenated at room temperature in the presence of 10% palladium–charcoal for 4 h. Removal of the catalyst and solvent gave dihydrodiplodialide-A (5) (10 mg) as a colourless oil (Found: M^+, 184.110 2. $C_{10}H_{16}O_3$ requires M, 184.109 9); $[\alpha]_D^{27}$ −130° (*c* 1.00, MeOH); $\nu_{max.}(CCl_4)$ 1 745 and 1 715 cm^{-1}; λ(MeOH) 210 (sh) nm (ε 767), $\lambda_{max.}$(MeOH + 1N NaOH) 277 nm (ε 12 500); $\delta(CDCl_3)$ 1.30 (3 H, d, *J* 6 Hz, CH–*Me*), 3.40 (2 H, s, CO–CH_2–CO_2), and 5.10 (1 H, m, O–C*H*–Me); *m/e* 184 (5%, M^+), 156 (5), 125 (13), 112 (8), 97 (21), 82 (17), 70 (25), 69 (25), 55 (100), 43 (75), and 41 (100).

Alkaline hydrolysis of diplodialide-A (1). Diplodialide-A (50 mg) in methanol (0.8 ml) and aqueous 0.8N potassium hydroxide (5 ml) was stirred at room temperature for 3.5 h. The solution was acidified with 1N hydrochloric acid to pH 2, saturated with sodium chloride, and extracted with ethyl acetate (5 × 50 ml). The organic layer was washed with brine and dried (Na_2SO_4).

Removal of solvent gave a colourless oil, which was treated with diazomethane and chromatographed on preparative t.l.c. [solvent ethyl acetate–n-hexane (1 : 1)] to give the methyl ester (6) as an oil (32 mg) (Found: [*M* − MeOH]$^+$ 140.083 5. $C_8H_{12}O_3$ requires 140.083 7); $\nu_{max.}(CCl_4)$ 3 625, 3 450, 1 730, and 1 660 cm^{-1}; λ(MeOH) 210 (sh) nm (ε 11 200); $\delta(CDCl_3)$ 1.20 (3 H, d, *J* 7 Hz, CH–*Me*), 1.48 (1 H, s, OH, exchanges with D_2O), 3.75 (3 H, s, CO_2Me), 3.80 (1 H, m, O–C*H*–Me), 5.88 (1 H, d, *J* 15 Hz, CH=C*H*–CO_2), and 7.00 (1 H, m, CH_2–C*H*=CH–CO_2).

Reduction of diplodialide-A (1). Sodium borohydride (15 mg) was added portionwise with stirring to a solution of (1) (24 mg) in tetrahydrofuran (5 ml) at 0 °C. After 1 h the mixture was allowed to warm to room temperature, stirred for an additional 1 h, treated with water, and extracted with ethyl acetate (3 × 30 ml). The organic layer was washed with brine and dried (Na_2SO_4). Removal of solvent gave an oil (17 mg), which was chromatographed on preparative t.l.c. [solvent acetone–dichloromethane (2 : 98)] to give the *alcohol* (2) as a colourless oil (8.8 mg); $[\alpha]_D^{30}$ −34.7° (*c* 0.438, $CHCl_3$); $\nu_{max.}(CCl_4)$ 3 550, 1 715, 1 255, 1 155, and 965 cm^{-1}; $\delta(CDCl_3)$ 1.20 (3 H, d, *J* 7 Hz, CH–Me), 2.20 (1 H, OH, exchanges with D_2O), 4.68 (1 H, m, HO–C*H*), 4.92 (1 H, m, O–C*H*–Me), and 5.50 (1 H, m), and 5.64 (1 H, m) (CH=CH); *m/e* 184 (6%, M^+), 166 (10), 142 (19), 124 (26), 112 (32), 107 (23), 95 (32), 83 (45), 81 (45), 70 (93), 55 (100), and 43 (87), identical in optical rotation, and i.r., u.v., n.m.r., and mass spectra, with authentic diplodialide-B. Identity was also shown by t.l.c. and g.l.c. behaviour; co-injection with authentic material on 5% OV-210 at 120 °C showed no peak separation.

Diplodialide-B (2).—This is a colourless oil (Found: M^+, 184.108 0; [*M* − H_2O]$^+$, 166.096 5. $C_{10}H_{16}O_3$ requires M, 184.109 9. $C_{10}H_{14}O_2$ requires 166.099 4); $[\alpha]_D^{27}$ −37.3° (*c* 0.938, $CHCl_3$); $\nu_{max.}(CCl_4)$ 3 550, 1 715, 1 255, 1 155, and 965 cm^{-1}; $\delta_H(CDCl_3)$ 1.20 (3 H, d, *J* 7 Hz, CH–*Me*), 2.80 (1 H, OH, exchanges with D_2O), 4.68 (1 H, m, HO–C*H*), 4.92 (1 H, m, O–C*H*–Me), and 5.50 (1 H, m) and 5.64 (1 H, m) (CH=CH); $\delta_C(CDCl_3)$ 21.687 (q), 27.921 (t), 32.466 (t), 35.193 (t), 44.803 (t), 67.918 (d), 72.593 (d), 128.044 (d), 132.590 (d), and 170.769 (s); *m/e* 184 (5%, M^+), 166 (13), 142 (32), 124 (48), 112 (56), 107 (35), 95 (53), 83 (43), 81 (70), 70 (100), 55 (64), and 43 (86).

Manganese dioxide oxidation of diplodialide-B (2). Activated manganese dioxide (400 mg) was added portionwise with stirring to a solution of (2) (18 mg) in dichloromethane (10 ml) at room temperature. After 1.5 h the reaction mixture was filtered and the filtrate was evaporated *in vacuo* to give the ketone (1) as a colourless oil (10 mg), identical in optical rotation, and i.r. and n.m.r. spectra, with authentic diplodialide-A. Identity was also shown by t.l.c. and g.l.c. behaviour; co-injection with authentic material on 5% OV-210 at 120 °C showed no peak separation.

Dipodialide-C (3).—This is a colourless oil (Found: M^+, 186.126 5; [*M* − H_2O]$^+$, 168.115 9. $C_{10}H_{18}O_3$ requires M, 186.125 6. $C_{10}H_{16}O_2$ requires 168.115 0); $[\alpha]_D^{28}$ −41.0° (*c* 0.610, $CHCl_3$); $\nu_{max.}(CCl_4)$ 3 650, 3 450, 1 730, 1 250, 1 145, and 1 030 cm^{-1}; $\delta_H(CDCl_3)$ 1.30 (3 H, d, *J* 7 Hz, CH–*Me*), 1.76 (1 H, OH, exchanges with D_2O), 2.36 [1 H, dd, *J* 16 and 10 Hz, CO–C$H_A$$H_B$–CH(OH)], 2.84 [1 H, dd, *J* 16 and 4 Hz, CO–CH$_A$$H_B$–CH(OH)], 4.38 (1 H, m, C*H*–OH), and 5.04 (1 H, m, O–C*H*–Me); $\delta_C(CDCl_3)$ 19.349 (q), 22.984 (t), 23.505 (t), 25.323 (t), 31.426(t), 36.881 (t),

43.634 (t), 66.100 (d), 73.112 (d), and 170.769 (s); *m/e* 168 (1.6%, $[M - H_2O]^+$), 150 (5.6), 140 (4.8), 127 (12), 124 (12), 109 (60), 98 (76), 89 (100), 82 (80), 70 (92), 67 (68), 56 (74), 55 (76), and 43 (68).

Catalytic Hydrogenation of Diplodialide-B (2).—Diplodialide-B (15 mg) in ethyl acetate (3 ml) was hydrogenated at room temperature in the presence of 10% palladium–charcoal for 2 h. Removal of the catalyst and solvent gave dihydrodiplodialide-B as a colourless oil (9.5 mg) (Found: M^+, 186.130 5; $[M - H_2O]^+$, 168.115 9. $C_{10}H_{18}O_3$ requires *M*, 186.125 6. $C_{10}H_{16}O_2$ requires 168.115 0); $[\alpha]_D^{27}$ −40.7° (*c* 0.473, $CHCl_3$); $\nu_{max.}(CCl_4)$ 3 650, 3 450, 1 730, 1 250, 1 145, and 1 030 cm^{-1}; $\delta(CDCl_3)$ 1.30 (3 H, d, *J* 7 Hz, CH–*Me*), 1.86 (1 H, OH, exchanges with D_2O), 2.36 [1 H, dd, *J* 16 and 10 Hz, CO–C*H*$_A$H$_B$–CH(OH)], 2.84 [1 H, dd, *J* 16 and 4 Hz, CO–CH$_A$*H*$_B$–CH(OH)], 4.38 (1 H, m, C*H*OH), and 5.04 (1 H, m, O–C*H*–Me), identical in optical rotation, and i.r. and n.m.r. spectra, with authentic diplodialide-C. Identity was also shown by t.l.c. and g.l.c. behaviour; co-injection with authentic material on 5% OV-210 at 125 °C showed no peak separation.

Ozonolysis of Diplodialide-B (2).—Ozonized oxygen was passed for 30 min through a solution of diplodialide-B (70 mg) in dichloromethane (30 ml) at −78 °C. After removal of excess of ozone and solvent, the residue was dissolved in dry tetrahydrofuran (30 ml) and refluxed with lithium aluminium hydride (76 mg) for 3 h. Excess of the hydride was decomposed with saturated aqueous sodium sulphate and the mixture was thoroughly extracted with ether. The organic layer was washed with brine and dried (Na_2SO_4). Removal of solvent and preparative t.l.c. (solvent ethyl acetate) afforded (−)-hexane-1,5-diol (7) as a colourless oil (17 mg) (Found: $[M - Me]^+$, 103.076 5: $[M - H_2O]^+$, 100.089 5. $C_5H_{11}O_2$ requires 103.075 9. $C_6H_{12}O$ requires 100.088 8); $[\alpha]_D^{26}$ −11° (*c* 0.41, MeOH); $\nu_{max.}(CH_2Cl_2)$ 3 600, 3 400, 1 380, 1 070, 1 040, and 940 cm^{-1}; $\delta(CDCl_3)$ 1.22 (3 H, d, *J* 6 Hz, CH–*Me*), 1.84 (2 H, 2 OH, exchanges with D_2O), 3.68 (2 H, t, *J* 6 Hz, HO–C*H*$_2$–CH$_2$), and 3.82 (1 H, m, HO–C*H*–Me) [lit.,[3] (*S*)-(+)-hexane-1,5-diol: $[\alpha]_D^{20}$ +8° (*c* 0.55, MeOH); $\nu_{max.}(CH_2Cl_2)$ 3 650, 3 500, 1 380, 1 080, 1 050, and 950 cm^{-1}; δ 1.17 (3 H, d, *J* 6 Hz), and 3.5—3.9 (3 H)].

p-*Nitrobenzoylation of* (−)-*Hexane*-1,5-*diol*.—To the stirred solution of the diol (7) (8 mg) in dry pyridine (2 ml) was added portionwise *p*-nitrobenzoyl chloride (105 mg) at 0 °C. After 1 h the mixture was warmed to room temperature, stirred for 20 h, water was added, and the reaction mixture extracted with ethyl acetate (5 × 20 ml). The organic layer was washed with 1N-hydrochloric acid, saturated aqueous sodium hydrogencarbonate, and brine, and dried (Na_2SO_4). Removal of solvent, preparative t.l.c. [solvent ethyl acetate–n-hexane (2 : 8)], and recrystallization from dichloromethane and n-pentane gave the di-*p*-nitrobenzoate (8) as colourless needles (14.8 mg), m.p. 110.0—110.5° (Found: C, 57.95; H, 4.90; N, 6.65 $C_{20}H_{20}O_8N_2$ requires C, 57.69; H, 4.84; N, 6.73%); $[\alpha]_D^{26}$ −39.7° (*c* 0.456, $CHCl_3$); $\nu_{max.}(CH_2Cl_2)$ 1 725, 1 610, 1 530, 1 352, 1 118, 1 015, 872, and 840 cm^{-1}; $\lambda_{max.}(CHCl_3)$ 260 nm (ε 27 800); $\delta(CDCl_3)$ 1.40 (3 H, d, *J* 6 Hz, CH–*Me*), 4.40 (2 H, t, *J* 6 Hz, ArCO$_2$–C*H*$_2$–CH$_2$), 5.24 (1 H, m, ArCO$_2$–C*H*–Me), and 8.24 (8 H, m, aromatic-H); *m/e* 416 (0.13%, M^+), 386 (3.1), 356 (1.6), 249 (23), 150 (100), and 82 (95) [lit.,[3] (*S*)-(+)-hexane-1,5-diol di-*p*-nitrobenzoate; m.p. 107—109 °C; $[\alpha]_D^{20}$ +43°; $\nu_{max.}(CH_2Cl_2)$ 1 705, 1 605, 1 530, 1 350, 1 120, 1 015, 880, and 850 cm^{-1}; $\lambda_{max.}(CHCl_3)$ 260.5 nm (ε 26 300); $\delta(CDCl_3)$ 1.40 (3 H, d, *J* 6 Hz), 4.40 (2 H), 5.22 (1 H), and 8.2 (8 H)].

Acetylation of Diplodialide-B.—To a stirred solution of diplodialide-B (172 mg) in dry pyridine (8 ml) was added dropwise acetic anhydride (0.6 ml) at 0 °C. After 0.5 h the mixture was warmed to room temperature, stirred for 15 h, water was added, and the reaction mixture extracted with ethyl acetate (5 × 20 ml). The organic solution was washed with 1N-hydrochloric acid, saturated aqueous sodium hydrogencarbonate, and brine, and dried (Na_2SO_4). Removal of solvent and preparative t.l.c. [solvent ethyl acetate–n-hexane (30 : 70)] gave the *acetate* (9) as a colourless oil (199 mg) (Found: M^+, 226.123 6; $[M - MeCO]^+$, 183.103 8; $[M - AcOH]^+$, 166.101 4. $C_{12}H_{18}O_4$ requires *M*, 226.120 5. $C_{10}H_{15}O_3$ requires 183.102 1. $C_{10}H_{14}O_2$ requires 166.099 3); $\nu_{max.}(CCl_4)$ 1 740, 1 230, 1 152, 1 030, and 960 cm^{-1}; $\delta(CDCl_3)$ 1.20 (3 H, d, *J* 7 Hz, C*H*–Me), 2.12 (3 H, s, Me–CO$_2$), 4.90 (1 H, m, O–C*H*–Me), and 5.0—5.6 (3 H, AcO–C*H*–C*H*=C*H*–CH$_2$).

Ozonolysis of Diplodialide-B *Acetate*.—Ozonized oxygen was passed for 30 min through a solution of the acetate (9) (180 mg) in dichloromethane (50 ml) at −78 °C. After removal of excess of ozone and solvent, the residue was dissolved in methanol (20 ml), 30% hydrogen peroxide (0.9 ml) and formic acid (0.9 ml) were added, and stirred at room temperature for 20 h. The mixture was treated with 10% palladium–charcoal (200 mg) for 1 h, and filtered. To the filtrate was added aqueous potassium hydroxide (3 g in 2 ml), the reaction was refluxed for 2 h, and then concentrated *in vacuo* to *ca.* 20 ml, and passed through an Amberlite IR-120 column (H^+ form, 100 g, 2 × 30 cm), eluting with water (200 ml). The eluate was concentrated *in vacuo* with the aid of ethanol, and the residue was treated with ethereal diazomethane and chromatographed on preparative t.l.c. [ethyl acetate–n-hexane (1 : 1)], R_F 0.2—0.5) to give a colourless oil (61 mg), which was dissolved in dry pyridine (20 ml) and treated with *p*-nitrobenzoyl chloride (700 mg). After 18 h the reaction mixture was worked-up as described above, and chromatographed on preparative t.l.c. [solvent ethyl acetate–n-hexane (20 : 80)] to give dimethyl (−)-2-*p*-nitrobenzoyloxysuccinate (10) (17 mg, R_F 0.26) and methyl (−)-5-*p*-nitrobenzoyloxyhexanoate (11) (24 mg, R_F 0.56). Dimethyl 2-*p*-nitrobenzoyloxysuccinate (10) is a colourless oil (Found: M^+, 311.062 0. $C_{13}H_{13}O_8N$ requires *M*, 311.064 1); $[\alpha]_D^{27}$ −5.1° (*c* 0.59, MeOH); $\nu_{max.}(CCl_4)$ 1 745, 1 610, 1 530, 1 440, 1 350, 1 270, 1 215, 1 170, 1 115, 1 100, 1 015, 870, and 720 cm^{-1}; $\delta(CDCl_3)$ 3.08 (2 H, d, *J* 6 Hz, CH–C*H*$_2$–CO$_2$), 3.76 (3 H, s, OMe), 3.84 (3 H, s, OMe), 5.76 [1 H, t, *J* 6 Hz, CH$_2$–C*H*(OCOAr)], and 8.08 (4 H, aromatic-H); *m/e* 311 (0.67%, M^+), 281 (1.3), 280 (2.4), 252 (10), and 150 (100), identical in t.l.c. behaviour, optical rotation, and i.r., n.m.r., and mass spectra, with authentic material, prepared from (*S*)-(−)-malic acid. Methyl (−)-5-*p*-nitrobenzoyloxyhexanoate (11) is a colourless oil (Found: M^+, 295.100 7. $C_{14}H_{17}O_6N$ requires *M*, 295.105 6); $[\alpha]_D^{28}$ −30.5° (*c* 1.16, $CHCl_3$); $\nu_{max.}(CCl_4)$ 1 730, 1 610, 1 530, 1 350, 1 275, 1 170, 1 115, 1 100, 1 015, 870, 840, and 715 cm^{-1}; $\delta(CDCl_3)$ 1.40 (3 H, d, *J* 7 Hz, CH–Me), 2.38 (2 H, m, CH$_2$–C*H*$_2$–CO$_2$), 3.70 (3 H, s, OMe), 5.20 (1 H, m, O–C*H*–Me), and 8.28 (4 H, aromatic protons); *m/e* 265 (4%, $[M - NO]^+$), 264 (3.2), 150 (100), and 128 (48).

Dimethyl (S)-(−)-2-p-*Nitrobenzoyloxysuccinate* (10).—To a stirred solution of authentic dimethyl (*S*)-(−)-malate (31 mg), prepared from (*S*)-(−)-malic acid and diazomethane),

in dry pyridine (5 ml) was added portionwise *p*-nitrobenzoyl chloride (183 mg) at 0 °C. After 1 h the mixture was warmed to room temperature, stirred overnight, worked-up as described above, and chromatographed on preparative t.l.c. [solvent ethyl acetate–n-hexane (30 : 70)] to give the *p*-nitrobenzoate (10) (56 mg) as a colourless oil.

Diplodialide-D (4).—This is a colourless oil (Found: M^+, 200.105 0. $C_{10}H_{16}O_4$ requires M, 200.104 8); $[\alpha]_D^{25}$ +0.8° (*c* 0.82, $CHCl_3$); $\nu_{max.}$(CCl_4) 3 450, 1 730, and 1 700 cm^{-1}; δ($CDCl_3$) 1.20 (3 H, d, *J* 6 Hz, CH–*Me*), 2.57 (2 H, d, *J* 5 Hz, CO–C*H*$_2$–CH), 2.60 [1 H, dd, *J* 14 and 4 Hz, CO–C*H*$_A$H$_B$–CH(OH)], 2.90 [1 H, dd, *J* 14 and 3 Hz, CO–CH$_A$*H*$_B$–CH(OH)], 3.23 (1 H, OH, exchanges with D_2O), 4.37 (1 H, m, C*H*–OH), and 4.56 (1 H, m, O–C*H*–Me).

Treatment of diplodialide-D with base and acid. The lactone (4) (4.5 mg) in methanol (0.5 ml) and aqueous 1N potassium hydroxide (1 ml) were stirred at room temperature for 2 h. The solution was acidified with 1N hydrochloric acid and extracted with ethyl acetate. The organic layer was washed with brine and dried (Na_2SO_4). Removal of solvent gave (12) as a colourless oil (4.09 mg) (Found: M^+, 182.093 8. $C_{10}H_{14}O_3$ requires M, 182.094 3); $\nu_{max.}$(CCl_4) 3 400, 1 650, and 1 610 cm^{-1}; $\lambda_{max.}$(MeOH) 263 nm (ε 11 800); δ($CDCl_3$) 1.36 (3 H, d, *J* 6 Hz, CH–Me), 1.3—1.8 (2 H, m), 2.0—3.0 (6 H, m), 4.16 (1 H, m, CH–O), and 4.30 (1 H, m, CH–O).

Treatment of (12) *with hydrochloric acid in methanol.* Compound (12) (0.5 mg) was dissolved in 1.75% hydrochloric acid in methanol (1 ml), refluxed for 1 h, and cooled to room temperature. The g.l.c. of the solution showed only one peak, and the u.v. and g.l.c.–mass spectra indicated the formation of a phenolic compound, (13) or (14); $\lambda_{max.}$(MeOH) 273 and 281 nm; *m/e* 178 (M^+ or [M − H_2O]$^+$), 137, and 136.

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REFERENCES

[1] T. Ishida and K. Wada, *J.C.S. Chem. Comm.*, 1975, 209.
[2] K. Wada and T. Ishida, *J.C.S. Chem. Comm.*, 1976, 340.
[3] H. P. Sigg, *Helv. Chim. Acta*, 1964, **47**, 1401.
[4] K. J. van der Merwe, P. S. Steyn, and L. Fourie, *J. Chem. Soc.*, 1965, 7083.
[5] A. Ballio, S. Barcellona, and B. Santurbano, *Tetrahedron Letters*, 1966, 3723.
[6] S. Nozoe, K. Hirai, K. Tsuda, K. Ishibashi, M. Shirasaka, and J. F. Grove, *Tetrahedron Letters*, 1975, 4665; D. Seebach, B. Seuring, H.-O. Kalinowski, and B. Renger, *Angew. Chem.*, 1977, **89**, 270.
[7] K. Nakanishi, 'Infrared Absorption Spectroscopy—Practical,' Holden-Day, San Francisco and Nankodo, Tokyo, 1962, p. 69; A. I. Scott, 'Interpretation of the Ultraviolet Spectra of Natural Products,' Pergamon Press, Oxford, 1964, p. 58.
[8] A. J. Birch, O. C. Musgrave, R. W. Rickards, and H. Smith, *J. Chem. Soc.*, 1959, 3146.
[9] W. B. Turner, 'Fungal Metabolites,' Academic Press, London, 1971, p. 116.