Metabolites of *Lasiodiplodia theobromae*

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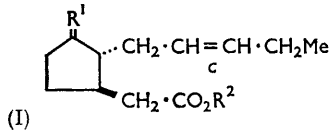
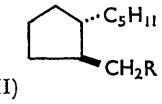
A plant-growth inhibitor produced by *Lasiodiplodia theobromae* has been shown to be jasmonic acid (Ia). Six other new fungal metabolites have been isolated from *L. theobromae*—lasiodiplodin (Va), de-*O*-methyl-lasiodiplodin (Vb), *cis*-4-hydroxymellein (XIIIa), indole-3-carboxylic acid, 3-formylindole, and ethyl hydrogen fumarate. (−)-Mellein (XIIIb) is also produced.

CULTURE filtrates from fermentations of a strain of *Lasiodiplodia theobromae* inhibited the growth of green plants. We have shown the active component to be jasmonic acid (Ia), which occurs as its methyl ester (Ib) in the essential oils of *Jasminium grandiflorum* [1] and *Rosmarinus officinalis*.[2] The isolation of an inseparable mixture of *N*-jasmonyl-isoleucine and *N*-dihydrojasmonyl-isoleucine from *Gibberella fujikuroi* has recently been reported,[3] but the free acid has not previously been isolated from a natural source. The strain of *L. theobromae* used in our experiments was number S22L from the Forest Products Research Laboratories, and we have used three cultures of this strain. The first, given the number ACC 2895 in our collection, initially produced 0·5 g./l. of jasmonic acid but the yield slowly fell during repeated culturing. A second culture, ACC 3183, again gave good yields at first, but again slowly lost the capacity to produce jasmonic acid, and a third culture, ACC 3589, produced only small quantities of jasmonic acid from the outset. Cultures from several other strains of *L. theobromae* (or the synonymous *Botryodiplodia theobromae*) and related species did not produce jasmonic acid.



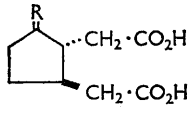
(I)

	R^1	R^2
a;	O	H
b;	O	Me
c;	H,OH	H



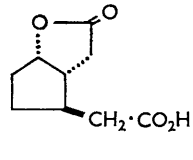
(II)

a; R = CO_2H
b; R = CH_2·OH



(III)

a; R = O
b; R = <OH, H
c; R = H_2



(IV)

During production of jasmonic acid we isolated a further six new fungal metabolites—lasiodiplodin (Va), de-*O*-methyl-lasiodiplodin (Vb), *cis*-4-hydroxymellein (XIIIa), indole-3-carboxylic acid, 3-formylindole, and ethyl hydrogen fumarate—as well as the known metabolite mellein (XIIIb). *L. theobromae* continued to produce these metabolites, under the appropriate conditions, when it was producing only low yields of jasmonic acid. Jasmonic acid was produced only in surface culture, whereas lasiodiplodin, desmethyl-lasiodiplodin, mellein, and indole-3-carboxylic acid were also obtained from submerged fermentations.

Chemistry of Jasmonic Acid.—The identity of the *L. theobromae* metabolite was confirmed by comparison of its methyl ester with a sample of racemic synthetic methyl jasmonate kindly provided by Dr. E. Demole. The compound from *L. theobromae* has the same absolute configuration as the jasmine product, shown to be as in (Ib).[4]

Before we had recognised our metabolite as jasmonic acid, and later in order to study structure-activity relationships in the series, we made several new derivatives of jasmonic acid. Thus Clemmensen reduction of dihydrojasmonic acid gave *trans*-2-pentylcyclopentaneacetic acid (IIa), which was reduced with lithium aluminium hydride to the corresponding alcohol (IIb). Ozonolysis of jasmonic acid followed by oxidation of the resulting aldehyde gave the keto-dicarboxylic acid (IIIa) which with sodium borohydride gave the alcohol (IIIb) and the lactone (IV). Clemmensen reduction of the keto-diacid (IIIa) gave (+)-*trans*-cyclopentane-1,2-diacetic acid, the (−)-isomer of which had previously been prepared;[5] the present derivation establishes the absolute stereochemistry of the compounds. Reduction of jasmonic acid with sodium borohydride gave the alcohol (Ic), shown by its n.m.r. spectrum to be a mixture of epimers.

Structures and Reactions of Lasiodiplodin and De-O-methyl-lasiodiplodin.—Lasiodiplodin (Va) $C_{17}H_{24}O_4$, and de-*O*-methyl-lasiodiplodin (Vb), $C_{16}H_{22}O_4$, form the same dimethyl ether (Vc). The n.m.r. spectrum of de-*O*-methyl-lasiodiplodin shows an AB system, *J* 2 Hz, characteristic of *meta*-benzenoid protons, and signals due to a bonded and a non-bonded phenolic group, a system MeCH·O·CO−, a benzylic methylene group, and 12 protons in the region τ 8—9. The spectrum of lasiodiplodin shows the same features except that the aromatic protons are magnetically equivalent and the signal due to the bonded phenolic group is replaced by one due to an *O*-methyl group. These properties lead to structure (Va) for lasiodiplodin which is consistent with its i.r. and u.v. spectra; the lactone carbonyl

[1] E. Demole, E. Lederer, and D. Mercier, *Helv. Chim. Acta*, 1962, **45**, 675.
[2] L. Crabalona, *Compt. rend.*, 1967, **264**, 2074.
[3] B. E. Cross and G. R. B. Webster, *J. Chem. Soc.* (*C*), 1970, 1839.
[4] R. K. Hill and A. G. Edwards, *Tetrahedron*, 1965, **21**, 1501.
[5] J. W. Barrett and R. P. Linstead, *J. Chem. Soc.*, 1935, 436.

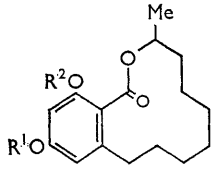
group of lasiodiplodin absorbs at 1690 cm.$^{-1}$ while that of de-*O*-methyl-lasiodiplodin absorbs at 1630 cm.$^{-1}$ as expected for the hydrogen-bonded system. Confirmation of the macrolide system was provided by hydrolysis of the dimethyl ether (Vc) to the hydroxy-acid (VIa). Under the forcing conditions required for this hydrolysis, some demethylation and decarboxylation occurred to give the phenol (VII).

Oxidation of the hydroxy-acid (VIa) with Jones' reagent gave the quinone (VIII). The formation of the quinone system which also occurs with di-*O*-methyl-orsellinic acid (IXa), could proceed by either of the routes indicated in formulae (X) and (XI). That route (X) is operative is shown by oxidation of *O*-ethyleverninic acid (IXb) to give 2-ethoxy-6-methylbenzoquinone, and of *O*-ethylisoeverninic acid (IXc) to give 2-methoxy-6-methylbenzoquinone. Similarly, oxidation of the hydroxy-acid (VIb), derived from lasiodiplodin ethyl ether (Vd), gave the quinone (VIII), a reaction which confirms the position of the *O*-methyl group in lasiodiplodin.

Chemical shifts (τ values) for protons in lasiodiplodin and derivatives [a]

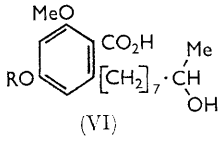
Compound	Ar*H*	C*H*OR	OCH_3	ArCH_2	CH_3	Miscellaneous
(Va)	3·88	4·78m	6·42	7·5m	8·72d	O*H*, 3·5
(Vb)	3·82d, 3·89d	4·8m		6·7m, 7·5m	8·73d	O*H*, −2·0 and 4·5
(Vc)	3·77	4·8m	6·29	7·4m	8·72d	
(Vd)	3·78	4·8m	6·29	7·4m	8·70d	OEt, 6·03q and 8·64t
(Ve)	3·52d, 3·58d	4·8m	6·31	7·4m	8·73d	OAc, 7·81
(VIa)	3·69d, 3·73d	*ca.* 6·2	6·22, 6·27	7·28t	8·88d	O*H*(2), 4·8
(VIb)	3·68	*ca.* 6·2	6·20	7·27t	8·83d	O*H*(2), 4·43; OEt, 5·98q and 8·62t
(VII)	3·67	*ca.* 6·2	6·26	7·52t	8·82d	
(VIII)	3·58m, 4·2d		6·27	7·64t	7·84s	

[a] Spectra were measured at 100MHz for deuteriochloroform solutions with tetramethylsilane as internal standard.



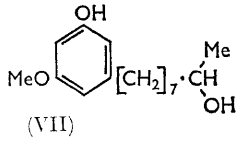
(V)

	R¹	R²
a;	H	Me
b;	H	H
c;	Me	Me
d;	Et	Me
e;	Ac	Me

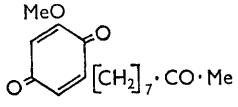


(VI)

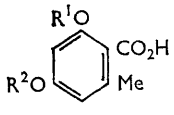
a; R = Me
b; R = Et



(VII)

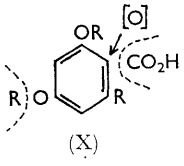


(VIII)

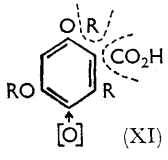


(IX)

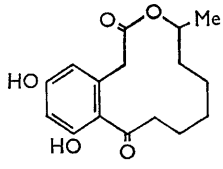
a; $R^1 = R^2 = Me$
b; $R^1 = Et$, $R^2 = Me$
c; $R^1 = Me$, $R^2 = Et$



(X)



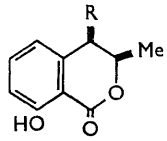
(XI)



(XII)

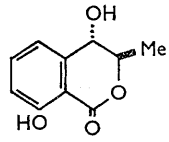
Lasiodiplodin could result from an alternative folding of an acyclic precursor of curvularin (XII).[6] Resorcylic acid lactones related to lasiodiplodin have been synthesized [7] and the appropriate physical properties of the synthetic compounds agree well with those of lasiodiplodin.

Structure of cis-4-*Hydroxymellein*.—The n.m.r. spectrum of *cis*-4-hydroxymellein (XIIIa), $C_{10}H_{10}O_4$, shows the presence of three aromatic protons, a hydrogen-bonded phenolic group, and the system ArCH(OH)·CH(Me)·O·CO·, while its i.r. spectrum shows a band at 1640 cm.$^{-1}$ characteristic of a hydrogen-bonded lactone carbonyl group. These features, together with the u.v. spectrum of the compound, are accommodated by the biosynthetically reasonable structure (XIIIa) which is supported by the formation of a diacetate with the expected physical properties. The *cis*-configuration is allocated to 3-H and 4-H on the basis of the coupling constant (2 Hz); the corresponding *trans*-isomer (XIV) has been isolated from *Apiospora camptospora* [8] and has $J_{3,4}$ 4 Hz. *L. theobromae* produces (−)-mellein while *A. camptospora* produces (+)-mellein [8] so that it is likely that the two hydroxy-compounds differ in the (unknown) absolute stereochemistry at C-3.



(XIII)

a; R = OH
b; R = H



(XIV)

EXPERIMENTAL

Unless otherwise stated, i.r. spectra of solids were determined for Nujol mulls and of liquids for chloroform solutions, u.v. spectra were measured for methanol solutions, and n.m.r. spectra for deuteriochloroform solutions. Silica gel for chromatography was Hopkin and Williams M.F.C. and light petroleum had b.p. 60—80°; t.l.c. was on Merck silica gel G. Mass spectra were determined on a Perkin-Elmer–Hitachi RMU 6E spectrometer and accurate mass measurements were made on an AEI MS9 spectrometer.

[6] A. J. Birch, O. C. Musgrave, R. W. Rickards, and H. Smith, *J. Chem. Soc.*, 1959, 3146.

[7] J. F. Bagli and H. Immer, *Canad. J. Chem.*, 1968, **46**, 3115.

[8] B. F. Burrows, unpublished result.

Isolation of the Metabolites.—(a) *Jasmonic acid, indole-3-carboxylic acid, and 3-formylindole. Lasiodiplodia theobromae* (ACC 3183) was grown as surface culture for 13 days in ceramic vessels each containing 1 l. of Czapek–Dox medium. The medium (76 l.) was filtered, acidified with hydrochloric acid, and extracted with ethyl acetate (1 × 15 l., 2 × 7·5 l.). The ethyl acetate solution was dried, concentrated, and extracted with aqueous sodium hydrogen carbonate to give an acidic (39·3 g.) and a non-acidic (3·6 g.) fraction. The acidic fraction was absorbed from benzene onto a column of silica gel (800 g.) made up in benzene, and the column was washed with benzene (2 l). Elution of the column with benzene–chloroform (1 : 1) gave a brown oil (21·9 g.) which was distilled at 160°/0·5 mm. (a bright red forerun was discarded) to give jasmonic acid.

Elution of the column was continued first with chloroform, which gave a brown gum (2·3 g.), and then with ethyl acetate which gave a dark gummy solid (8·8 g.) which was crystallized from acetone–light petroleum (charcoal) to give indole-3-carboxylic acid, m.p. 237—240° decomp.), identified by its i.r. and mass spectra.

The non-acidic fraction (5·8 g.) from a separate experiment was chromatographed on silica gel (350 ml.). Elution with light petroleum gave a gummy solid (130 mg.) which was crystallized from light petroleum to give sulphur, identified by its i.r. spectrum. Elution with light petroleum–chloroform (1 : 1) gave, first, fractions (490 mg.) containing lasiodiplodin, and then fractions (569 mg.) which were crystallized from acetone–light petroleum to give 3-formylindole, m.p. 189—192°, identified by its i.r. and n.m.r. spectra.

(b) *Lasiodiplodin. L. theobromae* (ACC 2895) was grown as surface culture for 13 days in ceramic vessels each containing 1 l. of Czapek–Dox medium. The mycelium from 98 flasks was washed with water and extracted with chloroform to give a brown oil (19·9 g.) which was extracted with hot, light petroleum leaving an insoluble residue (2·6 g.). When set aside overnight the light petroleum solution deposited crystals (1·2 g.) of *lasiodiplodin*. The mother liquor was evaporated and the residue was combined with the insoluble material above and chromatographed on silica gel (100 ml.). Elution with benzene and with benzene–chloroform (4 : 1) gave a yellow oil which was discarded. Elution with benzene–chloroform (1 : 1) gave a solid (2·2 g.) which was recrystallized from acetone–light petroleum to give further lasiodiplodin.

(c) *Mellein,* cis-4-*hydroxymellein, lasiodiplodin, and ethyl hydrogen fumarate. L. theobromae* (ACC 2895) was grown in milk bottles (1/3 pint capacity) each containing 30 ml. of Raulin–Thom medium. After 13 days the culture filtrate (1·5 l.) was extracted at the natural pH (7) with ethyl acetate to give a neutral/phenolic fraction (A) (206 mg.), acidified with hydrochloric acid, and again extracted with ethyl acetate to give an acid fraction (B) (763 mg.).

A solution of fraction (A) in ether was extracted with *N*-sodium hydroxide to give a phenolic fraction (120 mg.) which was chromatographed on silica gel (20 g.). Elution with benzene and with benzene containing successively 5, 10, and 20% chloroform gave traces of gum. Elution with benzene–chloroform (1 : 1) gave fractions (52 mg.) containing a mixture of three compounds, resolved by t.l.c. in chloroform–acetone (19 : 1) to give (i) (lowest R_F) mellein (5 mg.), m.p. 55—56°, $[\alpha]_D$ −92·5° (*c* 1%, EtOH), identified by its i.r. spectrum, (ii) (middle R_F) lasiodiplodin (14 mg.), and (iii) (highest R_F) cis-4-*hydroxymellein* (10·5 mg.).

A portion of the acidic extract (B) was triturated with acetone to give a solid with i.r. spectrum identical with that of fumaric acid. A second portion (120 mg.) of (B) was chromatographed on silica gel (15 g.). Elution with benzene–chloroform (1 : 1) gave a solid (81 mg.) which was recrystallized from light petroleum to give ethyl hydrogen fumarate as plates, m.p. 66—67° (Found: C, 50·0; H, 5·3%. Calc. for $C_6H_8O_4$: C, 50·0; H, 5·6%).

(d) *De-O-methyl-lasiodiplodin, lasiodiplodin, and mellein. L. theobromae* (ACC 3183) was grown as surface culture for 15 days in Thompson vessels each containing 1 l. of Czapek–Dox medium. The mycelium from 72 flasks was blended with methanol at pH 2; the filtered methanolic solution was concentrated *in vacuo* and the aqueous residue was extracted with ethyl acetate to give a gum (19·5 g.). The gum was absorbed onto silica gel (200 g.) by evaporation of an acetone solution and placed at the top of a column of silica gel (1 l.) made up in benzene. Elution with benzene (2 l.) gave a fraction (1·8 g.) which was further purified by t.l.c. in chloroform–acetone–formic acid (95 : 4 : 1) to give (a) (lower R_F *de-O-methyl-lasiodiplodin* (212 mg.) and (b) higher R_F) mellein (429 mg.). Continued elution of the above column with benzene–chloroform (3 : 1) gave lasiodiplodin.

Characterization of the Metabolites.—(a) Jasmonic acid (Ia) is an oil, n_D^{23} 1·486, $[\alpha]_D^{25}$ −73° (*c* 0·1 in MeOH) (Found: C, 68·5; H, 8·5%; *m/e*, 210. Calc. for $C_{12}H_{18}O_3$: C, 68·5; H, 8·6%; *M*, 210).

(b) *Lasiodiplodin* (Va) forms needles, m.p. 183—184° (Found: C, 70·0; H, 8·2%; *m/e* 292. $C_{17}H_{24}O_4$ requires C, 69·8; H, 8·3%; *M*, 292); $\nu_{max.}$ 3382, 1690, and 1610 cm.$^{-1}$; $\lambda_{max.}$ 219 (ε 12,900), 245 (4560), and 282 mμ (2860).

Lasiodiplodin forms an *acetate* (Ve), needles, m.p. 90° (Found: C, 68·6; H, 8·0%; *m/e* 334. $C_{19}H_{26}O_5$ requires C, 68·2; H, 7·8%; *M*, 334); $\nu_{max.}$ 1760, 1715, and 1591 cm.$^{-1}$; $\lambda_{max.}$ 226 (ε 5000) and 279 mμ (2300); a gummy *methyl ether* (Vc) (Found: *m/e*, 306·1810. $C_{18}H_{26}O_4$ requires *M*, 306·1831); $\nu_{max.}$ 1710 and 1600 cm.$^{-1}$; and a gummy *ethyl ether* (Vd) (Found: *m/e* 320·1955. $C_{19}H_{28}O_4$ requires *M*, 320·1987); $\nu_{max.}$ 1710, 1607, and 1587 cm.$^{-1}$.

(c) *De-O-methyl-lasiodiplodin* (Vb) forms prisms, m.p. 127—129° (Found: C, 69·0; H, 7·8%; *m/e* 278. $C_{16}H_{22}O_4$ requires C, 69·0; H, 8·0%; *M*, 278); $\nu_{max.}$ 3345, 1630, and 1585 cm.$^{-1}$; $\lambda_{max.}$ 224 (ε 14,800), 266 (9,800), and 303 mμ (4,500).

(d) cis-4-*Hydroxymellein* (XIIIa) forms prisms, m.p. 112—117° (Found: C, 62·0; H, 5·2%; *m/e*, 194. $C_{10}H_{10}O_4$ requires C, 61·85; H, 5·2%; *M*, 194); $\nu_{max.}$ 3400, 3100, 1640, 1610sh, and 1530sh, cm.$^{-1}$; $\lambda_{max.}$ 244 (ε 7820) and 312 mμ (4370); τ −0·85 (ArO*H*), 2·48—3·2 (3 Ar*H*), 5·4dq (MeC*H*OCO), 5·53d (C*H*OH) 7·58d (CHO*H*), and 8·47d (CH_3).

cis-4-Hydroxymellein forms a *diacetate*, m.p. 83—85° (Found: C, 60·7; H, 5·1. $C_{14}H_{14}O_6$ requires C, 60·4; H, 5·1%); $\nu_{max.}$ 1770, 1750, 1720, 1610, and 1590 cm.$^{-1}$; τ 2·36—2·93 (3 Ar*H*), 4·21d (C*H*OAc), 5·31 dq (MeC*H*OCO), 7·71 and 7·79 (COCH_3), and 8·59d (CH_3).

trans-2-*Pentylcyclopentaneacetic Acid* (IId).—A suspension of dihydrojasmonic acid [1] (600 mg.) in 6N-hydrochloric acid (15 ml.) was heated under reflux for 16 hr. with zinc amalgam (from zinc dust, 2·5 g.). The cooled mixture was extracted with chloroform to give a yellow oil (474 mg.) which was absorbed onto silica gel by evaporation of an

acetone solution, and placed at the top of a column of silica gel made up in light petroleum. Elution with light petroleum–chloroform gave *trans*-2-pentylcyclopentaneacetic acid (IIa) (275 mg.) as an oil (Found: *m/e* 198; Calc. for $C_{12}H_{22}O_2$: *M*, 198); $\nu_{max.}$ (liquid film) 3300—2500 and 1720 cm.$^{-1}$; *m/e* 198 (3), 180 (0·8), 169 (0·6), 138 (100), 110 (10), 109 (11), 83 (42), 81 (39), and 67 (73).

trans-1-(2′-*Hydroxyethyl*)-2-*pentylcyclopentane* (IIb).—Lithium aluminium hydride (90 mg.) was added portionwise with stirring to a solution of *trans*-2-pentylcyclopentaneacetic acid (85 mg.) in ether (3 ml.) and the mixture was stirred at room temperature for 1 hr. Excess hydride was decomposed with ethyl acetate, 2N–hydrochloric acid was added, and the organic layer was separated; the aqueous layer was extracted with ethyl acetate. The combined organic layers were evaporated to give an oil (83 mg.) which was chromatographed on silica gel (8 ml.). Elution with light petroleum (7 : 3) gave *trans*-1-(2′-hydroxyethyl)-2-pentylcyclopentane (IIb) (67 mg.) as an oil (Found: *m/e*, 183. Calc. for $C_{12}H_{24}O$: *M* − 1, 183); $\nu_{max.}$ (liquid film) 3400 cm.$^{-1}$; τ 6·3t (CH_2OH); *m/e* 138 (100), 110 (18), 109 (16), 95 (71), 83 (29), 82 (68), 81 (34), and 67 (60).

2-*Pentyl*-3-*carboxymethylcyclopentanol* (Ic).— A solution of jasmonic acid (400 mg.) and sodium borohydride (150 mg.) in 0·2N-sodium hydroxide (10 ml.) was set aside at room temperature for 2 hr., acidified with hydrochloric acid, and extracted with ethyl acetate to give a pale yellow oil (377 mg.) which was chromatographed on silica gel. Elution with chloroform and with chloroform–ethyl acetate (9 : 1) gave 2-*pentyl*-3-*carboxymethylcyclopentanol* (Ic) as an oil (Found: *m/e* 212·1404. $C_{12}H_{20}O_3$ requires *M*, 212·1412); $\nu_{max.}$ 3500—2500 and 1712 cm.$^{-1}$; τ 3·6 (*OH* and CO_2H), 4·5m (C*H*=C*H*), 5·75m and 6·05m. (epimeric C*H*OH), and 9·03t (CH_3); *m/e* 212 (0·9), 194 (32), 153 (26), 151 (18), 134 (100), 83 (68), and 79 (53).

trans-2,3-*Di*(*carboxymethyl*)*cyclopentanone* (IIIa).—A solution of jasmonic acid (1·0 g.) in methylene chloride (100 ml.) was treated during 0·5 hr. with an excess of ozonized oxygen and the resulting solution was added dropwise with stirring to a suspension of zinc dust in hot water (200 ml.) at such a rate that the methylene chloride evaporated smoothly. The mixture was filtered and extracted thoroughly with ethyl acetate to give an oil (773 mg.) which was taken up in acetone and treated with 8N-chromic acid (1·05 ml.). The mixture was poured into water and extracted with ethyl acetate to give a solid (635 mg.) which was recrystallized from ethyl acetate–light petroleum to give trans-2,3-*di*(*carboxymethyl*)*cyclopentanone* (IIIa) (421 mg.), m.p. 120—122° (Found: C, 53·8; H, 6·2%; *m/e* 200; $C_9H_{12}O_5$ requires C, 54·0; H, 6·0%; *M*, 200); $\nu_{max.}$ 3100—2600, 1735, and 1683 cm.$^{-1}$.

(+)-trans-*Cyclopentane*-1,2-*diacetic acid* (IIIc).—A solution of the keto-diacid (IIIa) (100 mg.) in 6N-hydrochloric acid (15 ml.) was heated under reflux for 16 hr. with zinc amalgam (from zinc dust, 2·5 g.). The cooled solution was extracted with ethyl acetate to give a gummy solid (94 mg.) which was chromatographed on silica gel (10 g.). Elution with chloroform–ethyl acetate (9 : 1) gave a solid (38 mg.) which was recrystallized from acetone–light petroleum to give (+)-trans-*cyclopentane*-1,2-*diacetic acid* (IIIc), m.p. 147—149°, $[\alpha]_D$ +38° (*c* 0·1, EtOH) (Found: C, 57·9; H, 7·6. $C_9H_{14}O_4$ requires C, 58·0; H, 7·6%). (−)-*trans*-Cyclopentane-1,2-diacetic acid has m.p. 151° and α_{5461} −63·5°.[5]

Reduction of the Keto-diacid (IIId).—Sodium borohydride (45 mg.) was added portionwise with stirring to a solution of the keto-diacid (IIIa) (150 mg.) in 0·5N-sodium hydroxide (3·2 ml.). The solution was set aside at room temperature for 1 hr., acidified with hydrochloric acid, and extracted thoroughly with ethyl acetate to give a gum (103 mg.) The product was absorbed onto silica gel (1 ml.) by evaporation of an acetone solution and placed at the top of a column of silica gel (4 ml.) made up in chloroform. Elution of the column with chloroform–ethyl acetate (9 : 1) gave the lactone (IV) (20 mg.) as a gum (Found: *m/e*, 184·0738. $C_9H_{12}O_4$ requires *M*, 184·0736); $\nu_{max.}$ 1773, 1728sh, and 1720 cm.$^{-1}$; τ 1·6 (CO_2H) and 5·1dt (*CH*OCO); *m/e* 184 (29), 166 (25), 138 (40), 125 (22), 124 (25), 96 (75), and 80 (100). Elution of the column with chloroform–ethyl acetate (3 : 2) gave the hydroxy-acid (IIIb) (54 mg.) (Found: *m/e* 184. Calc. for $C_9H_{14}O_5$: *M* − 18, 184); $\nu_{max.}$ 3500—2400 and 1710 cm.$^{-1}$; τ (in $^2[H]_6$acetone) 2·3 (*OH* and CO_2H) and 6·14m (C*H*OH); *m/e* 184 (1·2), 166 (2·4), 156 (3·8), 125 (50), 97 (31), and 55 (100).

Hydrolysis of Lasiodiplodin Methyl Ether.—A solution of the methyl ether (Vc) (600 mg.) and potassium hydroxide (2 g.) in ethylene glycol (20 ml.) was heated under reflux between 180 and 200° for 24 hr. The ethylene glycol was removed *in vacuo* and the residue was dissolved in water, acidified, and extracted with ethyl acetate. The ethyl acetate solution was extracted with aqueous sodium hydrogen carbonate to give an acid fraction (128 mg.) and a neutral fraction (333 mg.). Chromatography of the acid fraction on a column of silica gel (5 ml.) and elution with chloroform gave the *hydroxy-acid* (VIa) as a gum (101 mg.) (Found: *m/e* 324·1934. $C_{18}H_{28}O_5$ requires *M*, 324·1937); $\nu_{max.}$ 3400—2500, 1725, 1712, and 1605 cm.$^{-1}$.

The neutral fraction was chromatographed on a column of silica gel (15 ml.). Elution with benzene–chloroform (3 : 1) gave starting material (45 mg.); elution with benzene–chloroform (1 : 1) and with chloroform gave the *decarboxylated compound* (VII) as a gum (Found: *m/e* 264·1725. $C_{16}H_{24}O_3$ requires *M*, 264·1725); $\nu_{max.}$ 3590, 3320, 1615, and 1600 cm.$^{-1}$.

Hydrolysis of Lasiodiplodin Ethyl Ether.—The ethyl ether (Vd) (100 mg.) was hydrolysed as above to give an acid fraction (43 mg.) and a neutral fraction (63 mg.). Purification of the acid fraction by t.l.c. in chloroform–acetone–formic acid (95 : 4 : 1) (developing the plate twice) gave the *hydroxy-acid* (VIb) (27 mg.) as a gum (Found: *m/e* 338·2062. $C_{19}H_{30}O_5$ requires *M*, 338·2093); $\nu_{max.}$ 3500—3100, 1725, and 1610 cm.$^{-1}$.

Oxidation of the Hydroxy-acid (VIa).—8N-Chromic acid (0·1 ml.) was added at 0° to a solution of the hydroxy-acid (VIa) (72 mg.) in acetone (1·5 ml.). After 5 min. at 0° the mixture was allowed to come to room temperature and further chromic acid (0·1 ml.) was added. After a further 5 min. a third portion (0·1 ml.) of chromic acid was added to the mixture which was again set aside for 5 min., diluted with water, and extracted with ethyl acetate to give a gummy yellow solid (50 mg.). The product was dissolved in chloroform and filtered through a short column of silica gel to give a yellow solid (22 mg.) which was recrystallized from acetone–light petroleum to give the *quinone* (VIII) as yellow needles, m.p. 122—123: (Found: C, 69·1; H, 7·9%; *m/e* 278. $C_{16}H_{22}O_4$ requires C, 69·0; H, 8·0%; *M*, 278); $\nu_{max.}$ 1717, 1680, 1658, 1653, 1630, and 1604 cm.$^{-1}$; $\lambda_{max.}$ 268 (ε 15,000) and 365 mμ (ε 950).

Oxidation of the Hydroxy-acid (VIb).—8N-Chromic acid was added to a solution of the hydroxy-acid (VIb) (23 mg.)

in acetone (1 ml.). After 10 min., the mixture was worked up as above to give the quinone (VIII), identical with the material obtained from the hydroxy-acid (VIa).

Oxidation of Di-O-methylorsellinic Acid.—8N-Chromic acid (0·35 ml.) was added portionwise to the acid (IXa) (170 mg.) in acetone (1·2 ml.) at 0°. The reaction was worked up as above and the product (116 mg.) was washed with aqueous potassium hydrogen carbonate to remove starting material. The neutral fraction was recrystallized from light petroleum to give 2-methoxy-6-methylbenzoquinone, m.p. 147—149° (lit.,[9] m.p. 147—149°), τ 3·52, pentaplet, *J* 2 Hz (C*H*=CMe), 4·20d, *J* 2 Hz (C*H*=COMe), 6·27s (O*Me*), and 8·00d, *J* 2 Hz (CH=C*Me*).

Oxidation of O-Ethylisoeverninic Acid.—The acid (IXc) (42 mg.) in acetone (0·5 ml.) was oxidised as above with 8N-chromic acid to give 2-methoxy-6-methylbenzoquinone (10 mg.).

Oxidation of O-Ethyleverninic Acid.—The acid (IXb) (420 mg.) in acetone (1·5 ml.) was oxidized as above to give 2-ethoxy-6-methylbenzoquinone (80 mg.), m.p. 53—55° (lit.,[10] m.p. 54—56°), τ 3·4 m. (C*H*=C·Me), 4·09d, *J* 1·5 Hz (C*H*=COMe), 5·93q and 9·53t, *J* 7 Hz (O*Et*), and 7·92d, *J* 2·0 Hz (CH=C–*Me*).

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