Studies on the Antibiotic Nigericin (Polyetherin A)[1]

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Polyetherin A, isolated from cultures of *Streptomyces hygroscopicus* E-749, is shown to be identical with the antibiotic nigericin, after independent structure elucidations. The compound is established, by chemical degradation and spectroscopic evidence, to be a polycyclic polyether (Ia).

FROM cultures of *Streptomyces hygroscopicus* E-749, we have isolated [2] an antibiotic, m.p. 183—185°, $[\alpha]_D$ +36·2° ($CHCl_3$) [sodium salt, m.p. 245—255° (decomp.)], and named it polyetherin A. An amorphous antibiotic, nigericin, $C_{39}H_{69}O_{11}$ (sodium salt; m.p. 246—254°), was obtained by other workers from an unidentified *Streptomyces* isolated from a Nigerian soil.[3] The same structure has recently been assigned independently to polyetherin A [1] and nigericin,[4] and the identity of the specimens has been established by direct comparison. In view of the fact that there are several biochemical studies on nigericin,[5] we now abandon our name polyetherin A. We now describe details of studies on nigericin leading to the assignment of structure (Ia).

The molecular formula of nigericin was previously [2] represented as $C_{42-43}H_{72-74}O_{12}$; it is now corrected to $C_{40}H_{68}O_{11}$ (M 724) as a result of elemental analyses,

[1] Preliminary communication, T. Kubota, S. Matsutani, M. Shiro, and H. Koyama, *Chem. Comm.*, 1968, 1541.

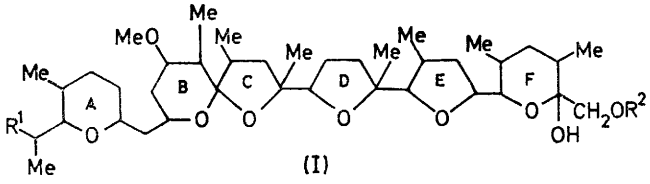
[2] J. Shoji, S. Kozuki, S. Matsutani, T. Kubota, H. Nishimura, M. Mayama, K. Motokawa, Y. Tanaka, N. Shimaoka, and H. Otsuka, *J. Antibiotics*, 1968, **21**, 402.

[3] R. L. Harned, P. H. Hidy, C. J. Corum, and K. L. Jones, *Antibiotics and Chemotherapy*, 1951, **1**, 594.

[4] L. K. Steinrauf, M. Pinkerton, and J. W. Chamberlin, *Biochem. Biophys. Res. Comm.*, 1968, **33**, 29.

[5] R. L. Harned, P. H. Hidy, C. J. Corum, and K. L. Jones, *Proc. Indiana Acad. Sci.*, 1950, **59**, 38; H. Lardy, D. Johnson, and W. C. McMurrany, *Arch. Biochem. Biophys.*, 1958, **78**, 587; S. N. Graven, S. Estrada-O, and H. Lardy, *Proc. Nat. Acad. Sci., U.S.*, 1966, **56**, 654; S. Estrada-O, S. N. Graven, and H. Lardy, *J. Biol. Chem.*, 1967, **242**, 2925.

osmometric molecular weight determination (in methanol), mass spectra of the methyl ester and other derivatives,* and *X*-ray diffraction analysis of the silver salt.[6] *Nigericin* has been characterised as a polycyclic polyether having vicinal primary and tertiary hydroxy-groups, a carboxy- and a methoxy-group, but no double bond. The remaining six oxygen atoms were assumed to exist as ether functions.[2]

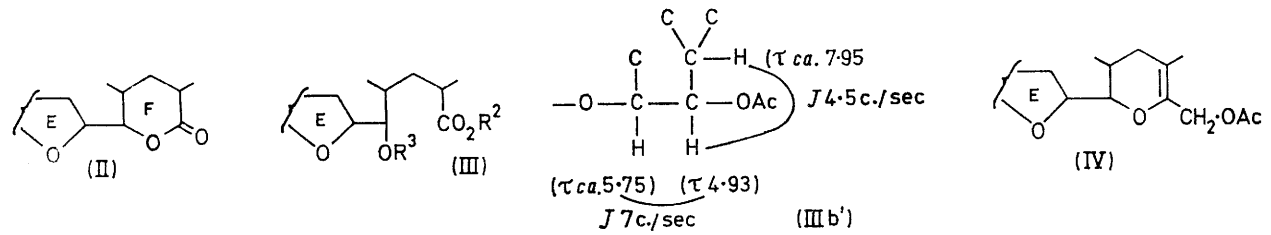


(I)

a; $R^1 = CO_2H$, $R^2 = H$
b; $R^1 = CO_2Me$, $R^2 = H$
c; $R^1 = CO_2Me$, $R^2 = Ac$
d; $R^1 = CO_2Et$, $R^2 = H$

The methyl ester (Ib), M^+ 738, on treatment with acetic anhydride–pyridine afforded a monoacetate (Ic), *m/e* 762 ($M - H_2O$), and on treatment with acetone at 1566 cm.$^{-1}$. Treatment of (IIIa) with diazomethane followed by acetylation yielded an acetoxy-dimethyl ester (IIIb), M^+ 780. The n.m.r. spectrum (100 Mc./sec. in C_6D_6) confirms the presence of two methoxycarbonyl (τ 6·58 and 6·38), an acetoxy- (τ 8·22), and a methoxy-group (τ 6·82), and a spin-decoupling study suggests that the environment of the acetoxy-group is as shown in (IIIb′).

Treatment of nigericin methyl ester-acetate (Ic) with thionyl chloride–pyridine afforded an anhydride (IV), M^+ 762. The n.m.r. spectrum exhibits no olefinic proton but implies the presence of a vinylic methyl [τ 7·95 (3H, s)] and an acetoxymethyl [τ 5·45 (2H, s)] group. This indicates that a methyl group is located on the carbon atom next to the tertiary hydroxy-bearing carbon in nigericin. This conclusion is supported by the fact that an anomeric proton of the acetal acetate (VIb) which was derived from reduction of the methyl ester (Ib) with lithium aluminium hydride followed by sodium periodate oxidation of the resulting tetrol (V) and acetylation, gave rise to an n.m.r. doublet

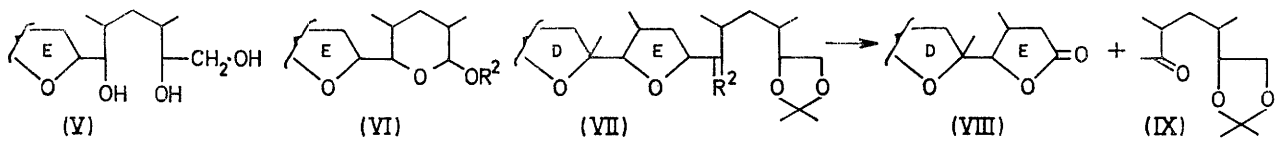


(II) (III) (IIIb′) (IV)

a; $R^1 = CO_2Me$
b; $R^1 = CO_2Et$

a; $R^1 = CO_2H$, $R^2 = R^3 = H$
b; $R^1 = CO_2Me$, $R^2 = Me$, $R^3 = Ac$

$R^1 = CO_2Me$



(V) (VI) (VII) (VIII) (IX)

$R^1 = CH_3{\cdot}OH$

a; $R^1 = CH_2{\cdot}OH$, $R^2 = H$
b; $R^1 = CH_2{\cdot}OAc$, $R^2 = Ac$

a; $R^1 = CO_2Me$, $R^2 = H,OH$
b; $R^1 = CO_2Me$, $R^2 = O$
c; $R^1 = Me_2C{\cdot}OH$, $R^2 = Me,OH$

$R^1 = Me_2C{\cdot}OH$

containing a catalytic amount of toluene-*p*-sulphonic acid yielded an acetonide, M^+ 778, which showed no i.r. hydroxy-absorption.[2] The methyl ester (Ib) reacted with sodium periodate (1 mol), giving an amorphous δ-lactone (IIa), with loss of formaldehyde (characterised as the dimedone derivative). The δ-lactone (IIa), M^+ 706, shows no aldehydic proton in the n.m.r. spectrum and no i.r. hydroxy-absorption but a band at 1735 cm.$^{-1}$. Saponification of (IIa) yielded a hydroxy-dicarboxylic acid (IIIa), of which the sodium salt exhibited no i.r. carbonyl band except for a carboxylate absorption (J 8·5 c./sec.) at τ 4·77. The mass spectrum of nigericin methyl ester (Ib), M^+ 738, exhibits a distinct fragment peak at *m/e* 579 (M — 159), which, in the light of comparison with other derivatives, is suggested to arise from fission between rings E and F.

Treatment of the methyl ester (Ib) with sodium borohydride gave a methyl ester-triol through reductive fission of the hemiacetal ring F. The triol on treatment with acetone and toluene-*p*-sulphonic acid yielded an acetonide (VIIa), M^+ 780, the remaining hydroxy-group of which was oxidised by dimethyl sulphoxide–acetic anhydride [7] to give an acetonide-ketone (VIIb),

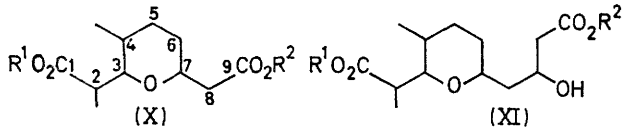
* The mass spectra of the free acid exhibit no peak for the molecular ion. The highest mass peak was at *m/e* 688 ($M - 2H_2O$).

[6] M. Shiro and H. Koyama, *J. Chem. Soc.* (*B*), 1970, 243.

[7] J. D. Albright and L. Goldman, *J. Amer. Chem. Soc.*, 1967, **89**, 2416.

M^+ 778, ($\nu_{max.}$ 1730 and 1710 cm.$^{-1}$). Reaction of the ketone (VIIb) with methylmagnesium iodide afforded a methyl carbinol (VIIc), M^+ 794, which shows no i.r. carbonyl absorption, with simultaneous conversion of the methoxycarbonyl group into a dimethyl carbinol. Treatment of (VIIc) with chromic acid in aqueous acetic acid at room temperature caused oxidative fission,[8] giving a γ-lactone (VIII), *m/e* 576 ($M - H_2O$), $\nu_{max.}$ 1775 cm.$^{-1}$ and a methyl ketone (IX), M^+ 214, $\nu_{max.}$ 1707 cm.$^{-1}$. The n.m.r. spectrum of (IX) shows the presence of an acetyl group [τ 7·87 (3H, s)], two secondary methyls [τ 8·92 and 9·02 (each 3H, *J* 10 and 9 c./sec.)], and an isopropylidenedioxy-group [τ 8·62 and 8·65 (each 3H, s)]. The n.m.r. spectrum of the γ-lactone (VIII) shows a doublet at τ 5·68 (1H, *J* 5 c./sec.) for a proton on the carbon bearing the oxygen atom in the lactone ring; the mass spectrum suggests that the lactone ring consists of $C_5H_7O_2$. These results establish the structure of the rings E and F in nigericin.

Oxidation of the methyl ester δ-lactone (IIa) with chromic acid in aqueous acetic acid at 80° yielded many products, from which, after methylation of the acidic fraction with diazomethane, a dimethyl ester (Xa), M^+ 258, [$\nu_{max.}$ 1740 and 1730 cm.$^{-1}$; no OH] was isolated. Saponification gave a crystalline diacid (Xc), $C_{11}H_{18}O_5$, m.p. 187·5—190°, $[\alpha]_D$ −32·2° (in EtOH). The results of n.m.r. spin decoupling (100 Mc./sec. in C_6D_6) of the dimethyl ester (Xa) [τ 6·58 and 6·55 (each

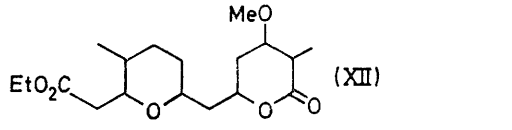


(X)

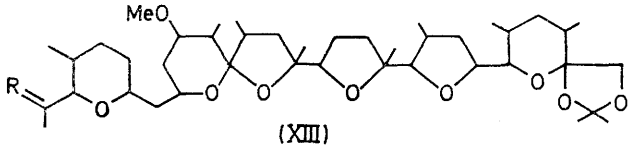
a; $R^1 = R^2 = Me$
b; $R^1 = Et, R^2 = Me$
c; $R^1 = R^2 = H$

(XI)

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



(XII)



(XIII)

a; R = H,CO_2Na
b; R = H,COCl
c; R = H,OH
d; R = O

3H, s, CO_2Me), 9·14 (d, *J* 7 c./sec., 2-Me), 9·28 (d, *J* 7 c./sec., 4-Me), 7·46 (dd, $J_{2,3}$ 10, $J_{2,2Me}$ 7 c./sec., 2-H), 6·32 (dd, $J_{3,2}$ 10, $J_{3,4}$ 2 c./sec., 3-H), 5·54 (partially resolved q, 7-H), and 7·60 and 7·22 (2H, ABX, J_{AB} 15, $J_{AX} = J_{BX}$ 7 c./sec., 8-H_2)] are in good agreement with the assigned structure. The carboxy-group in nigericin acetonide was converted into a hydroxy-group, by the carboxy-inversion reaction of acyl aroyl peroxides.[9] The resulting nor-alcohol (XIIIc) on oxidation with chromium trioxide in pyridine afforded a ketone (XIIId), M^+ 734, $\nu_{max.}$ 1710 cm.$^{-1}$, of which the n.m.r. spectrum exhibits a signal at τ 7·83 (3H, s, Ac); thus the carboxy-group of nigericin is shown to exist as $MeCH{\cdot}CO_2H$. In the light of this result, the 1-carboxy-group in the diacid (Xc) is suggested to be the one existing originally in nigericin.

The ethyl ester δ-lactone (IIb), derived from oxidation of nigericin ethyl ester (Id) with sodium periodate, was oxidised with chromium trioxide in acetic acid, to give a methyl ethyl diester (Xb), M^+ 272, which on saponification afforded the diacid (Xc). Comparison between the mass spectra of (Xa) (*m/e* 258, 185, and 171) and (Xb) (*m/e* 272, 199, and 171) confirms the carboxy-terminal structure in nigericin. Two additional oxidation products were obtained. One, isolated from methylation of an acidic fraction, was identified as (XIa), in which the hydroxy-group may be formed by hydrolysis during the isolation process, on the basis of the following spectral data: *m/e* 316, 199, 153, 125, and 103 (100%), $\nu_{max.}$ 3490, 1733, and 1720 cm.$^{-1}$, τ (C_6D_6) 6·60 (s, CO_2Me), 8·92 (t, *J* 7 c./sec., $CO_2{\cdot}CH_2CH_3$), 9·08 and 9·23 (each 3H, d, *J* 7 and 6·5 c./sec., 2 secondary Me), 5·87 (q, *J* 7 c./sec., 10-H), and 6·85br (exchangeable, OH). Alkaline hydrolysis gave the free acid (XIb), $C_{13}H_{22}O_6$, m.p. 158—161°. The other product was neutral $C_{19}H_{32}O_6$, m.p. 156—159°. The mass and n.m.r. (C_6D_6) spectra suggest the structure (XII): *m/e* 356, 199, 153, and 125; τ 6·95 (s, OMe), and 9·23, 9·10, and 8·63 (each 3H, d, *J* 7 c./sec., 3 secondary Me).

The isolation of (XII), as well as (Xb) and (XIa), proves the structure of rings A and B, containing the carboxy- and methoxy-group in nigericin.

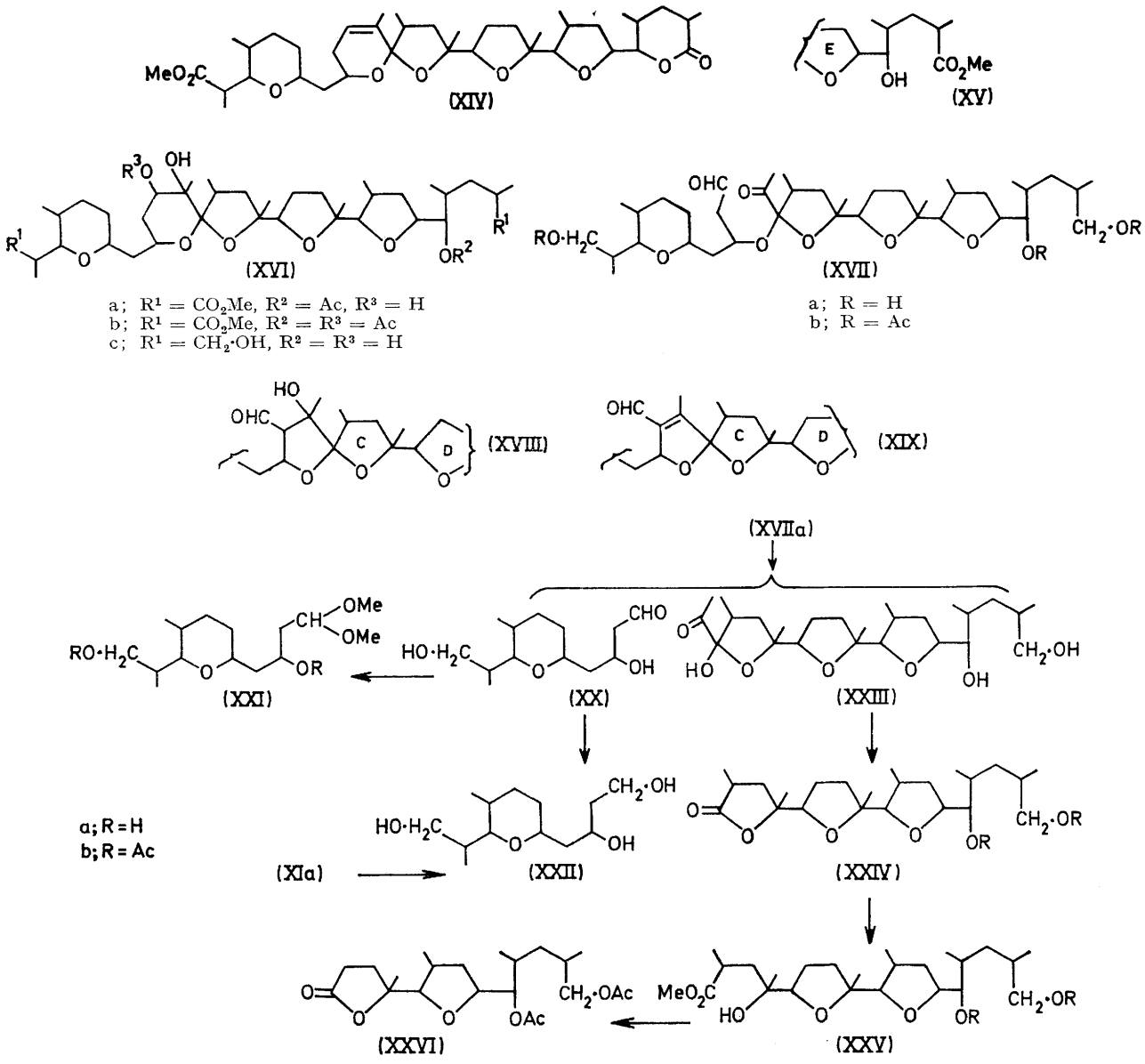
Treatment of the methyl ester δ-lactone (IIa) with aqueous 2N-sulphuric acid and methanol (1 : 10, v/v) at reflux afforded two products, (XIV), M^+ 674, and (XV), M^+ 706, in which the methoxy-group is missing. Both of their n.m.r. spectra (C_6D_6) reveal an olefinic proton (τ 4·33, $W_{\frac{1}{2}}$ 0·8 c./sec.) and a vinylic methyl group [τ 8·28 in (XIV); 8·33 in (XV)]. The former (XIV) has one methoxycarbonyl group [τ 6·32 (3H)], as existed originally in (IIa), but the latter (XV) has two [τ 6·32 and 6·35 (each 3H)] indicating that methanolysis of the δ-lactone ring has occurred. The former (XIV) on alkaline hydrolysis followed by methylation was converted into the latter (XV), as expected. Acetylation of (XV) followed by hydroxylation with osmium tetroxide afforded a *vic*-glycol (XVIa), which on acetylation gave a diacetate (XVIb), M^+ 824, having a free hydroxy-group [$\nu_{max.}$ 3600—3400 and 1730 cm.$^{-1}$, τ (C_6D_6) 8·23 and 8·17 (each 3H, s), 4·70 (1H, dd, *J* 6 and 12 c./sec.), and 4·88 (1H, dd, *J* 5 and 7 c./sec.)]. Reduction of (XVIa) with lithium aluminium hydride, followed by glycol fission of the resulting pentol (XVIc) with lead

[8] E. W. Warnhoff and C. M. M. Halls, *Canad. J. Chem.*, 1965, **43**, 3311.

[9] D. B. Denney and N. Sherman, *J. Org. Chem.*, 1965, **30**, 3760.

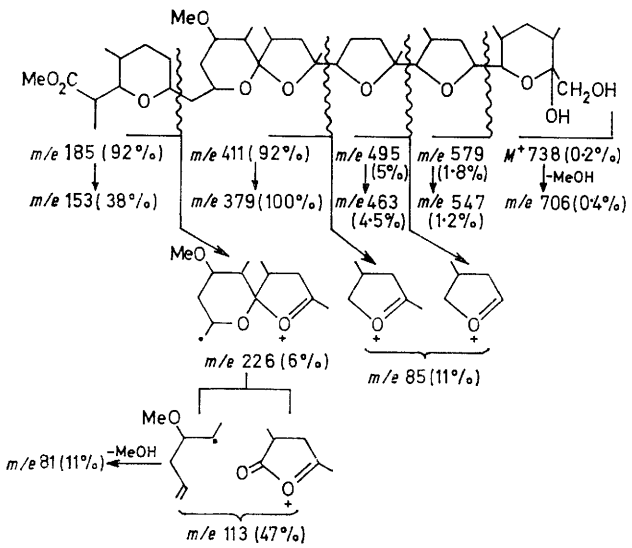
tetra-acetate afforded a keto-aldehyde (XVIIa), as shown by the n.m.r. spectrum [a singlet (3H) at τ 7·65 and a multiplet (1H) at τ 0·17]. Acetylation of (XVIIa) gave the triacetate (XVIIb), which shows no i.r. hydroxy-band. Attempts to purify (XVIIb) by preparative layer chromatography on silica gel resulted in intramolecular aldol condensation to give (XVIII), M^+ 808. The i.r. spectrum showed a hydroxy-absorption at 3460 cm.$^{-1}$, and in the n.m.r. spectrum, the methyl carbonyl absorption had disappeared; signals remained for three acetoxy-groups [τ 7·95 (9H)] and the aldehyde [τ *ca.* 0·3 (1H, m)]. Alkaline hydrolysis of (XVIII) afforded an αβ-unsaturated aldehyde (XIX), M^+ 664, [$\nu_{max.}$ 3500br and 1675 cm.$^{-1}$, $\lambda_{max.}$ (EtOH) 243 mμ]. Direct oxidation of (XVIa) with lead tetra-acetate gave the corresponding keto-aldehyde, which on alkaline treatment was converted into the αβ-unsaturated aldehyde, in a similar way to (XVIIb).

The smooth formation of the olefin (XIV) through elimination of methanol by treatment of the δ-lactone (IIa) with acid, coupled with the fact that the oxidation product (XII) was isolated, suggests the presence of a spiroacetal function at the β-position of the methoxy-bearing carbon. Treatment of the keto-aldehyde (XVIIa) with perchloric acid in dioxan caused hydrolysis of the acetal linkage, giving two compounds, an aldehyde (XX) [τ 0·18 (1H, t, J 1·5 c./sec.)] and a methyl ketone (XXIII), M^+ 456, [τ 7·68 (3H, s)]. The aldehyde (XX) on treatment with methanol and a catalytic amount of toluene-*p*-sulphonic acid gave a dimethyl acetal (XXIa), $C_{15}H_{30}O_5$ [*m/e* 291 (M + 1)], m.p. 93·5—95°, which on acetylation afforded a diacetate (XXIb), M^+ 374. Re-



duction of (XX) with sodium borohydride afforded a triol (XXII), M^+ 246, identical with the product derived from reduction of the hydroxy-diester (XIa) with lithium aluminium hydride.

Treatment of the methyl ketone (XXIII) with lead tetra-acetate in 90% acetic acid yielded a tricyclic γ-lactone (XXIVa), M^+ 412, $\nu_{max.}$ 3460 and 1758 cm.$^{-1}$, which on acetylation gave a diacetate (XXIVb), M^+ 496. The i.r. spectrum showed no hydroxy-absorption and the n.m.r. spectrum demonstrated the presence of two acetoxy-groups (τ 8·27 and 8·18), four secondary methyl groups [τ 9·28, 9·15, 9·10, and 8·90 (each 3H, d, J 7 c./sec.)], and two tertiary methyl groups (τ 9·05 and 8·85). Alkaline hydrolysis of (XXIVa) followed by treatment with diazomethane afforded a trihydroxy-acid methyl ester (XXVa), $C_{24}H_{44}O_7$ (M^+ 444), m.p. 105·5—107°, $[\alpha]_D$ −11·4°. Acetylation with acetic anhydride–pyridine afforded a diacetate (XXVb), M^+ 528, containing a free hydroxy-group ($\nu_{max.}$ 3440 cm.$^{-1}$). In n.m.r. spin decoupling, a doublet at τ 8·80 (J 7 c./sec.) due to one of the four secondary methyl groups, on irradiation at τ 7·30 (α-proton of the methoxycarbonyl group) was transformed into a singlet, and on irradiation at τ 8·80 the signal at τ 7·30 was transformed from a broad multiplet into a deformed quartet. This proves the presence of a secondary methyl group on the α-carbon of the methoxycarbonyl group in (XXVb). Treatment of (XXVb) with chromium trioxide in acetic acid resulted in oxidative fission, giving a bicyclic γ-lactone



SCHEME

Mass spectral fragmentation of nigericin methyl ester (Ib) (relative intensities in parentheses)

(XXVI), M^+ 398, $\nu_{max.}$ 1768 and 1733 cm.$^{-1}$. The n.m.r. spectrum shows the presence of two acetoxy-groups [τ 8·22 (6H)], three secondary methyl groups [τ 9·33, 9·10, and 9·05 (each 3H, d, J 7 c./sec.)], and one tertiary methyl (τ 8·98), but contains signals for one tertiary and one secondary methyl group less than the spectrum of (XXIVb). In view of the assigned structures of rings E and F, these experiments prove the structures of rings C and D in nigericin.

The structure of nigericin is thus as shown in formula (Ia); this is supported by the mass spectral fragmentation of nigericin methyl ester (Ib) (see Scheme). *X*-Ray diffraction studies of the silver salt[1,6] fully establish the complete structure of nigericin (Ia) including its stereochemistry.

EXPERIMENTAL

M.p.s were determined with a Monoscop VS hot plate. Unless otherwise specified, i.r. spectra were taken for solutions in chloroform and n.m.r. spectra were determined at 60 Mc./sec. with a Varian A60 instrument for solutions in deuteriochloroform with tetramethylsilane as internal standard. Mass spectra were recorded with a Hitachi RMU-6 spectrometer by direct insertion. All extracts were dried over anhydrous sodium sulphate. Preparative t.l.c. was performed on Merck silica gel G; spots were detected under u.v. light (254 mμ) after spraying with 0·1% morin in methanol. Homogeneities of amorphous compounds were confirmed by t.l.c. and spectra (i.r., mass, and n.m.r.).

Sodium Periodate Oxidation of Nigericin Methyl Ester (Ib).—A mixture of the methyl ester (Ib)[2] (1·24 g.) in dioxan (10 ml.) and sodium periodate (880 mg.) in water (4 ml.) was set aside in the dark at room temperature for 5·5 hr. with intermittent swirling. A stream of nitrogen was passed through the mixture into a trap containing a cold solution of dimedone (300 mg.) in ethanol (50 ml.); this was kept overnight at room temperature, then evaporated to dryness. The residue, after preparative t.l.c. [toluene–ethyl acetate–methanol (6 : 3 : 1)], gave formaldehyde-dimedone, m.p. 187—191° (from ethanol), identical with an authentic sample (Found: C, 70·0; H, 8·3. Calc. for $C_{17}H_{24}O_4$: C, 69·85; H, 8·25%).

The non-volatile product was diluted with water and extracted with ether. The extract was washed, dried, and evaporated to afford the *methyl ester* δ-*lactone* (IIa) (1·19 g.) as a foam, $\nu_{max.}$ 1735 cm.$^{-1}$, τ 6·70 (3H, s, OMe) and 6·27 (3H, s, CO_2Me).

Hydrolysis of the Methyl Ester δ-*Lactone* (IIa).—A solution of (IIa) (82 mg.) in 10% methanolic potassium hydroxide was refluxed for 1·5 hr. in a stream of nitrogen. After dilution with water and washing with ether, the alkaline layer was acidified with N-hydrochloric acid and extracted thoroughly with ether. The extract was washed and treated with diazomethane to give the *hydroxy-dimethyl ester* (IIIa) (82 mg.), $\nu_{max.}$ 3460 and 1730 cm.$^{-1}$.

Acetylation of (IIIa) (61 mg.) with acetic anhydride (1·6 ml.) and pyridine (2 ml.) at room temperature overnight gave an oil which was purified by preparative t.l.c. [toluene–ethyl acetate (3 : 1)] to afford the *acetoxy-dimethyl ester* (IIIb) (59 mg.), $\nu_{max.}$ 1730 cm.$^{-1}$, τ (100 Mc./sec. in C_6D_6) 8·22 (3H, s, OAc), 6·85 (3H, s, OMe), 6·58 and 6·38 (each 3H, s, 2 × CO_2Me), and 4·93 (1H, dd, J 4·5 and 7 c./sec., CH bearing OAc) [Found: M (mass spectrum), 780. $C_{43}H_{72}O_{12}$ requires M, 780].

Dehydration of Nigericin Methyl Ester–Acetate (Ic) *with Thionyl Chloride and Pyridine.*—To a solution of the methyl ester-acetate (Ic)[2] (70 mg.) in pyridine (6 ml.) cooled in ice, thionyl chloride (0·6 ml.) was added. The mixture was gradually warmed to room temperature and kept for 2 hr.

It was then poured on ice, acidified with 10% sulphuric acid, and extracted with ether. The extract was washed with 5% sodium hydrogen carbonate and water, dried, and evaporated to leave a coloured foam (66 mg.), which on t.l.c. [toluene–ethyl acetate (3 : 1)] showed at least four spots. Separation of the predominant fraction by preparative t.l.c. gave the *anhydride* (IV) (14 mg.) as a foam, $\nu_{max.}$ 1730 cm.$^{-1}$, τ 8·35 (3H, s, vinylic Me), 7·95 (3H, s, OAc), 6·70 (3H, s, OMe), 6·28 (3H, s, CO_2Me), and 5·45br (2H, s, CH_2 bearing OAc) [Found: *M* (mass spectrum), 762. $C_{43}H_{70}O_{11}$ requires *M*, 762].

Lithium Aluminium Hydride Reduction of Nigericin Methyl Ester (Ib).—A mixture of the methyl ester (Ib) (68 mg.) and lithium aluminium hydride (35 mg.) in dry ether (8 ml.) was set aside at room temperature overnight. The crude product (62 mg.) was isolated, in the usual way, by extraction with ether and purified by preparative t.l.c. [toluene–ethyl acetate–methanol (6 : 3 : 1)] to give the *tetrol* (V) (45 mg.).

Sodium Periodate Oxidation of the Tetrol (V).—To a solution of the tetrol (V) (45 mg.) in tetrahydrofuran (2·6 ml.), sodium periodate (38 mg.) in water (1 ml.) was added. The mixture was kept at room temperature overnight and evaporated *in vacuo*. The residue was dissolved in ether and washed with water. Removal of the ether left the crude *acetal* (VIa) (45·4 mg.), no i.r. carbonyl peak.

The acetal (VIa) was treated with acetic anhydride–pyridine and the product, isolated in the usual manner, was purified by preparative t.l.c. [toluene–ethyl acetate (2 : 1)] to give the *diacetate* (VIb) (38 mg.), $\nu_{max.}$ 1730 cm.$^{-1}$, τ 7·95 and 7·92 (each 3H, s, 2 × OAc), 6·70 (3H, s, OMe), and 4·77 (1H, dd, *J* 8·5 c./sec., anomeric H).

Sodium Borohydride Reduction of the Methyl Ester (Ib).—A mixture of the methyl ester (Ib) [2] (78 mg.) and sodium borohydride (40 mg.) in ethanol (3 ml.) was kept at room temperature overnight. Isolation of the product, in the usual way, by extraction with ether gave the *triol* (79 mg.) as a foam, which was treated with toluene-*p*-sulphonic acid (5 mg.) in acetone (5 ml.) at room temperature overnight. The product, isolated in the usual way, was purified by preparative t.l.c. [toluene–ethyl acetate (2 : 1)] to afford the *triol acetonide* (VIIa) (75 mg.) as a foam, $\nu_{max.}$ 3480, 3280, and 1730 cm.$^{-1}$ [Found: *M* (mass spectrum), 780. $C_{44}H_{76}O_{11}$ requires *M*, 780].

Oxidation of the Triol Acetonide (VIIa) *with Dimethyl Sulphoxide and Acetic Anhydride*.—A mixture of (VIIa) (88 mg.), dry dimethyl sulphoxide (5 ml.), and acetic anhydride (1·5 ml.) was kept at room temperature for 24 hr., and then concentrated *in vacuo* under nitrogen (bath temp. $<80°$). The resulting viscous oil was taken up in ether, washed with 10% potassium carbonate and water, dried, and evaporated to afford the *acetonide-ketone* (VIIb) (88 mg.), $\nu_{max.}$ 1730 and 1710 cm.$^{-1}$ [Found: *M* (mass spectrum), 778. $C_{45}H_{78}O_{11}$ requires *M*, 778].

Grignard Reaction of the Acetonide-ketone (VIIb).—To methylmagnesium iodide [from magnesium (72 mg.), methyl iodide (450 mg.), and dry ether (10 ml.)] the acetonide-ketone (VIIb) (85 mg.) in dry ether (10 ml.) was added dropwise with stirring. The mixture was refluxed for 5 hr., then cooled, saturated ammonium chloride solution (20 ml.) was added, and the product was extracted with ether. The ether layer was washed, dried, and evaporated to leave a foam (81 mg.), which was purified by preparative t.l.c. [toluene–ethyl acetate–methanol (6 : 3 : 1)], affording the *methyl carbinol* (VIIc) (53 mg.), $\nu_{max.}$ 3400 cm.$^{-1}$, τ 8·85 (15H, s, 5 × tertiary Me), 8·67 and 8·62 (each 3H, s, isopropylidenedioxy), and 6·68 (3H, s, OMe) [Found: *M* (mass spectrum), 794. $C_{46}H_{82}O_{10}$ requires *M*, 794].

Chromium Trioxide Oxidation of the Methyl Carbinol (VIIc).—A solution of (VIIc) (312 mg.) in glacial acetic acid (6 ml.) was added to a stirred solution of chromium trioxide (119 mg.) in glacial acetic acid (8 ml.) and water (0·05 ml.) cooled in ice. The mixture was gradually warmed to room temperature, kept for 4 hr., then diluted with ice–water, neutralised with 5% sodium hydrogen carbonate, and extracted with ether. The extract was washed, dried, and evaporated to give a residue (285 mg.), which was separated into two fractions by preparative t.l.c. [toluene–ethyl acetate (2 : 1); two developments]. The more mobile fraction (R_F 0·68; 24 mg.) was identified as the *methyl ketone* (IX), $\nu_{max.}$ 1706 cm.$^{-1}$, τ 9·02 and 8·92 (each 3H, d, *J* 9 and 10 c./sec., respectively, 2 × secondary Me), 8·65 and 8·62 (each 3H, s, isopropylidenedioxy), and 7·83 (3H, s, Ac), *m/e* 214 (M^+) and 199 (M — Me). The less mobile one (R_F 0·38; 74 mg.) was the *γ-lactone* (VIII), $\nu_{max.}$ 3400 and 1755 cm.$^{-1}$, τ 9·33—8·92 (15H, m, 5 × secondary Me), 8·85, 8·82, and 8·72 (6H, 3H, and 3H respectively, each s, 4 × tertiary Me), 6·70 (3H, s, OMe), and 5·68 (1H, d, *J* 5 c./sec., CH of γ-lactone).

Chromium Trioxide Oxidation of the Methyl Ester δ-Lactone (IIa).—To a solution of (IIa) (1·24 g.) in glacial acetic acid (18 ml.) cooled in ice, chromium trioxide (1·6 g.) in 66% aqueous acetic acid (53 ml.) was added dropwise with stirring. The mixture was warmed at 80° for 45 min., then diluted with water, and extracted thoroughly with ether. The extract was washed with 10% sodium hydroxide and water, dried, and evaporated to give a neutral fraction (179 mg.). The alkaline layer was acidified with 10% sulphuric acid, with cooling, and extracted with ether. The acidic product was treated with diazomethane in ether, giving a methyl ester (731 mg.) which was chromatographed on silica gel (35 g.). Fractions (195 mg.) eluted with light petroleum–ether (7 : 3, v/v; 180 ml. and 6 : 4, v/v; 300 ml.) were submitted to preparative t.l.c. [toluene–ethyl acetate (4 : 1)], giving the *dimethyl ester* (Xa) (52 mg.) as the most mobile fraction, $\nu_{max.}$ 1740 and 1730 cm.$^{-1}$, τ (100 Mc./sec. in C_6D_6) 9·28 and 9·14 (each 3H, d, *J* 7 c./sec., 2 × secondary Me), 7·60 and 7·22 (2H, AB part of ABX, *J* 7 and 15 c./sec.), 7·46 (1H, dd, *J* 7 and 10 c./sec.), 6·58 and 6·55 (each 3H, s, 2 × CO_2Me), 6·32 (1H, dd, *J* 2 and 10 c./sec.), and 5·54 (1H, partially resolved q), *m/e* 258 (M^+) 185 and 171.

The dimethyl ester (Xa) (43 mg.) in 1% potassium hydroxide (1·5 ml.) was refluxed for 30 min. in a stream of nitrogen to give a product (31 mg.) which afforded the *dicarboxylic acid* (Xc), m.p. 187·5—190° (from light petroleum–ether), $[\alpha]_D$ −32·2° (*c* 0·46 in EtOH), $\nu_{max.}$ 3500—2400, 1720, and 1710 cm.$^{-1}$ (Found: C, 57·5; H, 7·95. $C_{11}H_{18}O_5$ requires C, 57·4; H, 7·9%).

Nigericin Ethyl Ester (Id).—Treatment of nigericin (Ia) with an excess of diazoethane [10] in ether gave the *ethyl ester* as a foam, $\nu_{max.}$ 3560 and 1730 cm.$^{-1}$ [Found: C, 67·3; H, 9·8%; *M* (mass spectrum), 752. $C_{42}H_{72}O_{11}$ requires C, 67·0; H, 9·65%; *M*, 752].

The Ethyl Ester δ-Lactone (IIb).—To a solution of nigericin ethyl ester (Id) (7·8 g.) in chloroform (50 ml.), lead tetra-acetate (3·6 g.) was added. The mixture was

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stirred for 1 hr., poured on ice (30 g.), and extracted with ether. The extract was washed with 5% sodium hydrogen carbonate solution and water, dried, and evaporated to give the ethyl ester δ-lactone (IIb) (6·5 g.) as a foam. The sample purified by preparative t.l.c. showed $\nu_{max.}$ 1730 cm.$^{-1}$ [Found: *M* (mass spectrum), 720. $C_{41}H_{68}O_{10}$ requires *M*, 720].

Chromium Trioxide Oxidation of the Ethyl Ester δ-Lactone (IIb).—The ethyl ester δ-lactone (IIb) (6·5 g.) was dissolved in glacial acetic acid (50 ml.) and a solution of chromium trioxide (5·6 g.) in 66% aqueous acetic acid (150 ml.) was added dropwise with stirring and cooling. The mixture was warmed at 80° for 35 min., poured into ice–water (700 ml.), and extracted with ether (5 × 150 ml.). The combined extracts were washed with cold 10% sodium hydroxide (100 ml.) and water, dried, and evaporated, leaving a neutral fraction (1·85 g.). The alkaline layer was acidified to pH 3 with 10% sulphuric acid, with cooling, and extracted with ether to afford an acidic fraction (3·53 g.).

The neutral fraction was chromatographed on silica gel (60 g.). Elution with light petroleum–ether (7 : 3, v/v) gave an unidentified oil (557 mg.). Further elution with light petroleum–ether (1 : 1, v/v) yielded material (295 mg.) which gave the *lactone* (XII) (33 mg.), m.p. 156—159° (from n-hexane), $[\alpha]_D$ −57·1° (*c* 0·98 in $CHCl_3$), $\nu_{max.}$ 1730 cm.$^{-1}$, τ (C_6D_6) 9·23, 9·10, and 8·63 (each 3H, d, *J* 7 c./sec., 3 × secondary Me), 8·88 (3H, t, *J* 7 c./sec., $CO_2{\cdot}CH_2{\cdot}CH_3$), 6·95 (3H, s, OMe), 6·20 (1H, dd, *J* 2 and 10 c./sec.), and 5·88 (2H, q, *J* 7 c./sec., $CO_2{\cdot}CH_2Me$) [Found: C, 63·95; H, 9·1%; *M* (mass spectrum), 356. $C_{19}H_{32}O_6$ requires C, 64·0; H, 9·05%; *M*, 356]. Continued elution with light petroleum–ether (3 : 7 to 1 : 9, v/v) afforded a complicated mixture (862 mg.).

The acidic product was esterified with diazomethane and chromatographed on silica gel (170 g.). Elution with light petroleum–ether (1 : 0 to 7 : 3, v/v; 800 ml.) gave an unidentified oil (51 mg.). Further elution with light petroleum–ether (7 : 3, v/v; 600 ml. and 6 : 4, v/v; 600 ml.) gave material (662 mg.) which was purified by preparative t.l.c. [toluene–ethyl acetate (5 : 1); two developments] to afford the *methyl ethyl diester* (Xb) (183 mg.) as an oil, $\nu_{max.}$ (film) 1736 cm.$^{-1}$, τ 9·05 and 8·95 (each 3H, d, *J* 7 c./sec., 2 × secondary Me), 8·75 (3H, t, *J* 7 c./sec., $CO_2{\cdot}CH_2{\cdot}CH_3$), 6·33 (3H, s, CO_2Me), and 5·87 (2H, q, *J* 7 c./sec., $CO_2{\cdot}CH_2$-Me), *m/e* 272 (M^+), 199, and 171 (Found: C, 62·15; H, 8·95. $C_{14}H_{24}O_5$ requires C, 61·75; H, 8·9%). The methyl ethyl diester (Xb), when refluxed in 3% methanolic potassium hydroxide for 1 hr., gave the dicarboxylic acid (Xc), m.p. 187·5—190°, identical with the specimen obtained before.

After further elution of the above column with light petroleum–ether (6 : 4, v/v; 1000 ml. and 1 : 1, v/v; 600 ml.), elution with light petroleum–ether (1 : 1, v/v; 600 ml. and 4 : 6, v/v; 200 ml.) gave material (897 mg.) which was rechromatographed on silica gel (60 g.). After elution with light petroleum–ether (6 : 4, v/v; 560 ml. and 1 : 1, v/v; 420 ml.), the fractions eluted with light petroleum–ether (1 : 1, v/v; 490 ml.) were collected (220 mg.) and purified by preparative t.l.c. [toluene–ethyl acetate (5 : 1); two developments], affording the *hydroxy-diester* (XIa) (67 mg.) as an oil, $\nu_{max.}$ (film) 3490, 1733, and 1720 cm.$^{-1}$, τ (C_6D_6) 9·23 and 9·08 (each 3H, d, *J* 7 c./sec., 2 × secondary Me), 8·92 (3H, t, *J* 7 c./sec., $CO_2{\cdot}CH_2{\cdot}CH_3$), 6·17 (1H, dd, *J* 2·5 c./sec., 2-H), and 5·87 (2H, q, *J* 7 c./sec., $CO_2{\cdot}CH_2Me$), *m/e* 316 (M^+), 199, 153, 125, and 103 (100%). The hydroxy-diester (XIa) (47 mg.), when refluxed in 5% methanolic potassium hydroxide (3 ml.) for 1 hr., gave a free acid (35 mg.), which afforded the *hydroxy-diacid* (XIb) (18 mg.), m.p. 158—161° (from n-hexane) (Found: C, 56·8; H, 8·05. $C_{13}H_{22}O_6$ requires C, 56·9; H, 8·1%).

Degradation of Nigericin (Ia) *to the Nor-ketone* (XIIId).—A solution of nigericin (Ia) (70 mg.) and toluene-*p*-sulphonic acid (7 mg.) in acetone (7 ml.) was kept at room temperature overnight. It was then neutralised with sodium hydrogen carbonate solution, evaporated, and extracted with ether. The ether layer was shaken with 0·1N-sodium hydroxide, washed with water, dried, and evaporated to give the *acetonide sodium salt* (XIIIa) (69 mg.), which was dried *in vacuo* (P_2O_5).

To a solution of (XIIIa) in dry benzene (6 ml.), oxalyl chloride (0·04 ml.) in dry benzene (3 ml.) was added with stirring. The mixture was stirred at room temperature for 25 min. and evaporated *in vacuo* to dryness. The *acid chloride* (XIIIb) and *m*-chloroperbenzoic acid (30 mg.) were dissolved in dry benzene (3 ml.) and, to the solution, dry pyridine (0·5 ml.) in dry benzene (3 ml.) was added dropwise with stirring. The mixture was kept at room temperature overnight, then washed successively with 10% sodium thiosulphate, N-hydrochloric acid, 0·1N-sodium hydroxide, and water, and evaporated. The residue (74 mg.) was treated with 5% methanolic potassium hydroxide under reflux for 2 hr. The mixture was diluted with water, acidified with N-hydrochloric acid with cooling, and extracted with ether. The ether layer was washed with 3% sodium hydroxide and water, dried, and evaporated. The residue (54 mg.) was separated by preparative t.l.c. [toluene–ethyl acetate (1 : 1)] into the *nor-alcohol* (XIIIc) (18 mg.) and unchanged acetonide sodium salt (XIIIa) (27 mg.). Acetylation of (XIIIc) with acetic anhydride–pyridine yielded the *acetate*, $\nu_{max.}$ 1740 cm.$^{-1}$.

The nor-alcohol (XIIIc) (6·8 mg.) in pyridine (0·8 ml.) was added to chromium trioxide (17 mg.) in pyridine (0·1 ml.) and the mixture was set aside at room temperature overnight. The excess of oxidant was destroyed with methanol, and the mixture was then diluted with water, acidified with N-hydrochloric acid, and extracted with ether. The extract was washed with 5% sodium hydrogen carbonate and water, dried, and evaporated to give the *nor-ketone* (XIIId) (6·7 mg.), $\nu_{max.}$ 1718 cm.$^{-1}$, τ 8·60 and 8·50 (each 3H, s, isopropylidenedioxy), 7·83 (3H, s, Ac), and 6·70 (3H, s, OMe) [Found: *M* (mass spectrum), 734. $C_{42}H_{70}O_{10}$ requires *M*, 734].

Treatment of the Methyl Ester δ-Lactone (IIa) *with Sulphuric Acid.*—A solution of (IIa) (92 mg.) and 2N-sulphuric acid (0·8 ml.) in methanol (8 ml.) was refluxed for 100 min. It was then diluted with a saturated sodium chloride solution and extracted with ether. The extract was washed with 5% sodium hydrogen carbonate and water, dried, and evaporated to afford a residue which showed four spots on t.l.c. [toluene–ethyl acetate (3 : 1)]; the products were separated by preparative t.l.c. The most mobile fraction (14·7 mg.) was identified as the *unsaturated lactone* (XIV), $\nu_{max.}$ 1728 cm.$^{-1}$, τ 8·28 (3H, s, vinylic Me), 6·32 (3H, s, CO_2Me), and 4·33br (1H, s, $W_{\frac{1}{2}}$ 0·8 c./sec., olefinic H) [Found: *M* (mass spectrum), 674. $C_{39}H_{62}O_9$ requires *M*, 674]. The second most mobile fraction (17·5 mg.) was identified as the *unsaturated dimethyl ester* (XV), $\nu_{max.}$ 3500 and 1728 cm.$^{-1}$, τ 8·33 (3H, s, vinylic Me), 6·33 and 6·32 (each 3H, s, 2 × CO_2Me), and 4·88br (1H, s, $W_{\frac{1}{2}}$ 0·8 c./sec., olefinic H) [Found: *M* (mass spectrum), 706. $C_{40}H_{66}O_{10}$

requires *M*, 706). Alkaline hydrolysis of the former (XIV) with 5% methanolic potassium hydroxide followed by treatment with diazomethane in ether gave the dimethyl ester (XV). The third most mobile fraction (20·0 mg.) was not identified, but on treatment with 2N-sulphuric acid in methanol as described before it gave a mixture of (XIV) and (XV). The least mobile fraction (21·6 mg.) was recognised to be starting material (IIa) (t.l.c. and i.r. spectrum).

Osmium Tetroxide Oxidation of the Unsaturated Dimethyl Ester (XV).—Acetylation of (XV) (100 mg.) with acetic anhydride–pyridine at room temperature overnight gave the *acetate*, which was treated with osmium tetroxide (40 mg.) in dry benzene (10 ml.) and pyridine (0·1 ml.) at room temperature for 2 days in the dark. Hydrogen sulphide was passed through the mixture and a precipitate was filtered off. The filtrate was evaporated to dryness and the residue was purified by preparative t.l.c. [toluene–ethyl acetate (1 : 1)], giving the *triol monoacetate* (XVIa) (98 mg.). Acetylation of (XVIa) with acetic anhydride–pyridine afforded the *diacetate* (XVIb), τ (C_6D_6) 8·23 and 8·17 (each 3H, s, 2 × OAc), 6·57 and 6·30 (each 3H, s, 2 × CO_2Me), 4·88 (1H, dd, *J* 5 and 7 c./sec., CH bearing OAc), and 4·70 (1H, dd, *J* 6 and 12 c./sec., CH bearing OAc) [Found: *M* (mass spectrum), 824. $C_{44}H_{72}O_{14}$ requires *M*, 824].

To a suspension of lithium aluminium hydride (40 mg.) in dry ether (10 ml.), (XVIa) (90 mg.) in dry ether (5 ml.) was added dropwise at room temperature. The mixture was stirred for 2 hr. After careful addition of ethyl acetate and dilute hydrochloric acid, it was extracted with ether. The extract was washed with 5% sodium hydrogen carbonate and water, dried, and evaporated to leave a foam (79 mg.), which was purified by preparative t.l.c. [toluene–ethyl acetate–methanol (6 : 3 : 1)], affording the *pentol* (XVIc) (68 mg.), $\nu_{max.}$ 3440 cm.$^{-1}$.

Lead Tetra-acetate Oxidation of the Pentol (XVIc).—A solution of the pentol (XVIc) (35 mg.) in chloroform (4 ml.) was treated with lead tetra-acetate (45 mg.) at room temperature for 1 hr. The mixture was diluted with ether with cooling, washed with 5% sodium hydrogen carbonate and water, dried, and evaporated to give the *keto-aldehyde* (XVIIa) (35 mg.) as an oil, $\nu_{max.}$ 3480br and 1720 cm.$^{-1}$, τ 7·65 (3H, s, Ac) and 0·17 (1H, m, CHO).

Acetylation of (XVIIa) with acetic anhydride–pyridine afforded the *triacetate* (XVIIb), $\nu_{max.}$ 1720 cm.$^{-1}$. An attempt to purify (XVIIb) by preparative t.l.c. on silica gel gave an epimeric mixture of the aldols (XVIII), $\nu_{max.}$ 3460 and 1730 cm.$^{-1}$, τ 7·95 (9H, s, 3 × OAc) and *ca.* 0·3 (1H, m, CHO) [Found: *M* (mass spectrum), 808. Calc. for $C_{45}H_{76}O_{12}$: *M*, 808]. When (XVIII) was refluxed in 5% methanolic potassium hydroxide under nitrogen the product, after extraction with ether, afforded the *αβ-unsaturated aldehyde* (XIX), $\lambda_{max.}$ (EtOH) 243 mμ, $\nu_{max.}$ 3500br and 1675 cm.$^{-1}$ [Found: *M* (mass spectrum), 664. $C_{39}H_{68}O_8$ requires *M*, 664].

Treatment of the Keto-aldehyde (XVIIa) *with Perchloric Acid*.—A mixture of (XVIIa) (184 mg.) in 10% perchloric acid (6·5 ml.) and dioxan (10 ml.) was stirred for 3·5 hr. at room temperature. The mixture was diluted with water (100 ml.), saturated with sodium chloride, and extracted with ether. The extract was washed with 5% sodium hydrogen carbonate and saturated sodium chloride solution, dried, and evaporated to give an oil (157 mg.), which was separated into two fractions by preparative t.l.c. [toluene–ethyl acetate–methanol (6 : 3 : 1, v/v)]. The more mobile one (102 mg.) was identified as the *methyl ketone* (XXIII), $\nu_{max.}$ 3480 and 1720 cm.$^{-1}$, τ 7·68 (3H, s, Ac) [Found: *M* (mass spectrum), 456. $C_{25}H_{44}O_7$ requires *M*, 456].

The less mobile one (30 mg.) was identified as the *aldehyde* (XX), $\nu_{max.}$ 3474 and 1728 cm.$^{-1}$, τ 9·20 and 9·03 (each 3H, d, *J* 7 c./sec., 2 × secondary Me) and 0·18 (1H, t, *J* 1·5 c./sec.). Treatment of the aldehyde (XX) with toluene-*p*-sulphonic acid in methanol at room temperature overnight gave the *acetal* (XXIa), m.p. 93·5—95°, $[\alpha]_D$ −35° (*c* 0·92 in $CHCl_3$), $\nu_{max.}$ 3480 cm.$^{-1}$, τ 9·18 and 9·02 (each 3H, d, *J* 7 c./sec., 2 × secondary Me), 6·65 and 6·62 (each 3H, s, 2 × OMe), and 5·42 (1H, d, *J* 5·5 c./sec., acetal H), *m/e* 291 (*M* + 1). Acetylation of the acetal (XXIa) with acetic anhydride–pyridine at room temperature overnight afforded the *diacetate* (XXIb) as an oil, $\nu_{max.}$ 1732 cm.$^{-1}$, τ 9·13 and 9·05 (each 3H, d, *J* 7 c./sec., 2 × secondary Me), 7·98 and 7·97 (each 3H, s, 2 × OAc), and 6·70 (6H, s, 2 × OMe) [Found: *M* (mass spectrum), 374. $C_{19}H_{34}O_7$ requires *M*, 374].

The Triol (XXII).—(a) *From the dihydroxy-aldehyde* (XX). A solution of (XX) (34 mg.) and sodium borohydride (30 mg.) in methanol (5 ml.) was kept at room temperature overnight. After addition of dilute acetic acid, the mixture was concentrated *in vacuo* below 40°. The residue was diluted with saturated sodium chloride solution and extracted thoroughly with ether. The extract was washed with 5% sodium hydrogen carbonate and saturated sodium chloride solution, dried, and evaporated to give the triol (XXII) (5·2 mg.), $\nu_{max.}$ 3400br cm.$^{-1}$, identical with the sample obtained in (b) (t.l.c. and i.r. spectra).

(b) *From the hydroxy-diester* (XIa). To a solution of (XIa) (17 mg.) in dry ether (12 ml.), lithium aluminium hydride (17 mg.) was added in portions. The mixture was stirred at room temperature for 30 min. and then refluxed for 2 hr. The complex was decomposed with ethyl acetate and water, and the mixture was extracted continuously with hot ether. The extract was dried and evaporated to leave an oil (9 mg.), which was purified by preparative t.l.c. [toluene–ethyl acetate–methanol (6 : 3 : 1)] to give the triol (XXII) (4·5 mg.).

Lead Tetra-acetate Oxidation of the Methyl Ketone (XXIII).—A solution of (XXIII) (26 mg.) and lead tetra-acetate (26 mg.) in 90% aqueous acetic acid was stirred for 80 min. It was then diluted with water (40 ml.), saturated with sodium chloride, and extracted with ether. The extract was washed with 5% sodium hydrogen carbonate and water, dried, and evaporated, giving the *tricyclic γ-lactone* (XXIVa) (24 mg.), as an oil, $\nu_{max.}$ 3460 and 1760 cm.$^{-1}$ [Found: *M* (mass spectrum), 412. $C_{23}H_{40}O_6$ requires *M*, 412]. Acetylation of (XXIVa) with acetic anhydride–pyridine at room temperature overnight gave the *diacetate* (XXIVb), $\nu_{max.}$ 1755 and 1730 cm.$^{-1}$, τ (C_6D_6) 9·28, 9·15, 9·10, and 8·90 (each 3H, d, *J* 7 c./sec., 4 × secondary Me), 9·05 and 8·85 (each 3H, s, 2 × tertiary Me), 8·27 and 8·18 (each 3H, s, 2 × OAc), and 4·97 (1H, dd, *J* 5 and 7 c./sec., CH bearing OAc) [Found: *M* (mass spectrum), 496. $C_{27}H_{44}O_8$ requires *M*, 496].

Hydrolysis of the Tricyclic γ-Lactone (XXIVa).—A solution of the γ-lactone (XXIVa) (107 mg.) in 5% potassium hydroxide in water–methanol (4 : 1; 6 ml.) was refluxed for 1 hr. in a stream of nitrogen. After dilution with water, the solution was acidified with N-hydrochloric acid with cooling and extracted with ether. The extract was immediately esterified with diazomethane in ether. After

the usual work-up the product (107 mg.) gave the *γ-hydroxy-acid methyl ester* (XXVa) (80 mg.) as colourless needles, m.p. 105—107° (from ether–n-hexane), $[\alpha]_D$ −11·4 (*c* 1·00 in $CHCl_3$), $\nu_{max.}$ 3650, 3400, and 1732 cm.$^{-1}$, τ (C_6D_6) 9·35, 9·12, 8·90, and 8·72 (each 3H, d, *J* 7 c./sec., 4 × secondary Me) 9·03 and 8·63 (each 3H, s, 2 × tertiary Me), *ca.* 7·08 (1H, m), and 6·62 (3H, s, CO_2Me) [Found: C, 64·75; H, 10·0; *M* (mass spectrum), 444. $C_{24}H_{44}O_7$ requires C, 64·85; H, 10·0%; *M*, 444]. Acetylation of (XXVa) with acetic anhydride–pyridine at room temperature overnight gave the *diacetate* (XXVb), $\nu_{max.}$ 3440 and 1732 cm.$^{-1}$, τ (C_6D_6) 9·32, 9·15, 9·08, and 8·70 (each 3H, d, *J* 7 c./sec., 4 × secondary Me), 9·00 and 8·67 (each 3H, s, 2 × tertiary Me), 8·20 and 8·17 (each 3H, s, 2 × OAc), *ca.* 7·08 (1H, m), 6·58 (3H, s, CO_2Me), and 4·97 (1H, dd, *J* 7 and 5 c./sec., CH bearing OAc) [Found: *M* (mass spectrum), 528. $C_{28}H_{48}O_9$ requires *M*, 528].

The Bicyclic γ-Lactone (XXVI).—A solution of the γ-hydroxy-acid diacetate (XXVb) (764 mg.) in glacial acetic acid (5 ml.) was added to chromium trioxide (435 mg.) in glacial acetic acid (10 ml.) and water (0·1 ml.) cooled in ice. The mixture was gradually warmed to room temperature and kept for 5 hr. The resulting emerald-green solution was diluted with water, neutralised with 5% sodium hydrogen carbonate with cooling, and extracted with ether. The extract was washed, dried, and evaporated to give an oil (609 mg.), which was chromatographed on silica gel (60 g.). Elution with light petroleum–ether (7 : 3, v/v; 80 ml. and 1 : 1, v/v; 180 ml.) gave an unidentified oil (153 mg.); elution with light petroleum–ether (1 : 1, v/v; 600 ml.) then afforded a fraction (362 mg.) which was purified by preparative t.l.c. [toluene–ethyl acetate (3 : 1)], giving the bicyclic γ-lactone (XXVI) (140 mg.) as an oil, $\nu_{max.}$ 1768 and 1733 cm.$^{-1}$. τ (C_6D_6) 9·33, 9·10, and 9·05 (each 3H, d, *J* 7 c./sec., 3 × secondary Me), 8·98 (3H, s, tertiary Me), 8·22 (6H, s, 2 × OAc), 6·33 and 6·00 (2H, ABq, *J* 4 c./sec., CH_2 bearing OAc), and 5·02 (1H, dd, *J* 8 and 4 c./sec., CH bearing OAc) [Found: *M* (mass spectrum), 398. $C_{21}H_{34}O_7$ requires *M*, 398]. Successive elution with ether (360 ml.) and chloroform (300 ml.) afforded an oily mixture (85 mg.).

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