Structures of Three New C_{16} Terpenoids from an *Acrostalagmus* Fungus

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Three new C_{16} terpenoids, which may lie on the biosynthetic pathway for the antifungal metabolite LL-Z1271α(1), have been isolated from an *Acrostalagmus* fungus, and assigned the structures (3)—(5).

THE antifungal metabolite LL-Z1271α (1), obtained from *Acrostalagmus* NRRL-3 481,[1] has a structure similar to those of the inumakilactones,[2] nagilactones,[3] and podolactones,[4] plant-growth inhibitors isolated from

[1] G. A. Ellestad, R. H. Evans, M. P. Kunstmann, J. E. Lancaster, and G. O. Morton, *J. Amer. Chem. Soc.*, 1970, **92**, 5483.

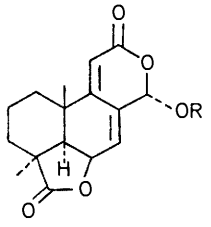
[2] S. Ito, M. Kodama, M. Sunagawa, T. Takahashi, H. Imamura, and O. Honda, *Tetrahedron Letters*, 1968, 2065.

[3] Y. Hayashi, S. Takahashi, H. Ono, and T. Sakan, *Tetrahedron Letters*, 1968, 2071.

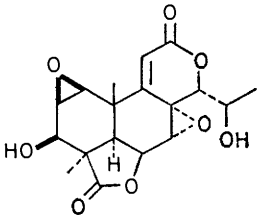
[4] M. N. Galbraith, D. H. S. Horn, and J. M. Sasse, *Chem. Comm.*, 1971, 1362.

the *Podocarpus* genus, and has been found to have a strong inhibitory activity on the growth of an *Avena* coleoptile section.

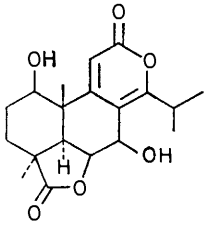
It has been suggested that this C_{16} terpenoid lactone (1) is biosynthesized from a diterpenoid precursor with



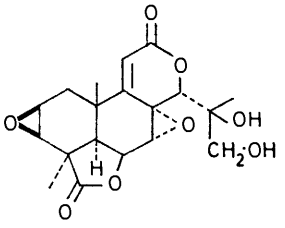
(1) R = Me
(2) R = H



inumakilactone A†



nagilactone A

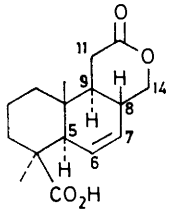


podolactone A†

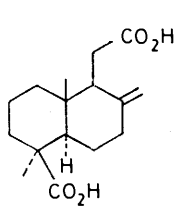
† Recent *X*-ray analyses have revised these structures as shown (S. K. Arora, R. B. Bates, P. C. C. Chou, W. E. Sanchez L, K. S. Brown, jun., and M. N. Galbraith, *J. Org. Chem.*, 1976, **41**, 2458).

loss of four carbon atoms.[5] As a part of a series of biosynthetic investigations of this C_{16} terpene, we have sought further metabolites of the fungus which could lie on the biosynthetic pathway to the lactone (1).

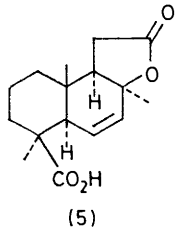
Repetition of the silica gel chromatographic separation of the crude mixture obtained from the culture fluid of



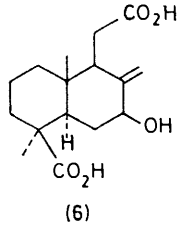
(3)



(4)



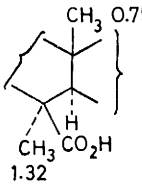
(5)



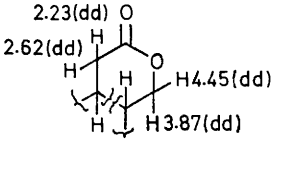
(6)

Acrostalagmus NRRL-3481 afforded three new metabolites, besides the known lactones, LL-Z1271α (1), γ (2), and β (6).[6] The new compounds have been designated acrostalidic acid (3), acrostalic acid (4), and iso-acrostalidic acid (5).[7]

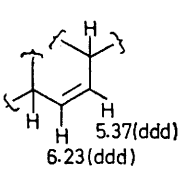
In acrostalidic acid, $C_{16}H_{22}O_4$, the four oxygen atoms were considered to be involved in a carboxylic acid and a lactone group ($\nu_{max.}$ 3 300—2 600, 1 740, and 1 690 cm^{-1}). The i.r. and n.m.r. spectra indicated the presence of the following features: (A) two tertiary methyl groups, one (δ 1.32) geminal and the other (δ 0.75) in a *cis*-1,3-diaxial relationship to the carboxy-group; (B) a lactone system bearing two methylene groups, deduced from the two ABX-type n.m.r. signals, one of which (centred at δ 2.4) is due to CH_2·CO and the other (centred at δ 4.2), to CH_2·O; and (C) an olefinic group, showing AB-type signals (δ 5.37 and 6.23, J 10.5 Hz) further split by strong couplings with two allylic protons (J 2, 3, 2, and 2.5). These three partial structures and the co-occurrence of this compound with the lactone (1) in the culture suggested the structure (3) for acrostalidic acid. This structure was confirmed by a decoupling experiment. Irradiation at the frequency of H-8 changes the H-6 and H-7 signals from broad doublets to doublets of doublets, and the H-14α and -14β signals from doublets of doublets to doublets. This indicates that the two partial structures (B) and (C), should be combined so as to share H-8.



(A)



(B)



(C)

The stereochemistry of the BC ring junction was identified as *trans* from the two diaxial coupling constants ($J_{8,14\alpha}$ 11.5, $J_{9,11\beta}$ 12.5 Hz). Two allylic coupling constants ($J_{5,7}$ 3, $J_{6,8}$ 2.5 Hz) and a very large homoallylic coupling constant ($J_{5,8}$ 4.5 Hz),[8] determined by irradiation at the H-6 and H-7 frequencies, revealed that H-5 and H-8 are oriented perpendicular to the plane of an olefinic bond. These relationships show the *trans-transoid-trans* stereochemistry of the ABC ring system in structure (3).

The ^{13}C n.m.r. spectrum exhibits sixteen carbon signals, of which two overlap at δ 35.2, thus supporting structure (3). The spectrum shows signals for CO_2H at δ 182.8, a lactonic group at 170.9 and 73.4, and an olefinic group at 131.0 and 120.3.[9]

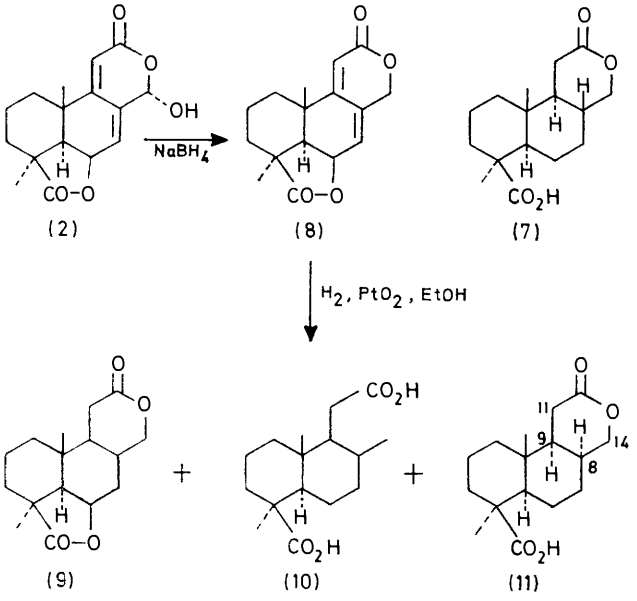
Acrostalidic acid (3) was converted into dihydroacrostalidic acid (7) by catalytic hydrogenation. The mass spectrum of the product (7) shows fragmentation patterns very similar to those of the BC *cis*-lactonic acid (11) derived from the lactol (2), corroborating the

[5] H. Kakisawa, M. Sato, T. I. Ruo, and T. Hayashi, *J.C.S. Chem. Comm.*, 1973, 802.
[6] G. A. Ellestad, R. H. Evans, and M. P. Kunstmann, *Tetrahedron Letters*, 1971, 497.
[7] M. Sato, T. I. Ruo, T. Hayashi, H. Kakisawa, T. Miyaki, H. Yamamoto, and K. Fujisawa, *Tetrahedron Letters*, 1974, 2183.
[8] S. Sternhell, *Quart. Rev.*, 1969, **23**, 236.
[9] G. C. Levy and G. L. Nelson, ' Carbon-13 Nuclear Magnetic Resonance for Organic Chemists,' Wiley–Interscience, New York, 1972.

carbon skeleton of acrostalidic acid (3). The mass spectral fragmentations of compounds (7) and (11) differ only in relative peak intensities, notably of the molecular ion and base peaks. The relative abundance of the molecular ion in the BC-*trans*-isomer (7) was higher than that of the BC-*cis*-isomer (11). The base peak of the *cis*-isomer appeared at *m/e* 221, but the *trans*-isomer showed the base peak at *m/e* 121, with a less intense peak at *m/e* 221. These differences may well be due to easier lactone ring cleavage as a result of steric strain in the *cis*-isomer.

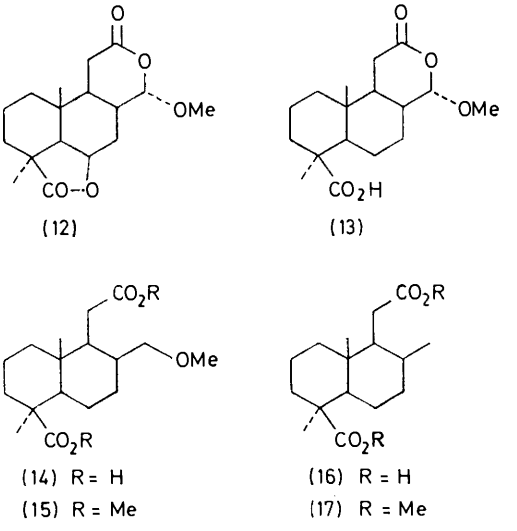
The BC *cis*-lactonic acid (11) was prepared from the lactol (2) by reduction with sodium borohydride to give the lactone (8), followed by catalytic hydrogenation. This gave a mixture of a dilactone (9), a diacid (10), and the desired lactonic acid (11). The stereochemistry of the ABC ring junctions of the lactonic acid (11) was identified as *trans-transoid-cis* from the four vicinal coupling constants ($J_{8,14}$ 7 and 12, $J_{9,11}$ 2.5 and 6.5 Hz).

The lactone (8) caused marked inhibition of plant growth at a concentration of 1 p.p.m. This activity is comparable to that of nagilactone F, which has the strongest activity in the *Podocarpus* lactone series.[10]

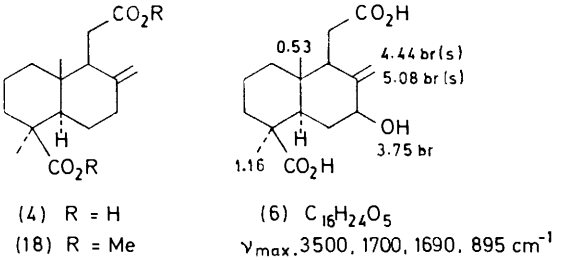


In connection with the catalytic hydrogenation of the deoxy-lactone (8), the hydrogenation of the lactone (1) in acetic acid was studied. The reaction afforded a dilactone (12), a lactonic acid (13), a methoxy-diacid (14), and a diacid (16) [identical with compound (10)], in the ratios 1.4 : 6.6 : 1.0 : 2.8. These products were identified by their i.r., n.m.r., and mass spectra and those of the methyl esters (15) and (17).

Acrostalic acid, $C_{16}H_{24}O_4$, $\nu_{max.}$ 3 300—2 600, 1 700, and 1 690 cm^{-1} (CO_2H), was converted into a dimethyl ester (18) by diazomethane. I.r. absorption at 890 cm^{-1} and n.m.r. signals at δ 4.55 and 4.74 (each a broad singlet) indicate the presence of a terminal methylene group in the acid. The n.m.r. spectrum also shows the presence of two tertiary methyl groups (δ 0.66 and 1.23). These spectral properties are similar to those of LL-Z1271β (6).



The differences between these two compounds are the number of oxygen atoms and the absence of both a C*H*·OH n.m.r. signal and OH i.r. absorption in the case of acrostalic acid. These properties suggest the structure (4) for this acid, which was supported by the mass. In agreement with the fragmentation patterns of bicyclic diterpenes with C-4 carboxy and C-8 terminal methylene groups, peaks at *m/e* 167(60%), 139(33%), and 121(100%) were observed. In the interpretation of the series of fragmentations from *m/e* 167 to 121 through 139 (*m** 116.5 and 105.5) found in these types of terpenoids, two pathways have been proposed by Enzell,[11] as shown in Scheme 1: *a*, initial elimination of carbonyl, followed by dehydration, or *b*, initial elimination of ethylene *via* a retro-Diels–Alder type fragmentation, followed by dehydration. These two pathways cannot be differentiated by low-resolution measurements, but a high-resolution spectrum, determining the composition of the ion *m/e* 121 as C_9H_{13}, verifies the pathway *a*.



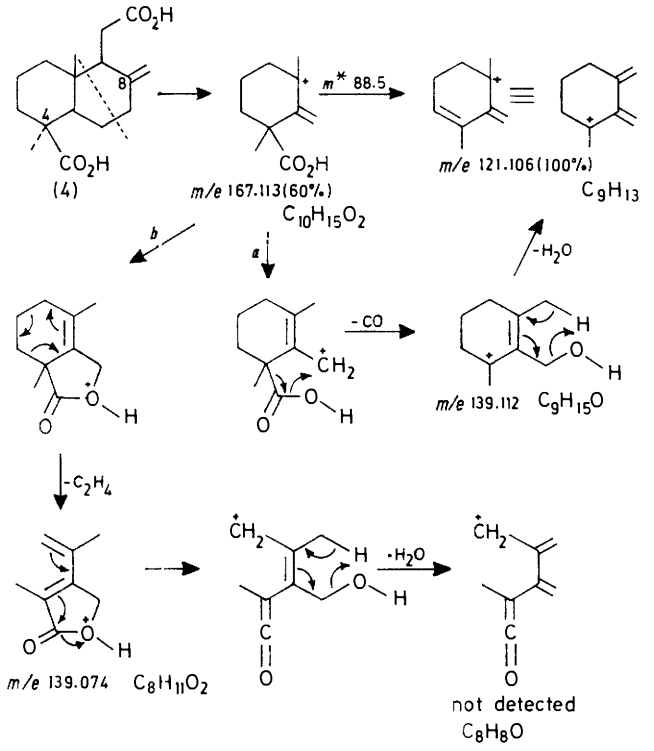
The structure (4) for acrostalic acid was confirmed by direct comparison of the dimethyl ester with the authentic compound (18), which had been synthesized from podocarpic acid by Bell.[12] This also defined the absolute stereochemistry of the structure (4).

[10] Y. Hayashi and T. Sakan, in 'Plant Growth Substances 1973,' ed. T. Furuya, Hirokawa, Tokyo, 1974, p. 525.

[11] C. R. Enzell and R. Ryhage, *Arkiv. Kemi*, 1964, **23**, 367.

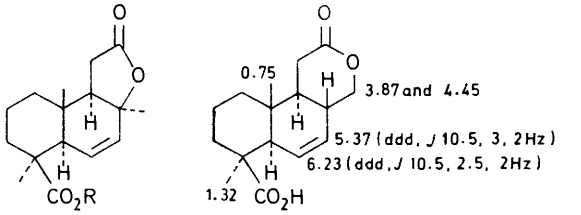
[12] R. A. Bell, M. G. Gravestock, and V. Y. Taguchi, *Canad. J. Chem.*, 1972, **50**, 3749.

Isoacrostalidic acid, $C_{16}H_{22}O_4$, $\nu_{max.}$ 3 300—2 600, 1 745, and 1 690 cm^{-1} (lactonic acid) differs from the other



SCHEME 1 Mass spectral fragmentation of acrostalic acid (4)

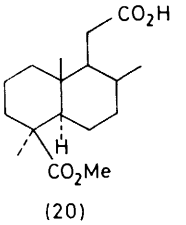
metabolites of this fungus in that three methyl groups are present (δ 0.73, 1.34, and 1.40). However, this acid not only has the same molecular composition as acrostalidic acid (3), but it also strongly resembles the latter in its spectral properties. However, isoacrostalidic acid has a γ-lactone i.r. band at 1 745 cm^{-1} (1 760 cm^{-1} in $CHCl_3$) and a methyl n.m.r. singlet at δ 1.40 instead of the δ-lactone band at 1 740 cm^{-1} and $O{\cdot}CH_2$ signals around δ 4.0 for acrostalidic acid. Furthermore, isoacrostalidic acid has two olefinic protons (δ 6.45 and 5.85) coupled with only one other proton. These properties led us to assign structure (5) to isoacrostalidic acid.



(5) R = H
(19) R = Me

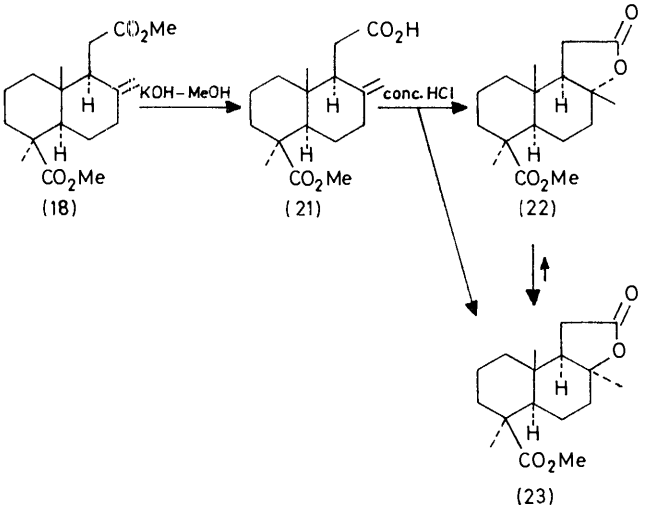
(3) $C_{16}H_{22}O_4$
$\nu_{max.}$ 3300—2600, 3030, 1720, 1690 cm^{-1}

To corroborate structure (5), the following reactions were performed. Catalytic hydrogenation of the lactonic ester (19) afforded a saturated ester acid (20), by hydrogenolytic cleavage of the lactone ring, which confirms the positional relation between the γ-lactone group and the olefinic double bond. The carbon skeleton and the AB ring junction stereochemistry were confirmed by the fact that partial hydrolysis of dimethyl ester (17) gave the same ester acid (20) as had been obtained from isoacrostalidic acid.



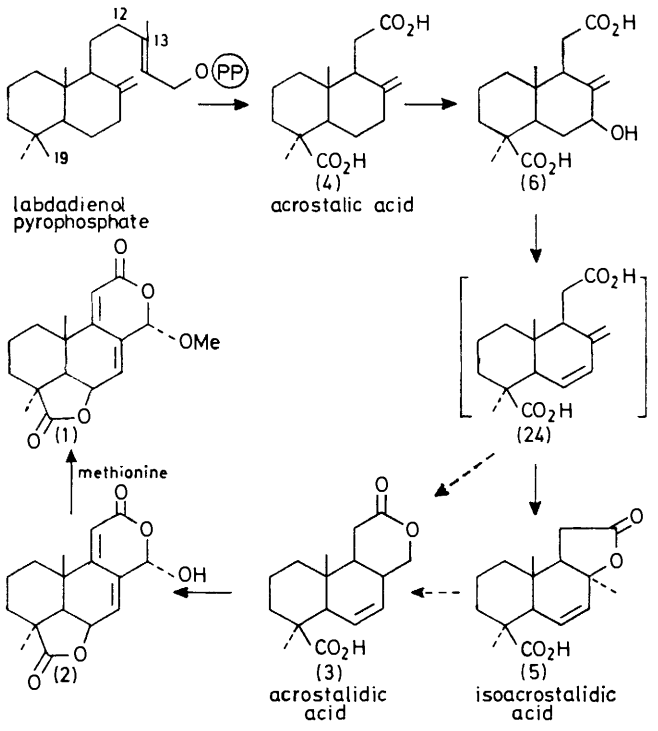
(20)

The stereochemistry of the junction between ring B and the lactone ring was identified as *cis* from the i.r. absorption at 1 745 cm^{-1}. Treatment of the monomethyl ester (21) with acid gave material showing $\nu_{max.}$ 1 770 cm^{-1} and a peak with retention time (t_R) 10 min in g.l.c. (together with a minor peak at t_R 9 min) which indicates the formation of a *trans*-lactone (22). On prolonged treatment with acid, this *trans*-lactone was slowly changed into a *cis*-lactone (23), which showed a g.l.c. peak at t_R 9 min, three methyl n.m.r. singlets (δ 0.74, 1.22, and 1.33), and a γ-lactone absorption at 1 745 cm^{-1}, in accord with the i.r. absorption of isoacrostalidic acid.



From the structures of these metabolites and the results of biosynthetic studies with [*methyl*-^{14}C]methionine, [2-^{13}C]acetic acid, [2-^{14}C, 5-$^{3}H_2$] mevalonic acid, and [2-^{14}C, 2-$^{3}H_2$]mevalonic acid,[5] the pathway for the biosynthesis of the lactone (1) may be presumed to involve conversion of a diterpenoid precursor such as labdadienol into acrostalic acid (4) by oxidative cleavage between C-12 and C-13, accompanied by oxidation at C-19. The resulting acid (4) could then

be successively transformed by oxidation and cyclization, as shown in Scheme 2.



SCHEME 2 Suggested pathway for biosynthesis of the lactone (1)

* The presence of compound (24) is assumed in view of the co-occurrence of lactones (3) and (5).

EXPERIMENTAL

M.p.s were determined for samples in sealed capillary tubes. I.r. spectra were recorded with a 215 Hitachi grating spectrophotometer and u.v. spectra with a Hitachi EPS-3T spectrophotometer. ^{1}H N.m.r. spectra were measured with a Hitachi H-60 and a Varian HA-100 instrument, and the ^{13}C spectrum was measured with a JEOL JNM-PFT-100 spectrometer (tetramethylsilane as internal reference). Mass spectra were measured with a Hitachi RMU-7L double focusing spectrometer; high resolution measurements were made with a CEC-110 B double-focusing spectrometer.

Fermentation.—A fermentation medium was prepared according to the prescription: molasses (20 g), glucose (10 g), bacto-peptone (5 g), streptomycin (5 mg), and water (to 1 000 ml). Before sterilization the pH of the medium was adjusted to 7.0 with sodium hydroxide. The medium was sterilized at 120 °C with steam for 45—60 min. The scraped spores from an agar slant of *Acrostalagmus* NRRL-3 481 were used to inoculate a 500 ml flask containing 100 ml of the above sterile medium. The flask was then placed on a rotary shaker and agitated vigorously for 3 days at 25 °C. The resulting inoculum was transferred to other 500 ml flasks each containing 100 ml of the sterile medium, and the flasks were further shaken similarly for 5—7 days. When the antifungal activity for *Cryptococcus neoformance* reached a maximum, the mash was harvested.

Isolation of Acrostalidic Acid (3).—The fermented mash (30 l) was adjusted to pH 2 with 2N-hydrochloric acid. The mash was filtered through Celite and the filter pad was washed with water. The filtrate was extracted with ethyl acetate and the organic layer was successively washed with water and saturated salt solution, dried (Na_2SO_4), and evaporated under reduced pressure to give an oily residue (17.8 g). This was washed with n-hexane, and the crude product was dissolved in ethyl acetate (40 ml) and precipitated with benzene (20 ml). The supernatant was evaporated to give an oil (7.5 g). Sufficient acetone was added to dissolve the oil and silica gel (10 g) was added to the solution. The solvent was removed under reduced pressure and the dry silica gel was placed on a silica gel (190 g) column slurry packed in benzene. The column was eluted successively with benzene (850 ml; fractions 1—16), benzene containing increasing amounts of chloroform (total 280 and 250 ml respectively; fractions 17—30), chloroform (300 ml; fractions 31—38), and chloroform containing increasing amounts of ethyl acetate (880 and 360 ml, respectively; fractions 39—65) (50 ml fractions). From fractions 44—47 [eluted by chloroform–ethyl acetate, (25 : 1)—(12 : 1)], fraction 58(1 : 1), and fraction 63(1 : 4), the lactone (1), the lactol (2), and the hydroxy-diacid (6) were isolated, respectively.

Further column chromatography of fractions 48—55 (1.04 g) [eluted by chloroform–ethyl acetate, (6 : 1)—(3 : 1)] was carried out on 100 g of silica gel by the usual method. The column was eluted successively with chloroform (1 100 ml; fractions 1—32) and chloroform containing an increasing proportion of ethyl acetate (1 300 and 150 ml, respectively; fractions 33—58) [50 ml fractions except for fractions 15—27 (10 ml portions)]. From fractions 42—44 [eluted by chloroform–ethyl acetate (12.5 : 1)], syringic acid was isolated. Crude *acrostalidic acid* (3) was obtained from fractions 37—40 [eluted by chloroform–ethyl acetate (25 : 1)]. Recrystallization from ethyl acetate gave a sample (20 mg), m.p. 210—211°; $\nu_{max.}$ (KBr) 3 030 (olefinic), $\nu_{max.}$ ($CHCl_3$) 1 720 and 1 690 cm^{-1}; δ_H ($CDCl_3$; 100 MHz) 2.03 (H-5), 2.33 (H-8), 1.57 (H-9), 2.23 (dd, *J* 18 and 12.5 Hz, H-11$_\beta$), 2.62 (dd, *J* 18 and 5 Hz, H-11$_\alpha$), 3.87 (dd, *J* 11.5 and 10.5 Hz, H-14$_\alpha$), 4.45 (dd, *J* 10.5 and 5 Hz, H-14$_\beta$), and 10.1 (CO_2H); δ_C ($CDCl_3$) 54.7, 46.1, 43.1, 37.0, 35.5, 35.2 (2 C), 30.6, 28.0, 18.9, and 10.7; *m/e* 278(M^+), 263, 260, 233 (intense), 217, 173, 145, and 105 (base); high resolution *m/e* 278.150 (M^+; $C_{16}H_{22}O_4$ requires *M*, 278.152), 263.126 ($C_{15}H_{19}O_4$, *M* − CH_3), 233.153 ($C_{15}H_{21}O_2$, *M* − CO_2H), 217.116 ($C_{14}H_{17}O_2$), 173.132 ($C_{13}H_{17}$), 145.102 ($C_{11}H_{13}$), and 105.070 (C_8H_9).

Isolation of Acrostalic Acid (4).—The second column fractions 45—50 [effluant chloroform–ethyl acetate (12.5 : 1)] gave crude *acrostalic acid* (4) (20 mg). Recrystallization from ethyl acetate gave material of m.p. 219—220°; $\nu_{max.}$ (KBr) 3 080, 1 645, and 890 cm^{-1} (=CH_2); δ_H [$(CD_3)_2CO$] 0.66 and 1.23 (s, 10- and 4-Me); *m/e* 280 (M^+), 262, 234, and 109; high resolution *m/e* 280.169 (M^+; $C_{16}H_{24}O_4$ requires *M*, 280.167), 262.150 ($C_{16}H_{22}O_3$, *M* − H_2O), 234.159 ($C_{15}H_{22}O_2$, *M* − HCO_2H), and 109.107 (C_8H_{13}) (Scheme 1).

Isolation of Isoacrostalidic Acid (5)—Elution of the second column with chloroform–ethyl acetate (50 : 1) (fraction 35) followed by recrystallization from ethyl acetate yielded *isoacrostalidic acid* (5) as needles (10 mg), m.p. 204—206°; $\nu_{max.}$ (KBr) 3 050 (olefinic), $\nu_{max.}$ ($CHCl_3$) 1 755 cm^{-1}; δ_H ($CDCl_3$) 5.85 (dd, *J* 10.5 and 3 Hz, H-7) and 6.45 (dd, *J* 10.5 and 2 Hz, H-6); high resolution *m/e* 278.152 (M^+ $C_{16}H_{22}O_4$ requires *M*, 278.152), 263.132 ($C_{15}H_{19}O_4$, *M* − CH_3), 232.146 ($C_{15}H_{20}O_2$), 217.120 ($C_{14}H_{17}O_2$), 189.127

($C_{13}H_{17}O$), 173.131 ($C_{13}H_{18}$), 135,115 ($C_{10}H_{15}$), 119.088 (C_9H_{11}), and 109.099 (C_8H_{13}).

Dihydroacrostalidic Acid (7).—Acrostalidic acid (3) was hydrogenated in ethyl acetate over platinum oxide. Evaporation gave dihydroacrostalidic acid (7), *m/e* 280 (M^+, 40%), 262 (M − 18), 234, 221, 220, 194, 135, 121 (base), 109, 107, 95, 93, and 81.

Preparation of the Lactone (8) *from the Lactol* (2).—The hydroxy-lactone (2) (101 mg) in ethanol (10 ml) was added to a solution of sodium borohydride (99 mg) in ethanol (15 ml). The mixture was stirred at room temperature for 1 h and acidified with 2N-hydrochloric acid. After removal of ethanol under reduced pressure, water was added and the aqueous solution was extracted with ether. The extract was washed with water and brine, and dried (Na_2SO_4). Evaporation afforded a crystalline solid (92 mg, 96%). Recrystallization from ethyl acetate yielded the lactone (8), m.p. 192.5—193.5°; $\lambda_{max.}$ (EtOH) 258.5 nm (ε13 500); $\nu_{max.}$ (KBr) 3 080, 3 030, and 1 610 (olefinic), 1 775 (γ-lactone), and 1 710 (δ-lactone), $\nu_{max.}$ ($CHCl_3$) 1 770 and 1 720 cm^{-1}; δ_H ($CDCl_3$) 1.19 and 1.35 (s, 10- and 4-Me), 1.99 (d, *J* 5 Hz, H-5), 4.95br (2 H, s, O·CH_2), 5.06 (t, *J* 5 Hz, CO_2·CH), 5.75 (d, *J* 2Hz, H-11), and 6.25 (m, H-7), δ_H (C_6D_6) 0.82 (Me), 0.87 (Me), 1.18 (H-5), 4.20 (H_2-14 and H-6), 5.33 (H-7), and 5.46 (H-11), δ_H ($CDCl_3$; 100 MHz) 4.95 (m, J_{gem} 16 Hz, H_2-14); *m/e* 274 (M^+), 246 (M − 28, *m** 221, base), 231, 228 (246—18, *m** 211.5), 200, 174, and 145.

Hydrogenation of the Lactone (8).—The lactone (8) (85 mg) in ethanol was hydrogenated over platinum oxide (19 mg). Filtration and evaporation afforded an oily residue (80 mg), which was chromatographed over silica gel (7 g) and eluted with chloroform to give three products: (i) the dilactone (9) (9 mg, 10.4%), m.p. 184.5—186° (from ethanol); $\nu_{max.}$ (KBr) 1 750 (γ-lactone) and 1 730sh (δ-lactone), $\nu_{max.}$ ($CHCl_3$) 1 760 and 1 745 cm^{-1}; δ_H ($CDCl_3$) 0.95 and 1.30 (s, 10- and 4-Me), 3.93 (dd, *J* 12 and 8 Hz, H-14), 4.35 (dd, *J* 12 and 5 Hz, H-14), and *ca.* 5.0 (m, H-6); *m/e* 279 (M^+ + 1), 278 (M^+), 263, 234 (base), 219 (intense), 159, and 136; (ii) the lactonic acid (11) (30 mg, 34.5%), m.p. 212.5—220° (from ethanol); $\nu_{max.}$ (KBr) 3 300—2 400 and 1 685 (carboxy), and 1 720 (δ-lactone), $\nu_{max.}$ ($CHCl_3$) 1 720 and 1 690 cm^{-1}; δ_H ($CDCl_3$) 0.89 and 1.28 (s, 10- and 4-Me), 2.37 and 2.74 (H_2-11), 4.25 (dd, *J* 12 and 7 Hz, H-14α), 4.50 (t, *J* 12 Hz, H-14β), and 9.9br (COOH), δ_H [$CDCl_3$–C_6D_6 (1 : 1)] 0.81 (Me), 1.18 (Me), 2.15 (dd, *J* 18 and 6.5 Hz, H-11), 2.59 (dd, *J* 18 and 2.5 Hz, H-11), and 4.07 and 4.27 (H_2-14); *m/e* 280 (M^+, 5%), 262 (M − 18), 234, 221 (base), 194, 135, 121, 109, 107, 95, 93, and 81; and (iii) the diacid (10) (9 mg, 10.3%), identical with that obtained from the lactone (1) (see below).

Hydrogenation of the Lactone (1).—The lactone (1) (501 mg) in acetic acid (33 ml) was hydrogenated over platinum oxide (101 mg). Filtration and evaporation afforded an oily residue (531 mg). Chromatography over silica gel (25 g) and elution with chloroform followed by gradient elution with chloroform and ethyl acetate gave four main products, further purified by recrystallization from ethanol. The *dilactone* (12) (33 mg, 6.5%) had m.p. 184.5—186°; $\nu_{max.}$ (KBr) 1 755 (γ-lactone and δ-lactone), $\nu_{max.}$ ($CHCl_3$) 1 760 and 1 750 cm^{-1}; δ_H ($CDCl_3$) 0.91 and 1.29 (s, 10- and 4-Me), 3.52 (3 H, s, OMe), and *ca.* 4.9 (m, H-6 and -14); *m/e* 308 (M^+), 277, 248, 206, 193, 161, and 147 (base); high resolution *m/e* 308.159 (M^+, $C_{17}H_{24}O_5$ requires *M*, 308.163) and 277.144 ($C_{16}H_{21}O_4$, M − CH_3). The *lactonic acid* (13) (158 mg, 30.9%), had m.p. 211—212°; $\nu_{max.}$ (KBr) 3 300—2 400 and 1 685 (carboxy) and 1 725 (δ-lactone), $\nu_{max.}$ ($CHCl_3$) 1 720 and 1 690 cm^{-1}; δ_H ($CDCl_3$) 0.81 and 1.29 (s, 10- and 4-Me), 3.59 (3 H, s, OMe), 5.25 (d, *J* 8.5 Hz, H-14), and 10.2br (CO_2H); *m/e* 310 (M^+), 292, 279, 208, 163 (base), and 109; high resolution *m/e* 310.175 (M^+; $C_{17}H_{26}O_5$ requires *M*, 310.178), 292.160 ($C_{17}H_{24}O_4$, M − H_2O), and 279.160 ($C_{16}H_{23}O_4$, M − OCH_3). The *diacid* (16) (61 mg, 13.1%) had m.p. 184—191°; $\nu_{max.}$ (KBr) 3 300—2 400 and 1 690 cm^{-1}; δ_H [$(CD_3)_2CO$] 0.82 and 1.21 (s, 10- and 4-Me), 0.98 (3 H, d, *J* 7 Hz, 8-Me), and 7.8br (2 CO_2H); *m/e* 282 (M^+), 264 (M − 18), 236, 177, 123, and 109 (base). The diacid (16) dissolved in methanol–ether was treated with ethereal diazomethane. The solvent was evaporated off to give the *dimethyl ester* (17), $\nu_{max.}$ ($CHCl_3$) 1 720 and 1 715 cm^{-1} (methyl ester); δ_H ($CDCl_3$) 0.68 and 1.19 (s, 10- and 4-Me), 0.96 (3 H, d, *J* 6.5 Hz, 8-Me), and 3.65 (6 H, s, 2 CO_2Me): *m/e* 310 (M^+), 278, 251 (base), 250, 237 (intense), 177, and 123; high resolution *m/e* 310.221 (M^+, $C_{18}H_{30}O_4$ requires *M*, 310.214), 278.190 ($C_{17}H_{26}O_3$), 251.204 ($C_{16}H_{27}O_2$), 250.197 ($C_{16}H_{26}O_2$), 237.189 ($C_{15}H_{25}O_2$), 177.161 ($C_{13}H_{21}$), and 123.128 (C_9H_{15}). The *methoxy-diacid* (14) (24 mg, 4.7%) had m.p. 234—238°; $\nu_{max.}$ (KBr) 3 300—2 400, 1 695, and 1 685 (carboxy), and 1 100 cm^{-1} (methoxy); δ_H [$(CD_3)_2SO$] 0.60 and 1.11 (s, 10- and 4-Me), 3.20 (3 H, s, OMe), and 3.15 (2 H, m, O·CH_2); *m/e* 312 (M^+), 294, 280, 262, 234, 167, 161, 121, and 109 (base); high resolution *m/e* 294.184 (M − H_2O; $C_{17}H_{26}O_4$ requires 294.183), 280.162 ($C_{16}H_{24}O_4$, M − CH_3OH), and 262.155 ($C_{16}H_{22}O_3$). Treatment of compound (14) with ethereal diazomethane afforded a *dimethyl ester* (15), $\nu_{max.}$ ($CHCl_3$) 1 715 cm^{-1} (CO_2Me); δ_H ($CDCl_3$) 0.66 and 1.18 (s, 10- and 4-Me), 3.24 (2 H, m, O·CH_2), 3.30 (3 H, s, OMe), and 3.62 and 3.65 (s, CO_2Me); *m/e* 340 (M^+), 308, 248, 161, 157, and 121 (base); high resolution *m/e* 340.227 (M^+; $C_{19}H_{32}O_5$ requires *M*, 340.225), 308.200 ($C_{18}H_{28}O_4$, M − CH_3OH), 248.184 ($C_{16}H_{24}O_2$), 161.133 ($C_{12}H_{17}$), 157.087 ($C_8H_{13}O_3$), and 121.110 (C_9H_{13}).

The Dimethyl Ester (18) *from Acrostalic Acid* (4).—An ethereal solution of acrostalic acid (4) was treated with an excess of diazomethane. Evaporation and recrystallization from n-hexane gave the *dimethyl ester* (18), m.p. 106—107°; $\nu_{max.}$ (KBr) 3 075, 1 640, and 890 (=CH_2), and 1 735 and 1 715 cm^{-1} (CO_2Me), identical with authentic material (m.p. 108—108.5°) obtained from podocarpic acid (i.r. absorptions, g.l.c. retention time, and mixed m. p.).

Conversion of Isoacrostalidic Acid (6) *into the Monomethyl Ester* (20).—A solution of isoacrostalidic acid (6) was treated with an excess of diazomethane. Evaporation gave the methyl ester (19), $\nu_{max.}$ (KBr) 3 050 (olefinic), 1 750 (γ-lactone), and 1 720 (CO_2Me), $\nu_{max.}$ ($CHCl_3$) 1 760 and 1 720 cm^{-1}. This methyl ester (19) in ethyl acetate was hydrogenated over platinum oxide. Removal of the solvent afforded the ester (20), $\nu_{max.}$ (KBr) 3 300—2 500 (CO_2H) and 1 710 cm^{-1} (CO_2H and CO_2Me), identical (i.r. spectrum) with the compound obtained from partial hydrolysis of the dimethyl ester (17). Treatment of this ester (20) with diazomethane afforded a dimethyl ester, identical with the dimethyl ester (17) (g.l.c. retention time, mass spectral fragmentations, and high resolution mass spectrum).

Preparation of the γ-Lactone (23) *from the Dimethyl Ester* (18).—The dimethyl ester (18) (19 mg) dissolved in methanol was treated with methanolic N-potassium hydroxide. The solution was refluxed for 1 h, the solvent

was removed under reduced pressure, and water was added. The mixture was extracted with ether, and the organic layer was washed with water and brine, dried (Na_2SO_4), and evaporated to give the monomethyl ester (21), $\nu_{max.}$ (KBr) 3 090, 1 640, and 890 (=CH_2), 3 300—2 500 and 1 710 (CO_2H), and 1 715 cm^{-1} (CO_2Me); δ_H ($CDCl_3$) 0.52 and 1.20 (s, 10- and 4-Me), 3.60 (3 H, s, OMe), and 4.52br (s) and 4.79br (s) (C=CH_2). Compound (21), slurried in tetrahydrofuran, was treated with concentrated hydrochloric acid for 1 h. Water was added, and the mixture was extracted with ether. Work-up as usual gave a crude product showing two peaks at t_R 9 (minor) and 10 min (major) in g.l.c. (1.5% SE-30; 190 °C), and $\nu_{max.}$ (KBr) 1 770 (*trans*-γ-lactone) and 1 720 cm^{-1}, $\nu_{max.}$ ($CHCl_3$) 1 760 and 1 715 cm^{-1}. Further treatment of this product with acid gave an oily solid, g.l.c. of which showed the same two peaks as before but in reverse order of intensities. Recrystallization from ethanol yielded a γ-lactone (23), $\nu_{max.}$ (KBr) 1 745 (*cis*-γ-lactone) and 1 720 cm^{-1}, $\nu_{max.}$ ($CHCl_3$) 1 760 and 1 710 cm^{-1}; δ_H ($CDCl_3$) 3.63 (3 H, s, OMe).

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