The Structure of Penduliflaworosin, a New Furanoid Diterpene from *Croton penduliflorus*

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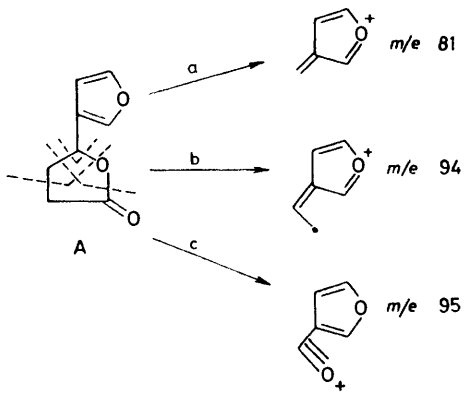
A clerodane-type diterpene has been isolated from the roots of *Croton penduliflorus* Hutch. (Euphorbiaceae). Spectroscopic evidence and chemical degradation have shown its structure to be *ent*-(12*R*)-methyl-15,16-epoxy-9,10-friedolabda-5(10),13(16),14-trien-19-oate 20,12-lactone (4).

CROTON PENDULIFLORUS Hutch. (Euphorbiaceae) is an important medicinal plant in southern Nigeria where it is known as *aworoso* by the Yorubas. It is used extensively as a remedy for several stomach complaints and the seeds are sold in market places.

The petroleum extract of the rootbark was chromatographed on silica gel. Ether eluted a white crystalline solid, penduliflaworosin, m.p. 100—101 °C, $[\alpha]_D$ +111.8°. Mass spectral and elemental analysis showed the molecular formula of penduliflaworosin to be $C_{21}H_{26}O_5$. Its i.r. absorptions at 1 490 and 875 cm^{-1} and positive Ehrlich test [1] suggested the presence of a furan ring. The n.m.r. multiplets at δ 6.34 and 7.34 were assigned to one β-proton and two α-protons respectively of a β-substituted furan ring. The i.r. absorption at 1 725 cm^{-1} and the three-proton singlet at δ 3.62 were assigned to a methoxycarbonyl group. The assignment was confirmed by mild alkaline hydrolysis of penduliflaworosin when the monoacid was obtained on acidification. The i.r. of the monocarboxylic acid showed a new carbonyl absorption at 1 690 cm^{-1} and a disappearance of that at 1 725 cm^{-1}, while the n.m.r. showed a broad one-proton signal centred at δ 9.6, exchangeable with D_2O, the δ 3.62 three-proton singlet being absent. Thus three of the five oxygen atoms in the molecule are accounted for.

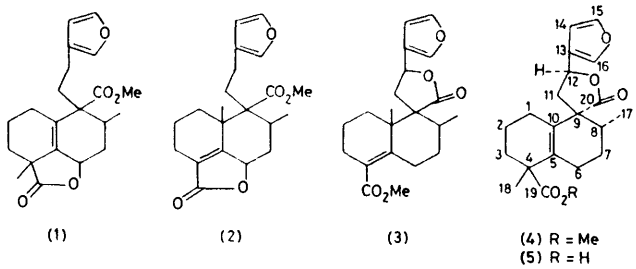
The remaining two oxygen atoms are contained in a γ-lactone whose presence was suggested by the i.r. absorption at 1 761 cm^{-1}. Vigorous alkaline hydroly is and careful acidification of the product gave a diacid. The i.r. of the diacid had a hydroxy-group while the only carbonyl absorption was at 1 695 cm^{-1}. The molecular ion of the diacid was barely detectable, and the rest of the spectrum was very similar to that of the monoacid. The ready loss of 18 daltons, most of which must have taken place in the ionization chamber, is in agreement with the observation that the diacid was readily transformed into the monoacid by heating.

The relative position of the γ-lactone to the β-substituted furan was determined in this way. The one proton triplet at δ 5.2 (J 8 Hz) was taken to indicate that the terminus of the lactone was allylic to the furan as in many similar furanoid lactones.[2] A partial structure (A) based on this premise has support from the mass spectral fragmentations as shown in Scheme 1 which is in accord with observations in similar systems.[3] Further support for the partial structure was provided by catalytic hydrogenation which gave a mixture of the hexahydro- and the tetrahydro-derivatives (10) and (9), respectively, in which the δ 5.3 proton (lactonic) disappeared. Such catalytic hydrogenolysis is well documented.[2]



SCHEME 1

Penduliflaworosin has one secondary methyl (δ 1.05, d, J 5 Hz) and one tertiary methyl (δ 1.34, s). The methoxycarbonyl, two methyl groups, the β-furan and the γ-lactone (minus its α-carbon) all add up to $C_{11}H_{15}O_5$. When due allowance is made for the groups identified, the rest of the molecule must have three double-bond equivalents and these were assigned to two rings and one double bond. The double bond should be tetra-substituted in view of its resistance to catalytic hydrogenation, and the absence of an olefinic proton signal in the n.m.r. spectrum.

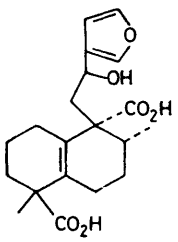


(1) (2) (3) (4) R = Me (5) R = H

Of the four possible structures (1), (2), (3), and (4) (stereochemistry unspecified) that could be written for the diterpene, (1) and (2) were ruled out since the n.m.r. of the tetrahydro-derivative did not show the one-

proton triplet at *ca.* δ 5.3 even though the lactone and the double bond were still intact. Furthermore in similar compounds with αβ-unsaturated γ-lactone like teucvin,[4] H-6 resonates at *ca.* δ 4.7.

The ^{13}C n.m.r. spectrum of penduliflaworosin supported structure (4), minus stereochemistry, rather than (3) in which the carbon of the tetra-substituted olefin which is β to the carbonyl would be expected at *ca.* δ 162. The signals were assigned with the aid of off-resonance multiplets and by analogy with mallotucin (8) [5] and other clerodanes like teucvin, and diasin [6] as appropriate.



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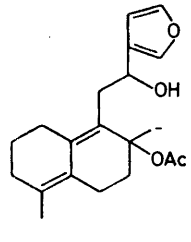

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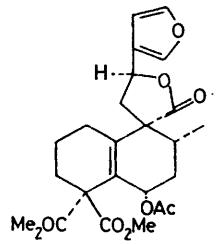

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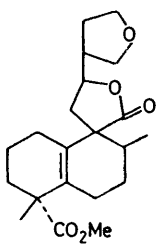

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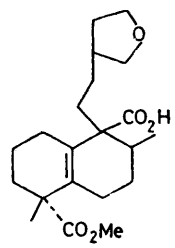

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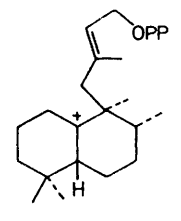
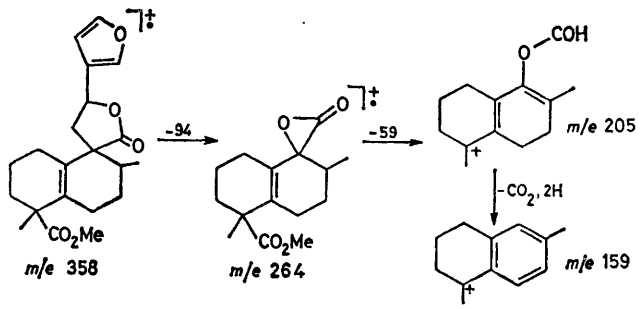


(11)

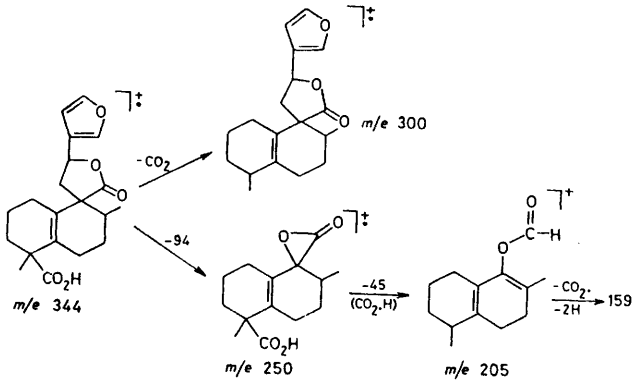
PP ≡ Pyrophosphate

Chemical evidence in support of (4) came from bis-decarboxylation of the diacid (6) with lead tetra-acetate. The reaction product was a complex mixture from which a compound M^+ 330 was separated as an oil and identified as the heteroannular diene (7). The diene showed



SCHEME 2

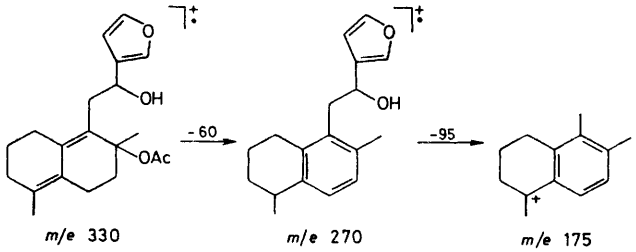
the expected spectral characteristics. The i.r. spectrum revealed the presence of a hydroxy-group at (3 450 cm^{-1}), an acetate (1 730 and 1 245 cm^{-1}), and a β-substituted furan (1 495 and 872 cm^{-1}). The u.v. had an absorption maximum at 240 nm. There were also one tertiary methyl (δ 1.27, s), a methyl on a double bond (δ 1.70, s) and an acetate (δ 2.03). A one-proton multiplet at 6.40 and a two-proton multiplet at 7.30 were attributed to a β-furan while H-12 at the base of the allylic –OH was located at δ 5.0.



SCHEME 3

The gross structure of penduliflaworosin [(4), minus stereochemistry] was supported by its mass spectral fragmentations and those of its derivatives (see Schemes 2—4). It is noteworthy that the base peak of the monoacid (5) is at *m/e* 159 rather than at *m/e* 94 which is diagnostic of furanoid diterpenes with a C-9 carboxylic acid group.[7] It therefore provides additional reason against the placement of the methoxycarbonyl at C-9.

Penduliflaworosin is a new member of the clerodanes which like the closely related mallotucin (8) has a $\Delta^{5,10}$ double-bond. Clerodanes are postulated to be



SCHEME 4

biosynthesized by a sequence of methyl migrations from labdane-type precursors by the ' backbone '-type rearrangement.[8] Penduliflaworosin provides additional evidence for this hypothesis, itself possibly having been formed from a hypothetical ion like (11). The stereochemistry as in (4) is therefore proposed on biogenetic grounds.

EXPERIMENTAL

M.p.s were taken on a micro hot-stage and are uncorrected. I.r. spectra were recorded on a Perkin-Elmer 137 spectrometer and refer to Nujol mulls unless otherwise stated. U.v. spectra were recorded for solutions in 95% ethanol solutions. The ^1H n.m.r. spectra were recorded at 60 MHz on a Varian T-60 instrument while tetramethylsilane was used as internal standard as it was with the ^{13}C n.m.r. spectrum which was run on a JEOLCO PS-100 instru-

ment. All n.m.r. measurements were made in deuteriochloroform solutions. Mass spectra were run on CEC-103 and Varian MAT-112S instruments.

Isolation of Penduliflaworosin (4).—Plant materials were collected by Mr. G. Adesida and identified by the Federal Department of Forestry Research, Ibadan, where a herbarium specimen FHI 91302 is filed. The ground root bark (2 kg) was extracted in a Soxhlet with light petroleum for 18 h and the extract chromatographed over silica gel. Diethyl ether eluted a white solid (1.1 g) which crystallised as needles from methanol m.p. 100—101 °C (Found: C, 70.05; H, 7.1. $C_{21}H_{26}O_5$ requires C, 70.37; H, 7.31%), $[\alpha]_D^{22}$ +111.8° (*c* 0.007 in $CHCl_3$); $\nu_{max.}$ 1 761, 1 725, 1 490, and 875 cm^{-1}; δ(^{1}H n.m.r.) 1.05 (3 H, d, *J* 5.0 Hz, 8-Me). 1.34 (3 H, s, 4-Me), 3.62 (3 H, s, CO_2Me), 5.42 (1 H, t, *J* 8 Hz, 12-H), 6.34br (1 H, s, 14-H), and 7.34 (2 H, m, 15- and 16-H); *m/e* (rel. intensity) 358 (M^+, 22), 264 (43), 205 (100), 187 (97), 159 (84), 105 (70), 95 (87), 94 (37), 91 (55), 81 (97), 55 (75), 44 (64), 43 (72), and 41 (74); $\lambda_{max.}$ 208 nm (ε 9 710); δ(^{13}C n.m.r.) 16.3 (q, C-17) 19.0 (t, C-2),* 22.9 (q, C-18), 26.5 (t, C-3),* 26.7 (t, C-7),* 35.0 (t, C-11),* 37.7 (d, C-8), 41.3 (t, C-1 and C-6), 47.5 (s, C-9), 51.8 (q, OCH_3), 53.2 (s, C-4), 72.1 (d, C-12), 108.2 (d, C-14), 125.9 (s, C-13), 128.7 (s, C-10), 134.7 (s, C-5), 139.2 (d, C-16), 144.0 (d, C-15), 177.3 (s, C-20), and 178.0 (s, C-19).

Mild Acid Hydrolysis.—Penduliflaworosin (200 mg) in methanol (25 cm^3) and sulphuric acid (1 cm^3) were refluxed for 2½ h. The solution was concentrated to *ca.* 10 cm^3 and water was added. The product on work-up was starting material.

Mild Alkaline Hydrolysis of Penduliflaworosin to give the Monoacid (5).—Penduliflaworosin (180 mg) in methanol (20 cm^3) was refluxed for 2½ h. The acidified product was filtered and washed several times to give a white solid (160 mg), m.p. 140—142 °C (from methanol) (Found; C, 69.38; H, 7.35. $C_{20}H_{24}O_5$ requires C, 69.75; H, 7.02%), M^+ 344; $\nu_{max.}$ 1 761 (γ-lactone), 1 690 (CO_2H), and 1 490 and 872 cm^{-1} (β-furan); δ 1.12 (3 H, d, *J* 5 Hz 8-Me), 1.32 (3 H, s, 4-Me), 5.44 (1 H, t, *J* 8 Hz, 12-H), 6.42 (1 H, d, *J* 1.5 Hz, 14-H), 7.50 (2 H, m, 15- and 16-H), 9.6br (1 H, s, disappeared with D_2O, CO_2H); *m/e* 344 (34), 300 (71), 299 (26), 250 (62), 205 (86), 159 (100), 95 (96), 91 (73), 81 (73), and 77 (68).

More Vigorous Alkaline Hydrolysis to give the Diacid (6).—Penduliflaworosin (350 mg) in ethanol (5 cm^3) was refluxed for 4 h with sodium hydroxide (5 g) in water (15 cm^3). Acidification of the cooled solution gave a white solid (340 mg), which crystallised from methanol, m.p. 190—192 °C (Found: C, 66.25; H, 7.5. $C_{20}H_{26}O_6$ requires C, 66.28; H, 7.23%); $\nu_{max.}$ 3 650 (OH), 1 695 (CO_2H), 1 495, and 876 cm^{-1} (β-furan).

Bisdecarboxylation of the Diacid.—The diacid (6) (220 mg) in dry benzene (3 cm^3) was refluxed with lead tetra-acetate (600 mg) for 3 h after the addition of pyridine (0.5 cm^3). After cooling, the product was filtered and washed with benzene. The combined filtrate and washings were washed successively with water, dilute sodium hydroxide, dilute hydrochloric acid, and water. The benzene solution was dried ($MgSO_4$) and evaporated to give a dark brown oil. Chromatography on silica gel and elution with diethyl ether–light petrol (1 : 4) gave a pale yellow oil (7) (74 mg) pure by t.l.c. (Found: C, 72.4; H, 8.3. $C_{20}H_{26}O_4$ requires C, 72.70; H, 7.93%); *m/e* 330 (M^+, 10), 312 (10.5), 270 (35), 252 (37), 175 (100), 95 (45), and 81 (43); δ 1.27 (3 H, s, 8-Me), 1.70 (3 H, s, 4-Me), 2.03 (3 H, s, O–$COCH_3$), 5.0br (1 H, t, 12-H), 6.40 (1 H, m, 14-H), 7.30 (2 H, m, 15- and 16-H); $\lambda_{max.}$ 240 nm; $\nu_{max.}$ (film) 3 450 (OH), 1 730, 1 245 ($OCOCH_3$), and 1 495, and 872 cm^{-1} (β-furan).

Action of Heat on the Diacid.—The diacid (100 mg) was heated at 210 °C (above its m.p.) for 2 min. The oily product (95 mg) which later solidified was shown to be identical in all respects with the monoacid (5).

Hydrogenation of Penduliflaworosin.—Penduliflaworosin (120 mg) in EtOH (25 cm^3) was hydrogenated over Pd–C (10%, 50 mg) at 25 °C and atmospheric pressure. After 30 min no more absorption of hydrogen was noticed. On work-up a gum was obtained. T.l.c. and mass spectroscopy showed it to be a mixture of two compounds, the tetra- and the hexa-hydro-derivatives. The hexahydro-derivative, an acid (10) was extracted from mixture by sodium carbonate while the neutral compound the tetrahydro-derivative (9) was taken in ethyl acetate and separated as an oil (21 mg), one spot on t.l.c.; M^+ 362. The only significant low-field signal in the n.m.r. spectrum was at δ 3.62 (3 H, s, CO_2Me). The hexahydro-derivative (10) was a solid (68 mg) crystallised from methanol, m.p. 174 °C (Found: C, 69.11; H, 8.9. $C_{21}H_{32}O_5$ requires C, 69.20; H, 8.85%), $\nu_{max.}$ 1 725 (CO_2Me) and 1 695 (CO_2H); δ 3.65 (3 H, s, CO_2Me), 7.80br (1 H, s, disappeared with D_2O, CO_2H); *m/e* 364 and 320.

* The assignments can be interchanged.

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