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DITERPENOIDS OF *PODOCARPUS NUBIGENA**

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Abstract—From the leaves and stems of *Podocarpus nubigena* Lindl. have been isolated rimuene, nagilactone-C, a new norditerpene, nubilactone-A (IV), daucosterin, sitosterol and cyanidin.

THE PRESENCE of insect moulting hormones, such as crustecdysone, have been detected in many plants including several species of *Podocarpus*.[1] A test on *P. nubigena* for the presence of insect moulting hormones proved negative;† however, in connection with our general screening programme on the Chilean flora,[2] a brief phytochemical study of the constituents of the leaves and twigs of this plant was made. Previous work on this family has shown the presence of diterpenes,[3] phenolic pigments,[4] the polyhydroxylated steroids,[5] and oxygenated diterpenes.[6] Except for the insect hormones, examples of each of these classes of compounds were isolated.

Acid hydrolysis of a portion of the dried, powdered leaves and twigs from the plant, followed by chromatography, indicated the presence of cyanidin. Examination of the neutral benzene extract afforded sitosterol and a hydrocarbon fraction. Separation of the latter material by TLC (10% $AgNO_3$–SiO_2) gave one major component and a minor substance. The major component was identified as rimuene (I)[7] by analysis of its MS

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† We thank Dr. D. H. S. HORN, Chemistry Research Laboratory, Division of Applied Chemistry, CSIRO, Melbourne for carrying out this test.

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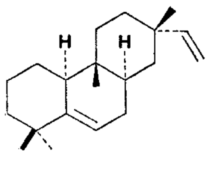
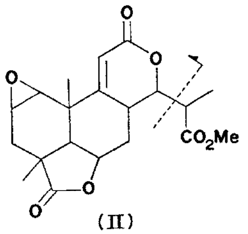
fragmentation pattern and, eventually, direct comparison.* The minor component, only isolated in small amounts, was isomeric with rimuene. Although not fully characterized its MS was similar to that of isoatisirene,[8a] however, its optical rotation was not the same. (Found: $[\alpha]_D^{20}$ +94°, isoatisirene[8b] $[\alpha]_D^{20}$ −74°.)



(I)



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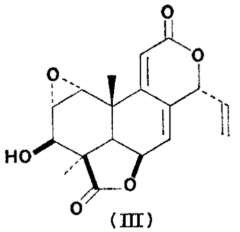

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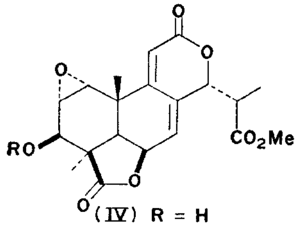
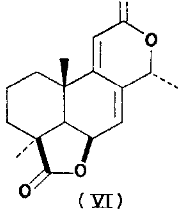


(IV) R = H
(V) R = Ac



(VI)

Extraction of the benzene isoluble material with ethyl acetate and subsequent purification afforded daucosterin,[9] whilst extraction of the residue with ethanol afforded two further products. The first was identified, both by direct comparison† and by examination of its spectra, as nagilactone-C.[10] The other product, named nubilactone-A, analysed as $C_{20}H_{22}O_8$. This material showed strong bands in its IR spectrum at ν_{max} 3525, 3320 (hydroxyl), 1755 (lactone), 1740 (ester), and 1710 and 1608 cm^{-1} (conjugated ester). Its UV absorption, λ_{max}^{EtOH} 263 nm (ϵ 23 000), suggested the presence of a conjugated diene lactone group. The MS fragmentation pattern showed the major loss from the parent ion (M^+ 390) to be 87 units leading to the ion at *m/e* 303. Accurate mass measurements indicated that this was due to the loss from the parent ion of $C_4H_7O_2$ (*a*) Me–$\dot{\mathrm{C}}$H–COOMe. Such a fragmentation was similar to that recently observed for the lactone (II), isolated from *Podocarpus saligna*[11] and due to the indicated fragmentation.

The NMR spectrum of nubilactone-A showed a very close similarity to that of podolactone-E (III)[12] (see Table 1), except that nubilactone-A contained a secondary methyl group and the carbomethoxy group associated with the fragment (*a*), rather than the vinyl group present in podolactone-E. Thus, assuming that the relative stereochemistry for

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† We thank Dr. T. Sakan, Department of Chemistry, Univerrity of Osaka, Japan for an authentic sample.

[8] (a) Appleton, R. A. and McCormick, A. (1968) *Tetrahedron* **24,** 633; (b) Kapadi, A. H. and Dev, S. (1964) *Tetrahedron Letters* 2751; Kapadi, A. H., Sobti, B. R. and Dev, S. (1965) *Tetrahedron Letters* 2729.

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nubilactone-A is similar to that of nagilactone-C, nubilactone-A can be allocated structure (IV). Nubilactone-A gave a monoacetate and the NMR spectrum of this was also consistent with its formulation (V) (see Experimental). Furthermore, lanthanide shift experiments with the Eu(fod)$_3$ reagent[13] allowed appropriate assignments to all of the protons in the acetate.

TABLE 1. CHEMICAL SHIFTS (τ) AND COUPLING CONSTANTS OF DITERPENE LACTONES*

	Podolactone-E	Nubilactone-A		Podolactone-E	Nubilactone-A
1-H	6·31*d* (4·5)	6·40 (*m*)	11-H	3·49*d* (1·8)	3·58*d* (1·5)
2-H	6·44*dd* (4·5, 6·0)	6·50 (*m*)	18-H$_3$	8·53*s*	8·62*s*
3-H	5·31*d* (6·0)	5·42*d* (6·0)	20-H$_3$	8·48*s*	8·54*s*
5-H	7·89*d* (5·5)	7·92*d* (5·5)	14-H	4·44*m*	4·70*m*
6-H	4·94*m*	4·98*m*	15-H	3·91*m*	6·70*m*
7-H	3·76*m*	3·60*m*	16-H	4·47 and 4·52	8·58*d* (6)

* In $^2H_5$-pyridine, with tetramethylsilane as internal reference.

Nubilactone-A is a novel member of the recently isolated abietane class of norditerpenes. The presence of the C-17 as a carboxyl group allows several speculations as to the biosynthetic pathways leading from this structure, via decarboxylation, to the podolactone and inumakilactone classes. Because of the close correlation between the structure of nubilactone-A with that of podolactone-E and to the antibiotic LL-Z1271α (VI)[14] it might be expected to possess plant growth inhibiting properties. Tests to ascertain this possibility are under way and will be reported on separately.

EXPERIMENTAL

M.ps were determined on a Kofler block and are uncorrected. NMR spectra were measured in CDCl$_3$ with tetramethylsilane as internal reference. Preparative GLC was effected with a Varian model 700 machine. Chromatography was carried out using Merck alumina or silica gel (0·05–0·20 mm).

Extraction. Powdered leaves and twigs from *P. nubigena* (dry wt 12 kg) were exhaustively extracted with benzene in a Soxhlet. The residue was then extracted with EtOH and this fraction evaporated and then re-extracted, using CHCl$_3$ and then EtOAc. The benzene extract weighed 480 g, whilst the EtOH extract weighed 2·25 kg.

Benzene fraction. This fraction was re-extracted with light petrol. before chromatrographing through alumina (grade III). The initial eluates (light petrol.) consisted mainly of one fraction, which was purified by GLC (5% SE52 on chromosorb W) and by preparative TLC (10% AgNO$_3$–SiO$_2$, solvent cyclohexane). The major compound had m.p. 55°, $[\alpha]_D^{20}$ +13° (*c* 1·55, CHCl$_3$); an authentic sample of rimuene had m.p. 54–55°, $[\alpha]_D^{20}$ +10·3° (*c* 1·09, CHCl$_3$) and the same R_f on TLC (0·11). The MS showed peaks at *m/e* 272 (M$^+$), 257 (base peak), 121, 105, 91, 80, 55 and 41 identical to that of an authentic sample.[7] Its NMR spectrum was also similar to that reported in the literature.[15] The less polar, minor component (R_f 0·68) had m.p. 84–85°, $[\alpha]_D^{20}$ +94·3° (*c* 4·3, CHCl$_3$), but was not further characterized. Its MS showed *m/e* 272, 257, 216, 201, 109, 94, 81. Elution of the alumina column with Et$_2$O–light petrol. (1:1) afforded sitosterol, m.p. 135°, ν_{max}^{Nujol} 3425, 2950, 1461, 1376, 1052, 800 cm^{-1}. Its acetate had m.p. 120–122°. Both the alcohol and its acetate had the same physical properties as authentic samples.

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EtOH fraction. A portion (600 g) of the EtOH extract was separated between $CHCl_3$ and water followed by EtOAc. The $CHCl_3$ extract (3·3 g) was purified by column chromatography on silica to give two compounds. The first, eluted with C_6H_6–EtOAc, crystallized as needles from EtOH. This material was shown to be identical to an authentic sample of nagilactone-C and had m.p. 325°, $[\alpha]_D^{20}$ + 99·5° (*c*, 0·5, pyridine), λ_{max}^{EtOH} 300 nm (ϵ 6200), ν_{max}^{Nujol} 3525, 3320, 1770, 1710, 1640, 1550 cm^{-1}. The second compound, also eluted with C_6H_6–EtOAc had m.p. 288–290° (from EtOH), $[\alpha]_D^{20}$ +43·5° (*c*. 0·2, pyridine), λ_{max}^{EtOH} 263 (ϵ 23 000), ν_{max}^{Nujol} 3525, 3320, 1755, 1740, 1710, 1608 cm^{-1} (for NMR see Table 1), MS bands at *m/e* 390 (10%), 375 (2), 372 (2), 362 (2), 358 (1), 303 (95), 275 (10), 201 (20), 182 (60), 115 (60), 91 (100). (Found: C, 61·3; H, 5·7; $C_{20}H_{22}O_8$ requires: C, 61·6; H, 5·7%.) This compound was called *nubilactone-A*. Acetylation of nubilactone-A afforded the *monoacetate*, m.p. 240° (from EtOH), ν_{max}^{Nujol} 1760 (strong), 1710, 1608 cm^{-1}, τ, 3·91 (1Hz, 7-H), 3·90 (1H, *d*, *J* 1·5 Hz, 11-H), 4·58 (1H broadened *d*, *J* 5·1 Hz, 3-H), 4·76 (1H, broadened *d*, *J ca*. 6 Hz, 14-H), 5·15 (1H, *ddd*, *J* 5·0, 4·0, 0·3 Hz, 6-H), 6·34 (3H, *s*, MeO), 6·54 (2H, *m*, 1-H, 2-H), 6·85 (1H, *dq*, *J* 7·0, 3·9 Hz, 15-H), 7·88 (1H, *d*, *J* 5·0 Hz, 5-H), 7·90 (3H, *s*), 8·47 (3H, *s*), 8·62(3H, *d*, *J* 7·0 Hz, 16-H), 8·87 (3H, *s*); MS bands at *m/e* 432, 400, 390, 385, 373, 345, 303. The EtOAc extract (28 g) was also chromatographed on silica gel (400 g) to give, as the major constituent, daucosterin, eluted with EtOAc. This had m.p. 275–278°, gave an acetate, m.p. 165–170°, both of which were identical in their physical properties to authentic specimens. Hydrolysis of a portion of the daucosterin[9] afforded sitosterol and D-glucose.

Detection of cyanidin. A portion of the dried, powdered leaves and twigs from *P. nubigena* (200 g) was heated with 2N HCl at reflux for 40 min. The solution was cooled, extracted with amyl alcohol and the residue chromatographed on Whatman paper No. 3 in Forestal. On the basis of its R_f, its UV and visible absorption spectra, and the changes of these with acid and base, the principal phenolic pigment was identified as cyanidin.