Cytotoxic Norditerpene Lactones from Ileostylus micranthus

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CYTOTOXIC NORDITERPENE LACTONES FROM *ILEOSTYLUS MICRANTHUS*

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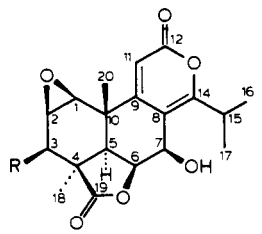
ABSTRACT.—Three new compounds **2–4** and two known compounds **1** and **5** have been isolated from the cytotoxic fraction of an extract from a New Zealand mistletoe, *Ileostylus micranthus*. Compounds **1–5** are shown to be norditerpene lactones and have strong cytotoxicity. The compounds are proposed to have been assimilated by the mistletoe from the host tree, *Podocarpus totara*.

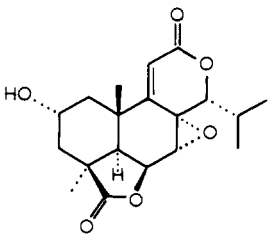
As part of a systematic survey of the New Zealand flora for new bioactive constituents, we have examined one of the New Zealand large leafy mistletoes, *Ileostylus micranthus* (Hook.f.) Tieghem (Loranthaceae). Our interest in this species was aroused when extracts of leaf material showed considerable cytotoxic activity. *I. micranthus* parasitizes a wide variety of trees and shrubs, with no particular preference for any one host. This particular sample of mistletoe was collected from an evergreen conifer *Podocarpus totara* D. Don (Podocarpaceae). Extracts of *P. totara* are cytotoxic, and this activity has been shown to be due to the presence of norditerpene lactones (1). It was interesting then to determine the nature of the cytotoxicity in the mistletoe extract.

Fractionation of the crude defatted extract by reversed-phase chromatography and analysis of individual fractions indicated the active constituents were uv-active, relatively non-polar (tlc, SiO_2), and amenable to Si gel chromatography. Flash Si gel cc (eluent $CHCl_3$↦20% MeOH in $CHCl_3$) gave a series of similar complex fractions from which only four major compounds could be purified by reversed-phase hplc in sufficient quantity for structural studies.

The structural formulae obtained for these compounds from the ms and ^{13}C-nmr



1 R=OH
2 R=H



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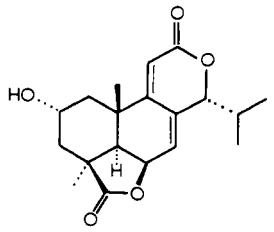

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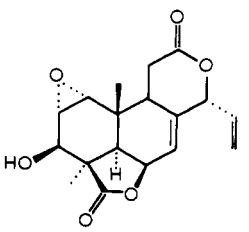

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spectral data suggested polycyclic structures with varying degrees of oxygenation. The observation of signals in the ^{13}C-nmr spectra consistent with δ-lactones and the presence of nineteen carbon signals led to the conclusion that these compounds were norditerpene lactones. This was confirmed by comparison of the spectral data with that for known norditerpene lactones (2,3).

Compound **1** had a molecular formula of $C_{19}H_{22}O_7$. The ^{13}C-nmr data indicated the presence of two double bonds and an epoxide. An isopropyl group was clearly evident in the ^{1}H-nmr spectrum (2 Me doublets and a 1H septet at 3.25 ppm). The absence of further coupling of the isopropyl proton indicated C-14 was fully substituted. Analysis of the proton coupling for the epoxy and alcoholic protons allowed the remaining substitution pattern to be determined. The doublet at 2.05 ppm could be assigned to H-5, and this proton was coupled to H-6 (4.94 ppm, dd), which in turn was also coupled to H-7 (5.35 ppm). The three-proton spin system comprising the epoxide protons and the proton resonating at 4.48 ppm was therefore located at the 1, 2, and 3 positions of the molecule. At this stage comparisons with known norditerpene lactones were made and the similarity with nagilactone C (4) was noted. Indeed, detailed comparison of the ^{13}C- and ^{1}H-nmr spectral data indicated the identity of **1** and nagilactone C, which has an epoxiode at C-1, -2 and a hydroxyl group at C-3.

The exact match of the ^{13}C-nmr spectrum (C_5D_5N) with that reported for nagilactone C (3) and the same sign of the optical rotation confirmed the stereochemistry as that reported for this compound.

The second compound was obviously similar to **1** in many of its spectral features. A molecular formula of $C_{19}H_{22}O_6$ for **2** corresponded to **1** with one less oxygen. The general appearance of many of the signals in the ^{1}H-nmr spectrum was similar but with the following differences. The signal corresponding to H-3 in **1** was missing in **2**, and two new signals were present at 1.86 and 2.44 ppm consistent with a methylene group coupled to the epoxide proton resonating at 3.50 ppm. Thus **2** is 3-deoxynagilactone C.

The substitution pattern of the third compound **3** ($C_{19}H_{24}O_6$) was somewhat more difficult to determine. The ^{1}H-^{1}H COSY spectra run in $CDCl_3$ and C_5D_5N were helpful in resolving the overlapping signals in the 1.5–2.5 ppm region. A hydroxy group sandwiched between two methylene groups could be established for the C-1↦3 positions. The methylene signals could be assigned on the evidence of a correlation between the C-20 methyl group (1.29 ppm, shifts given for $CDCl_3$) and one of the methylene protons (2.16 ppm) in the ^{1}H-^{1}H NOESY nmr spectrum. A 5 Hz coupling between this latter H-1β proton and H-2 indicates that the relative stereochemistry of the hydroxyl group at C-2 is α. A larger trans-diaxial 13 Hz coupling between H-1α and H-2 is consistent with this assignment. The characteristic doublet due to H-5 showed coupling to a broad doublet at 4.93 ppm, which was further coupled to a proton resonating at 3.96 ppm. This latter signal was clearly that of an epoxide proton since the ^{13}C-nmr spectrum had signals for one epoxide and only one double bond, the latter with chemical shifts characteristic of an unsaturated δ-lactone. The remaining ^{1}H-nmr signals could be assigned to the isopropyl group, a proton at C-14 (4.42 ppm, d), and the vinylic proton at C-11. The sharp singlet signal of the latter proton is a characteristic feature of the spectra of many of these compounds. Thus **3** is 2,3-dihydro-2-hydroxypodolide. The only previous report of **3** is as a mass spectral substrate (5).

Spectra of the fourth compound **4** ($C_{19}H_{24}O_5$), bore a marked resemblance to those of **3**. The presence of a second double bond in **4** and the absence of an epoxide group were clear from the ^{13}C-nmr spectrum. Comparison of ^{1}H-nmr spectra established **4** as having a double bond at C-7, -8, but the remaining substitution pattern was identical to that of **3**. The extension of the unsaturation is also evident in the uv spectrum of **4**. Comparison of the ^{1}H- and ^{13}C-nmr spectra of **3** and **4** with those of known compounds

TABLE 1. ^{1}H-nmr Data for Compounds **1–5**.[a]

Proton	Compound				
	1	**2**	**3**	**4**	**5**
H-1	3.57, d, J = 4	3.23, d, J = 3.7	2.16, t, J = 13 1.93, dd, J = 13, 5	2.24, t, J = 13 1.95 (obsc.)	3.55, d, J = 4
H-2	3.49, dd, J = 4, 6	3.50, m	4.06, m	4.11, m	3.50, dd, J = 6, 4
H-3	4.48, d, J = 6	2.44, dd, J = 14.5, 2.3 1.86, brd, J = 14.5	2.36, dd, J = 14, 8 1.56, dd, J = 14, 5	2.45, dd, J = 14, 9 1.59, dd, J = 14, 7	4.51, d, J = 6
H-5	2.05, d, J = 6.5	17.2, d, J = 5.8	1.87, d, J = 5	1.95, d, J = 5	2.15, d, J = 5
H-6	4.94, dd, J = 8.6, 6.5	4.98, dd, J = 8.8, 5.8	4.93, dd, J = 5, 1	5.08 ddd, J = 5, 5, 1.5	
H-7	5.35, d, J = 8.6	5.29, d, J = 8.8	3.96, br s	6.20, ddd, J = 5, 1.5	6.21, m
H-11	6.29, s	5.90, s	6.03, s	5.83, d, J = 1.6	6.20, s
H-14			4.42, d, J = 3.4	4.87, d	5.43, d, J = 7.7
H-15	3.27, sept, J = 6.8	3.25, sept (obsc.), J = 6.8	1.90, m	2.25, m	5.99, ddd, J = 17, 10, 7.7
H-16, -17	1.35, d, J = 6.8 1.27, d, J = 6.8	1.33, d, J = 6.8 1.25, d, J = 6.8	1.15 (6H), d, J = 5.5	0.98, d, J = 6 1.20, d, J = 6	5.62, d, J = 10 5.56, d, J = 17.2
H-18	1.49, s	1.51, s	1.38, s	1.42, s	1.50, s
H-20	1.49, s	1.55, s	1.29, s	1.28, s	1.28, s

[a]300 MHz, $CDCl_3$. Presented as chemical shift (ppm), multiplicity, coupling constants (Hz).

(3,6) establishes the relative stereochemistry for the C-4 to C-14 segments to be that shown. The absolute stereochemistry shown is consistent with other members of this class of compounds.

Attempts to obtain more of compounds **1–4** from an additional collection of plant material were frustrated by an apparent variation in the levels and nature of the norditerpene lactones present. This second, much larger extract yielded additional small amounts of **3** and **4** as well as a fifth compound which could be readily identified as podolactone E [**5**] (7).

The cytotoxicity of norditerpene lactones, previously only isolated from *Podocarpus* species, is well documented (2). Compounds **1–5** demonstrate varying levels of in vitro cytotoxicity in the P-388 leukemia screen, with podolactone E the most active (see Experimental).

Because we have now confirmed that the cytotoxicity of the host and parasite are due to the same class of compounds, it would appear logical to suggest that these compounds are assimilated from the host. To confirm this, we collected *Ileostylus micranthus* from an alternative host, the widespread shrub *Coprosma propinqua* Cunn. (Rubiaceae), and as expected the extract of this mistletoe was inactive. This assimilation phenomenon has been recorded for some other mistletoe species; for example, mistletoes growing on an Australian plant, *Nerium oleander*, have been shown to extract cardiac glycosides from the host plant (8).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Reversed-phase cc (9) and Si gel chromatography (10) were performed according to literature methods. Nmr spectra were recorded at 300 MHz for ^{1}H and 75 MHz for ^{13}C on a Bruker AC300 spectrometer. Mass spectra were recorded on a VG70-250S mass spectrometer. Mp's are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 Polarimeter. Voucher specimens of plants sampled have been deposited in the DSIR Herbarium, Lincoln, New Zealand (CHR).

EXTRACTION AND ISOLATION OF **1–5**.—Air-dried leaves and stems of *I. micranthus* (100 g) (host *P. totara*) collected in September 1988 from Peel Forest Park, Canterbury, New Zealand (CHR 459361) were extracted by soaking in EtOH-H_2O (80:20). The crude extract was defatted with hexanes, and 4 g of this material was subjected to reversed-phase flash chromatography. The fraction containing compounds **1–4** [eluted with H_2O-MeOH (25:75)] was chromatographed on Si gel [MeOH-$CHCl_3$ (2:98→10:90)] followed by reversed-phase hplc [H_2O-MeOH (1:1)] to give pure **1** (4.0 mg), **2** (5.0 mg), **3** (0.8 mg), and **4** (0.7 mg).

A second collection (755 g) of *I. micranthus* (host *P. totara*) from the same area was made in April 1989 (CHR 461059). This extract was treated in the same manner to yield additional amounts of **3** (2.0 mg) and **4** (1.7 mg) was well as **5** (3.4 mg). A small sample of *I. micranthus* [host *C. propinqua* (CHR 465844)] was collected and extracted as above.

Nagilactone C [**1**].—Needles from MeOH: dec above 280° [lit. (4) 290° dec]; $[\alpha]_D$ +43° (c = 0.1, MeOH) [lit. (4) +111°]; λ max (EtOH) 320 nm (5688), 212 nm (9825); hreims 362.1374 ($C_{19}H_{22}O_7$ requires 362.1365); ^{1}H nmr see Table 1; ^{13}C nmr see Table 2.

3-Deoxy nagilactone C [**2**].—Needles from MeOH: mp 272° (dec); $[\alpha]_D$ +83° (c = 0.16, MeOH); λ max (EtOH) 300 nm (9000), 220 nm (9340); hreims 346.1416 ($C_{19}H_{22}O_6$ requires 346.1416); ^{1}H nmr see Table 1; ^{13}C nmr see Table 2.

2,3-Dihydro, 2-hydroxy podolide [**3**].—Fine needles form C_6H_6/MeOH/hexane: mp 275–277°; $[\alpha]_D$ +41° (c = 0.1, MeOH); λ max (EtOH) 224 nm (11900); hreims 348.1584 ($C_{19}H_{24}O_6$ requires 348.1573); ^{1}H nmr see Table 1; ^{13}C nmr see Table 2.

2-Hydroxy nagilactone F [**4**].—Fine needles from MeOH: mp 224°; $[\alpha]_D$ −32° (c = 0.1, MeOH); λ max (EtOH) 262 nm (6400); hreims 332.1630 ($C_{19}H_{24}O_5$ requires 332.1624); ^{1}H nmr see Table 1; ^{13}C nmr see Table 2.

Podolactone E [**5**].—Fine needles from MeOH: slowly dec above 250° [lit. (3) 261–262°]; $[\alpha]_D$ −203° (c = 0.1, MeOH); λ max (EtOH) 264 nm (11100); hreims 330.1110 ($C_{18}H_{18}O_6$ requires 330.1103); ^{1}H nmr see Table 1; ^{13}C nmr see Table 2.

TABLE 2. ^{13}C-nmr Data for Compounds **1–5**.[a]

Carbon	Compound				
	1	**2**	**3**	**4**	**5**
C-1	58.2	60.3	39.6	40.2	55.5
C-2	51.5	52.3	64.4	64.7	50.1
C-3	67.9	33.8	37.2	36.5	68.1
C-4	50.2	44.1	41.9	42.4	48.3
C-5	51.2	49.2	42.2	45.7	49.0
C-6	73.7	73.7	72.5	72.0	71.2
C-7	60.1	60.3	53.6	121.6	122.6
C-8	111.8	108.8	57.3	133.8	135.0
C-9	170.4	171.8	158.5	158.3	155.6
C-10	38.2	35.3	36.6	35.9	36.4
C-11	107.4	104.8	118.1	112.8	113.3
C-12	162.1	162.0	163.5	165.0	163.3
C-14	165.0	164.5	82.7	83.0	80.6
C-15	29.9	29.5	26.8	29.7	131.3
C-16	20.1	20.7	16.3	15.1	123.1
C-17	20.8	22.0	21.3	19.6	—
C-18	26.3	24.6	29.0	27.9	25.3
C-19	177.7	176.2	180.3	180.6	178.0
C-20	19.2	20.1	23.0	23.4	19.0

[a]75 MHz, C_5D_5N (**1**) or $CDCl_3$ (**2–5**).

CYTOTOXICITY.—In vitro cytotoxicity vs. P388 leukemia cells for compounds **1–5**: IC_{50} (μg/ml) **1** 0.33, **2** 0.65, **3** 0.06, **4** 0.06, **5** <0.01. Testing was performed at Chemistry Department, University of Canterbury.

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