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CHEMICAL CONSTITUENTS OF *NEOLITSEA PARVIGEMMA*

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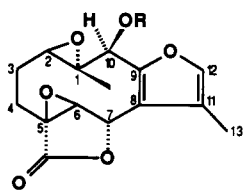
ABSTRACT.—Four known furanosesquiterpene lactones, zeylanidine [**1**], zeylanicine [**2**], linderane [**3**], and linderalactone [**4**], and a new naturally occurring one, deacetylzeylanidine [**5**], along with β-sitosterol, were isolated from the root of *Neolitsea parvigemma*. The stereochemistry of C-1, C-2, and C-10 of zeylanidine and zeylanicine was determined by the NOESY experiments, 1-Me and 10-OAc are in β configurations, and H-2 is in the α configuration.

Neolitsea parvigemma Kan. & Sas. (Lauraceae) is a small tree growing in the central and southern mountain regions in Taiwan (1). In continuation of our investigation of the biologically active chemical constituents of plants endemic to Taiwan, four known furanosesquiterpene lactones, zeylanidine [**1**], zeylanicine [**2**], linderane [**3**], and linderalactone [**4**], and a new naturally occurring one, deacetylzeylanidine [**5**], along with β-sitosterol, were isolated from the roots of this species.

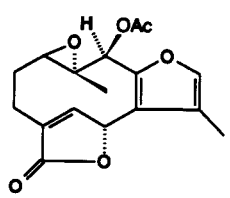
β-Sitosterol was identified by comparison of its tlc, mp, and ^{1}H-nmr spectrum with those of an authentic sample.

The five structurally related compounds belong to the germacrane-type furan sesquiterpenes, which contain a ten-membered monocyclic sesquiterpene lactone having a furan moiety in the molecules. The known compounds were identified by comparison of the mp, [α]D, ir, and ^{1}H-nmr data with those reported for zeylanidine (2,3), zeylanicine (2,3), linderane (2, 4–6) and linderalactone (2,4,5), and further confirmed by COSY and HETCOR experiments.

The stereochemistry of C-1, C-2, and C-10 in zeylanidine and zeylanicine has not been reported previously. In order to determine the stereochemistry of these chiral centers in these two compounds, NOESY experiments were performed. The salient common nOe interactions between H-7 and Ac, 1-Me and Ac, H-6 and Ac, 1-Me and H-6, H-6 and H-7,



1 R=Ac
5 R=H



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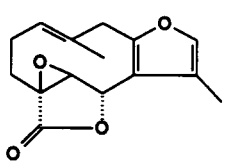

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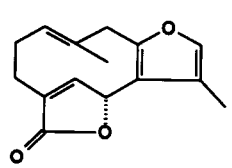

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TABLE 1. ^{1}H-nmr Data[a] for Compounds **1–5**.

Proton	Compound				
	1	**5**	**2**	**3**	**4**
H-2	3.66 (1H, dd, J = 9.0, 0.9)	3.72 (1H, dd, J = 11.1, 2.1)	3.36 (1H, dd, J = 12.0, 1.8)	5.37 (1H, br d, J = 15.0)	4.92 (1H, br t, J = 6.9)
H-3	1.50–1.65 (1H, m)	1.53–1.69 (1H, m)	1.49–1.70 (1H, m)	2.21–2.33 (1H, m)	2.08–3.35 (3H, m)
H-3	2.22 (1H, ddd, J = 14.0, 1.1, 9.5)	2.26–2.34 (1H, m)	2.22–2.30 (1H, m)	2.65–2.80 (2H, m)	
H-4	1.83 (1H, ddd, J = 15.0, 8.5, 1.1)	1.82 (1H, ddd, J = 15.0, 9.3, 1.5)	2.62 (1H, ddd, J = 14.1, 9.0, 1.1)		
H-4	3.02 (1H, ddd, J = 15.0, 9.5, 9.9)	3.00 (1H, ddd, J = 15.0, 9.3, 9.3)	2.86 (1H, ddd, J = 14.1, 9.6, 9.6)	1.66–1.81 (1H, m)	2.88–2.91 (1H, m)
H-6	3.96 (1H, s)	3.95 (1H, s)	7.05 (1H, s)	3.92 (1H, s)	6.81 (1H, d, J = 1.5)
H-7	5.36 (1H, s)	5.38 (1H, s)	5.85 (1H, s)	5.31 (1H, s)	5.82 (1H, s)
H-10	5.99 (1H, s)	5.00 (1H, s)	5.98 (1H, s)	3.51 (2H, s)	3.38 (1H, d, J = 15.0) 3.59 (1H, d, J = 15.0)
H-12	7.25 (1H, br s)	7.24 (1H, s)	7.22 (1H, br s)	7.14 (1H, s)	7.11 (1H, s)
H-13	2.06 (3H, br s)	2.07 (3H, s)	2.09 (3H, br s)	2.01 (3H, s)	2.08 (3H, br s)
H-14	1.14 (3H, s)	1.17 (3H, s)	1.00 (3H, s)	1.58 (3H, s)	1.25 (3H, s)
Ac	2.03 (3H, s)		2.01 (3H, s)		

[a]Recorded on Varian VXR-300 in $CDCl_3$ solution.

H-4 and H-4′, H-3 and H-3′ were clearly observed. In addition, the weak nOe interaction between 1-Me and H-10 also appeared in both cases, thus emphasizing the conformationally flexible nature of the ten-membered germacrane ring system which allows these groups to approach each other. These results indicate the stereochemistry of zeylanidine and zeylanicine should be expressed as structures **1** and **2**, respectively.

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

Carbon	Compound		
	1	**5**	**2**
C-1	60.9 s	60.7 s	60.0 s
C-2	56.7 d	56.4 d	58.5 d
C-3	21.0 t	21.2 t	19.2 t
C-4	21.3 t	21.1 t	23.0 t
C-5	61.5 s	62.6 s	132.0 s
C-6	60.4 d	61.9 d	147.2 d
C-7	72.6 d	72.8 d	73.6 d
C-8	116.6 s	115.7 s	117.6 s
C-9	150.6 s	152.9 s	150.0 d
C-10	68.8 d	69.5 d	68.7 d
C-11	121.6 s	121.8 s	121.3 s
C-12	139.0 d	138.6 d	139.0 d
C-13	8.3 q	8.4 q	8.4 q
C-14	16.4 q	16.2 q	16.2 q
C-15	172.0 d	171.4 s	174.5 d
Ac	169.3 s		169.3 s
Ac	20.5 q		20.5 q

[a]Recorded on Varian VXR-300 in $CDCl_3$ solution.

Most ^{1}H-nmr data of **5** are very close to those of **1** except that a singlet for H-10 was shifted upfield to δ 5.00 and the three-proton singlet for the acetyl protons was absent. The ^{13}C-nmr spectrum showed a doublet at δ 69.5 for C-10. The ir spectrum showed a broad absorption at 3500 cm^{-1}. These data clearly indicated that there was a hydroxyl group located at position 10 instead of an acetoxy group as in **1**. This inference was confirmed by hydrolysis of **1** with methanolic KOH to give a hydrolyzed product which had the same tlc, mp, and ^{1}H-nmr characteristics as those of **5**. The structure of **5** was in good agreement with the COSY experiment and ^{13}C-nmr data as well as the mp and ir data reported for deacetylzeylanidine (3).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Fisher-Johns digital melting point analyzer model 355 and were not corrected. Ir spectra were taken a KBr pellets, $CHCl_3$ solutions, or Nujol on a Perkin-Elmer 281 B spectrometer. Specific rotations

were obtained on a Perkin-Elmer 141 automatic polarimeter using $CHCl_3$ solutions. Low resolution mass spectra were obtained on a Finnigan 3200 GC/MS mass spectrometer coupled to an INCOS data system or Hewlett Packard model 5985, both *in ei mode at* 70 eV. ^{1}H-*nmr spectra* were recorded either on a Varian EM-390 (90 MHz) operating in cw mode or on a Varian VXR-300 (300 MHz) operating in ft mode. The ^{13}C-nmr and COSY, HETCOR, and NOESY experiments were determined on a Varian VXR-300 (300 MHz). *Cc was carried out on MN Si gel 60* (70–270 mesh ASTM) or Merck Si gel 60. Tlc analysis was performed by utilizing MN or Merck precoated plates, and detection of compounds was done by spraying with *p*-anisaldehyde reagent (7). All solvents used for chromatography purposes were AR grade. Solvents used for measuring specific rotation were spectroscopic grade.

PLANT MATERIAL.—The roots of *N. parvigemma* were collected at the Experimental Forest Station of National Pingtung Institute of Agriculture, Taiwan, Republic of China, *in* 1983. A voucher specimen is deposited in the Herbarium, Department of Forestry of the Institute. Whole roots were cut into pieces, air-dried, and ground in a Wiley mill.

EXTRACTION AND CRUDE FRACTIONATION.—The powdered root (2.5 kg) was defatted by percolation with *n*-hexane (10 liters in total) to obtain 5.4 g of oil. The marc was percolated exhaustively with 95% EtOH (16 liters). During the concentration of the EtOH extract (98.9 g), a pinkish white crystalline material precipitated (6.42 g) and was collected by filtration. The precipitate was recrystallized from warm MeOH to provide 4.14 g of colorless prisms. Tlc analysis showed it contained two components.

The EtOH extract (98.9 g) was mixed with Si gel (250 g) and eluted with $CHCl_3$, EtOAc, and MeOH successively to obtain a $CHCl_3$-soluble fraction (46.3 g), an EtOAc-soluble fraction (9.0 g), and an MeOH-soluble fraction (22.4 g).

CHROMATOGRAPHY OF THE $CHCl_3$-SOLUBLE FRACTION.—A batch of the $CHCl_3$-soluble fraction (40 g) was chromatographed on Si gel and eluted with $CHCl_3$. Fractions of 300 ml were collected. Fractions were pooled into 9 fractions, I–IX, according to tlc analysis.

ZEYLANIDINE [**1**] AND ZEYLANICINE [**2**].—A column was packed with Si gel 60 (100 g) in *n*-hexane–EtOAc (7:3). The crystalline precipitate obtained during concentration of the original extract (3.42 g) was chromatographed on the column and was eluted with *n*-hexane–EtOAc (7:3). Fraction 2 was recrystallized from hot MeOH to yield colorless prisms (1.3 g) of **1**: mp 223–224°, $[\alpha]D$ +161° (c = 0.76, $CHCl_3$); ir (KBr) ν max 3160, 3070 (furan), 1781 (γ-lactone), 1745, 1225 (acetate), 1380, 1365 cm^{-1}; ms *m/z* (relative abundance) $[M]^+$ 334 (3.31), 292 (2.9), 274 (5.5), 135 (base peak).

Fraction 4 was recrystallized from hot MeOH to obtain colorless prisms (160 mg) of **2**: mp 231–233°, $[\alpha]D$ +146° (c = 0.57, $CHCl_3$); ir (KBr) ν max 3145, 1645, 1580, and 885 (furan), 1760 (γ-lactone), 1745 and 1230 (acetate), 1380, 1370 cm^{-1}; ms *m/z* (relative abundance) $[M]^+$ 318 (11.6), 276 (20.0), 258 (5.5), 105 (base peak).

LINDERANE [**3**] AND LINDERALACTONE [**4**].—Fraction II was shown to be a mixture of two major substances with R_f values 0.83 and 0.73, respectively (10% EtOAc in $CHCl_3$). The residue (1.40 g) of this fraction was chromatographed *over Si gel* (42 g) *eluting* with C_6H_6-$CHCl_3$ (1:1), and 50-ml fractions were collected. Fraction 3 was further chromatographed on Si gel (3 g) using C_6H_6-$CHCl_3$ (1:1) as eluent to obtain **3** (16 mg): mp 184–186°, $[\alpha]D$ +162° (c = 0.44, $CHCl_3$); ir (KBr) ν max 3130, 3060, 1555 (furan), 1775 and 1330 (γ-lactone), 1615 cm^{-1} (double bond); ms *m/z* (relative abundance) $[M]^+$ 260 (23.9), 159 (59.5), 145 (93.7), 91 (base peak).

Fraction 5 was further purified by preparative tlc using 5% MeCN in C_6H_6 as developing solvent to yield **4** (60.8 mg), mp 137–139°, $[\alpha]D$ +100° (c = 0.57, $CHCl_3$); ir (KBr) ν max 3120, 3100, 1645, and 1560 (furan), 1745 cm^{-1} (γ-lactone); ms *m/z* (relative abundance) $[M]^+$ 244 (19.5), 200 (2.8), 77 (51.6), 39 (base peak).

β-SITOSTEROL.—Fraction IV was evaporated in vacuo leaving a brown gummy substance (0.88 g) which showed a major compound having R_f 0.38 (10% EtOAc in $CHCl_3$). It was purified by chromatographing on Si gel (30 g) and eluted with *n*-hexane–EtOAc (7:3). Fraction 3 (348.7 mg) from this column was further purified by another cc to yield 121 mg of β-sitosterol.

DEACETYLZEYLANIDINE [**5**].—Fraction VII showed a complicated pattern on tlc but there appeared a discernible spot with R_f 0.18 (10% EtOAc in $CHCl_3$). The residue (8.10 g) was chromatographed on Si gel (400 g) with *n*-hexane–EtOAc (7:3) as eluent. Fraction 8 was rechromatographed on another Si gel column and eluted with *n*-hexane–EtOAc (5:1) to obtain **5** (24.2 mg): mp 207–209°; $[\alpha]D$ +62° (c = 0.36, $CHCl_3$); ir (KBr) ν max 3500 (OH), 3130, 3090, 1655, and 1560 cm^{-1} (furan), 1770 (γ-lactone); ms *m/z* (relative abundance) $[M]^+$ 292 (2.3), 135 (42.1), 77 (19.5), 28 (base peak).

To a solution of **1** (200 mg) in MeOH (10 ml) was added 5% methanolic KOH solution (3 ml). The mixture was left overnight at room temperature. The reaction solution was acidified to pH 2–3 with HCl (5%) and then extracted with CH_2Cl_2

(3×30 ml). The CH_2Cl_2 extract was washed with saturated aqueous $NaHCO_3$ followed by H_2O and then the CH_2Cl_2 evaporated to leave a residue (106 mg) which was chromatographed on Si gel (5 g) and eluted with 10% EtOAc in $CHCl_3$ to obtain the hydrolyzed product. The hydrolyzed product has R_f value on tlc, mp, and ^{1}H-nmr characteristics identical to those of **5**.

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