Sesquiterpene Lactones from the Root of Neolitsea acutotrinervia

Wen-Shyong Li

J. Nat. Prod., **1992**, 55 (11), 1614-1619• DOI:
10.1021/np50089a008 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 55, No. 11, pp. 1614-1619, November 1992

SESQUITERPENE LACTONES FROM THE ROOT OF *NEOLITSEA ACUTOTRINERVIA*

WEN-SHYONG LI

Department of Environmental Protection, National Pingtung Polytechnic Institute, Pingtung, Taiwan, Republic of China

ABSTRACT.—An EtOH extract of *Neolitsea acutotrinervia* has yielded four known furanogermacranolides, linderane [**1**], (+)-lindera dine [**2**], zeylanane [**3**], and zeylanine [**4**]; one known germacranediolide, pseudoneoliacine [**6**]; and four new germacranediolides, acutotrine [**5**], zeylaninone [**7**], acutotrinone [**8**], and acutotrinol [**9**]. Structures of the compounds were determined on the basis of spectroscopic properties.

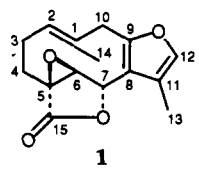
Investigation of the cytotoxic components from plants of the genus *Neolitsea* endemic in Taiwan, *Neolitsea parvigemma* (1), *Neolitsea buisanensis* (2), and *Neolitsea villosa* (3), has revealed numerous sesquiterpene lactones. In a continuation of this program, an EtOH extract obtained from the roots of *Neolitsea acutotrinervia* Kan. and Sas. (Lauraceae) by solvent partitioning and chromatographic purification has led to isolation of nine sesquiterpene lactones. Four of these were known furanogermacranolides, linderane [**1**], (+)-lindera dine [**2**], zeylanane [**3**], and zeylanine [**4**]; the remaining five compounds have been identified as germacranediolides, acutotrine [**5**], pseudoneoliacine [**6**], zeylaninone [**7**], acutotrinone [**8**], and acutotrinol [**9**]. Of the five germacranediolides, only compound **6** was reported previously in the literature.

DISCUSSION

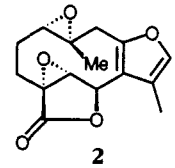
The identities of known compounds were verified by comparing their spectroscopic data with literature data for linderane (4–11), (+)-lindera dine (3), zeylanane (10–13), zeylanine (10,13,14), and pseudoneoliacine (3).

Each germacranediolide possesses two lactone functions, both or one of which is an α,β-unsaturated γ-lactone as revealed by ir and supported by ^{13}C nmr. Acutotrine [**5**] crystallized as plates from Me_2CO/*n*-hexane. Its molecular formular was established as $C_{15}H_{16}O_6$ by hrms, *m/z* 292.0941. The 10-membered macrocyclic moieties of both compounds **5** and **2** contain the same functionalities. It was found, however, that a singlet at δ 7.11 for H-12 in **2** disappeared and a double doublet at δ 5.22 for H-9 was observed in compound **5**. One proton at C-10 was seen as a double doublet at δ 2.79 and the other appeared as multiplet between δ 1.30 to 1.24 in compound **5**, apparently coupling to the adjacent proton, H-9. The above comparison along with the singlets at δ 155.03, 131.00, and 171.73 in the ^{13}C nmr clearly indicated that compound **5** contained an α,β-unsaturated γ-lactone group instead of a furan ring system as in compound **2** and thus established the structure of acutotrine as **5**.

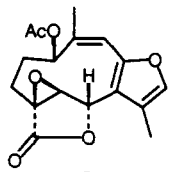
Compound **7** analyzed for $C_{17}H_{18}O_6$ from the mass of 318.1105 in the hrms. The structural relationship between compounds **7** and **4** is the same as that between compounds **5** and **2**. The same rationale used for establishing the structure of compound **5** is also used for this compound. The configuration of H-9 in compound **7** is uncertain but it is most probably in the β configuration from the observed coupling constant and examination of Dreiding model. The stereochemistry of the double bond at 1(10), was assigned the *Z* form by NOESY experiments in which strong nOe between Me-14 and H-10 was observed. Signals for the protons on C-3 and C-4 appeared as complicated patterns in three groupings. To avoid ambiguities and to differentiate these overlapping signals, selective spin decoupling experiments were conducted. Irradiation of the carbinolic proton (H-2) at δ 5.18 simplified the signals at δ 1.88–2.01 and 2.21–2.41.



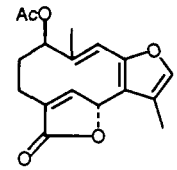
1



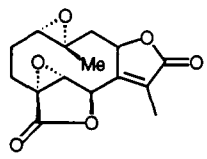
2



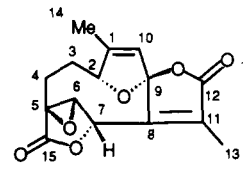
3



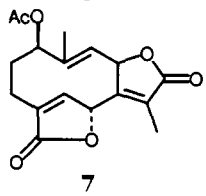
4



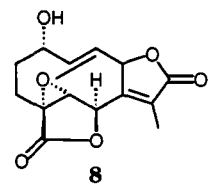
5



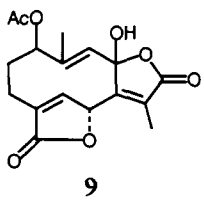
6



7



8



9

These results confirmed the assignments and further supported the structure of compound **7**.

The molecular formula, $C_{15}H_{16}O_6$, of compound **8** was determined from the observation of the $[M]^+$ ion at 292.0940 by hrms. The secondary hydroxy group at C-2 was indicated by ir absorption bands at ν max 3535 and 3497 cm^{-1} and supported by a doublet at δ 62.93 in the ^{13}C nmr. Two doublets at δ 4.85 and 4.81 were proved to be due to H-2 and the olefinic H-10, respectively, by selective spin decoupling and 2D HETCOR experiments. Based on the multiplicity, coupling constant, and study of Dreiding model OH-2 should possess the α configuration. The configuration of the 14-methyl group was not determined at this stage. The existence and configuration of the epoxy ring between C-5 and C-6 was deduced by comparing the corresponding spectroscopic data of compound **5**. The chemical shifts and coupling constants of H-9 (C-9) and H-10 (C-10) are very close to those of compound **7**. The above information and comparison of spectral data with other related compounds provided sufficient credible evidence to establish the structure **8** for this dilactone, acutotrinone.

Compound **9** crystallized as plates from Me_2CO/*n*-hexane. It had a mol wt of 334.1052 consistent with molecular formula $C_{17}H_{18}O_7$. The ^1H-nmr spectrum re-

TABLE 1. ^{1}H-nmr Spectral Data.[a]

Proton	Compound						
	2	**3**	**4**	**5**	**7**	**8**	**9**
H-2	2.79 (1H, d, J = 10.2)	5.31 (1H, dd, J = 11.0,6.0)	4.89 (1H, dd, J = 11.5,5.0)	3.00 (1H, dd, J = 11.2,2.0)	5.18 (1H, dd, J = 11.0,5.5)	4.85 (1H, d, J = 9.4)	5.89 (1H, dd, J = 10.3,5.0)
H-3	2.19 (1H,m)	2.05–2.33 (2H,m)	1.88 (1H,m)	2.15 (1H, ddd, J = 13.5,7.0, 2.0)	1.88–2.01 (1H,m)	2.43–2.36 (1H,m)	2.07–1.95 (1H,m)
H-3			2.20 (1H,m)	1.66–1.55 (1H,m)		1.74–1.90 (3H,m)	2.33–2.21 (1H,m)
	1.64 (2H,m)				2.21–2.41 (2H,m)		
H-4		2.54 (1H, dd, J = 14.0,6.0)	2.34 (1H, ddd, J = 12.5, 12.0,7.0)	1.77 (1H, ddd, J = 14.0, 13.0,7.0)			2.47 (1H, ddd, J = 14.5,8.5, 8.4)
H-4	2.79 (1H,m)	1.40 (1H, ddd, J = 14.0, 13.5,7.0)	2.68 (1H, dd, J = 12.5,7.0)	2.58 (1H, dd, J = 14.0,6.0)	2.63–2.80 (1H,m)		2.85–2.75 (1H,m)
H-6	4.18 (1H,s)	4.09 (1H,s)	7.10 (1H,s)	4.62 (1H,s)	6.85 (1H,s)	4.65 (1H,s)	6.89 (1H,br s)
H-7	5.37 (1H,s)	5.32 (1H,s)	5.70 (1H,s)	5.81 (1H,s)	5.94 (1H,s)	5.59 (1H,s)	5.94 (1H,t, J = 1.5)
H-9				5.22 (1H,dd, J = 12.0,4.0)	5.96 (1H,d, J = 10.0)	6.07 (1H,d, J = 10.0)	
H-10	3.47 (1H, d, J = 16.2) 2.53 (1H,d, J = 16.2)	6.34 (1H,s)	6.23 (1H,s)	2.79 (1H,dd, J = 13.5,4.0) 1.30–1.24 (1H,m)	5.01 (1H,d, J = 10.0)	4.81 (1H,d, J = 10.0)	5.24 (1H,d, J = 1.5)
H-12	7.11 (1H,s)	7.25 (1H,s)	7.22 (1H,s)				
H-13	1.99 (3H,s)	2.05 (3H,s)	2.11 (1H,s)	2.00 (3H,s)	2.03 (3H,d, J = 0.9)	1.97 (3H,s)	2.40 (3H,s)
H-14	1.04 (3H,s)	1.90 (3H,s)	1.88 (1H,s)	1.28 (3H,s)	1.78 (3H,d, J = 1.5)	1.74 (3H,d, J = 1.0)	1.78 (3H,d, J = 1.5)
Ac		1.91 (3H,s)	1.89 (3H,s)		2.01 (3H,s)		2.07 (3H,s)

[a]Compounds **5** and **8** were recorded in Me_2CO-d_6 solution, whereas the other compounds were in $CDCl_3$ solution. HETCOR 2D nmr spectra were recorded for compounds **3**, **4**, **5**, and **8**, and selective proton decoupling experiments were performed for compounds **3**, **4**, **5**, and **9** to confirm the assignments.

vealed one broad singlet at δ 6.89 for a vinyl proton (H-6) and a doublet at δ 5.24 for another one (H-10). The existence of two trisubstituted double bond systems was supported by signals at δ 130.27 (s, C-5), 149.33 (d, C-6), 129.27 (d, C-10), and 138.90 (s, C-1). A one-proton double doublet at δ 5.89 in the ^{1}H-nmr spectrum was assigned to H-2. The configuration of Me-14 was determined by the same arguments used for compound **7**. Resonances for H-3 and H-4 were assigned by comparing with those for corresponding protons in compound **4**. The singlet at δ 105.66 was assigned to the bioxygenated C-9 (15,16). The existence of a hydroxy group was demonstrated by the absorption band at 3450 cm^{-1}. Thus, the structure of compound **9**, named acutotrinol, was established.

Since the furan ring is vulnerable to oxidation, the possibility that the five germacranediolides are the oxidation products of other furanogermacranolides cannot be ruled out. The assumption was partially confirmed by analyzing the autooxidized products of **2**, from which compounds **5** and **8** along with villosine (3) were isolated. The presence of these oxidized products prompted a search for germacranediolides in the fresh plant. Only small amounts of germacranediolides were detected when the fresh extract was subjected to tlc analysis. However, quantities of these dilactones increased as the material was stored for a long period of time.

TABLE 2. ^{13}C-nmr spectral data.[a]

Carbon	Compound						
	2	**3**	**4**	**5**	**7**	**8**	**9**
C-1 s	58.04	141.97	139.80	57.62	137.42	145.83	138.90
C-2 d	62.42	69.25	70.46	64.09	68.73	62.93	70.24
C-3 t	21.79	25.79	24.50	22.26	21.94	24.43	23.26
C-4 t	23.43	20.45	20.94	23.30	20.79	underneath the signal for CD_3COCD_3	21.02
C-5 s	60.55	58.41	130.57	60.80	129.94	58.92	130.27
C-6 d	67.21	63.21	149.73	64.55	149.81	63.42	149.33
C-7 d	73.19	71.30	74.07	76.29	74.27	71.90	74.69
C-8 s	114.75	117.95	120.36	155.03	152.71	151.73	148.92
C-9	149.78 s	146.58 s	146.93 s	78.68 d	75.82 d	76.49 d	105.66 s
C-10	38.12 t	122.24 d	122.28 d	45.46 t	128.73 d	124.19 d	129.27 d
C-11 s	122.40	121.00	120.65	131.00	130.85	132.07	133.42
C-12	137.58 d	139.53 d	139.65 d	170.36 s	171.65 s	170.92 s	169.66 s
C-13 q	8.04	7.86	8.09	9.32	9.25	8.93	9.14
C-14 q	16.07	17.44	17.60	17.01	20.37	17.07	20.21
C-15 s	170.58	169.82	172.08	171.73	172.41	173.19	172.49
Ac q		20.69	20.86		17.30		17.84
Ac s		168.73	168.83		169.40		172.40

[a]Compounds **5** and **8** were recorded in Me_2CO-d_6 solution, whereas the other compounds were in $CDCl_3$ solution. HETCOR 2D nmr spectra were recorded for compounds **3**, **4**, **5**, and **8**, and selective proton decoupling experiments were performed for compounds **3**, **4**, **5**, and **9** to confirm the assignments.

EXPERIMENTAL

PLANT MATERIAL.—The roots of *N. acutotrinervia* were collected in Pingtung, Taiwan in 1989. A voucher specimen is deposited in the Herbarium, Department of Forest Resources Management and Technology, National Pingtung Polytechnic Institute. Whole roots were cut into pieces, air-dried, and ground to a coarse powder.

METHODOLOGY.—Melting points were determined on a Yamamoto melting point apparatus model J-2 and were not corrected. Ir were taken as KBr pellets or in $CHCl_3$ solution on a Perkin-Elmer 882 IR spectrometer. Ms spectra were obtained on a VG 70-25 gc-ms in the electron impact mode at 70 eV. ^{1}H-nmr, ^{13}C-nmr, and 2D nmr spectra were taken on a Varian VXR-300 (300 MHz) using TMS as internal standard, and the chemical shifts are expressed in δ (ppm). Cc was carried out on Si gel 60 (Merck, 70–270 mesh). Tlc was performed by using Merck precoated plates and detection of compounds was done by spraying with *p*-anisaldehyde reagent. All solvents used for chromatography were hplc grade (Fisher).

EXTRACTION AND FRACTIONATION.—The powdered root (3.2 kg) was extracted with 95% EtOH (18.0 liters) to obtain 81.2 g of dark brown extract. The extract was partitioned between $CHCl_3$ and H_2O to obtain $CHCl_3$ solubles (52.6 g) and H_2O solubles.

The $CHCl_3$ solubles (32 g) were chromatographed on Si gel (1.10 kg). Elution of the column was started with 20% EtOAc in *n*-hexane, and a gradient of EtOAc was added up to 100%, which was followed by eluting with 100% Me_2CO. Fractions of 400 ml were collected and pooled into 11 fractions (I–XI) based on tlc analysis.

Linderane [**1**].—Fraction II (3.82 g) contained **1** and β-sitosterol with R_f values 0.71 and 0.64, respectively. This fraction was chromatographed on Si gel 60 (120.0 g) using 10% EtOAc in *n*-hexane as the eluting solvent. Fraction 2 (2.7 g) was further chromatographed using same adsorbent and eluting system to yield **1**, colorless plates from EtOAc/*n*-hexane, mp 180–182°; ir (KBr) ν max 3125, 3060, and 1550 (furan), 1770 and 1325 (γ-lactone), 1610 (double bond), 1460, 1380 cm^{-1}; eims *m/z* (rel. abundance) $[M]^+$ 260 (21.0), 159 (62.1), 145 (91.2), 91 (base peak).

(+)-Linderadine [**2**].—Fraction V (1.7 g) contained two major substances which correspond to compounds **2** and **3** with R_f 0.51 and 0.47 (40% EtOAc in *n*-hexane), respectively. This fraction was chromatographed on Si gel 60 (70 g) using 20% EtOAc in *n*-hexane as the eluting solvent. Compound **2** was crystallized as plates from EtOAc and *n*-hexane, mp 139–141°; ir (KBr) ν max 3140, 3060, and 1620 (furan), 1565, 1470, 1380, 1120, 920, 820 cm^{-1}; eims *m/z* (rel. abundance) $[M]^+$ 276 (49.0), 219 (42.3), 161 (46.2), 145 (46.6), 135 (33.0), 105 (30.0), 91 (base peak).

Zeylanane [**3**].—Fraction 3 from the Si gel column of fraction V was rechromatographed to obtain zeylanane [**3**]: needles from EtOAc/*n*-hexane; mp 177–179°; ir (KBr) ν max 3126, 3068, 1614, and 1559 (furan), 1657, 840, and 804 (trisubstituted double bond), 1775 (γ-lactone), 1749 and 1228 (acetate), 1449, 1385, 1375 cm^{-1}; eims *m/z* (rel. abundance) $[M]^+$ 318 (49.4), 276 (29.6), 189 (46.0), 175 (60.5), 159 (53.7), 147 (base peak).

Zeylanine [**4**].—Fraction VII (3.2 g) showed a major spot on tlc with R_f 0.45 (40% EtOAc in *n*-hexane) which was separated by cc on Si gel 60 (130 g) developing with 30% EtOAc in *n*-hexane. Colorless needles were obtained: mp 170–172°; ir (KBr) ν max 1751, 1740, 1240, 1647, 1540, 890 cm^{-1}; eims *m/z* (rel. abundance) $[M]^+$ 302 (29.0), 242 (43.4), 215 (30.0), 185 (base peak), 119 (89.4).

Acutotrine [**5**].—Fraction IX gave 1.30 g of residue which on cc with 30% EtOAc in *n*-hexane gave fractions IX-1, IX-2, and IX-3. Repeated cc of IX-2 (0.42 g) using the above conditions gave **5** (21.0 mg): mp 240–242°; ir (KBr) ν max 1777, 1764, 1752, 1671, 1444, 1346, 1188, 1121 cm^{-1}; hrms found 292.0941, calcd 292.0947.

Pseudoneoliacine [**6**].—Fraction X was evaporated in vacuo to a dark brown residue (3.84 g). Tlc analysis indicated the presence of at least four compounds. This fraction was chromatographed over Si gel 60 (150 g), using 40% EtOAc in *n*-hexane as the eluting solvent to separate it into four fractions: X-1, X-2, X-3, and X-4.

Fraction X-1 was rechromatographed on a Si gel column eluted with 15% iPrOH in *n*-hexane to obtain **6** (15.0 mg): mp 270–272°; ir (KBr) ν max 3085, 1774, 1770, 1659, 1540, 1140 cm^{-1}; eims *m/z* (rel abundance) $[M]^+$ 290 (18.0), 246 (51.2), 218 (42.0), 146 (18.0), 133 (base peak), 120 (30.0), 105 (6.0), 91 (30.4).

Zeylaninone [**7**].—Fraction X-4 (0.6 g) was mixed with celite (2.0 g) and rechromatographed on Si gel 60 (18.0 g) using 35% EtOAc in *n*-hexane as eluting solvent system. Fraction 2 was rechromatographed repeatedly to obtain **7** (42.0 mg): plates; mp 206–208°; ir (KBr) ν max 1765, 1718, 1654, 1560, 1448, 1373, 1292, 1246, 849, 791 cm^{-1}; hrms found 318.1105, calcd 318.1103.

Acutotrinone [**8**].—Fraction XI (1.5 g) contained two major spots on tlc and was chromatographed on Si gel 60 (100.0 g) developed with EtOAc–*n*-hexane (1:1) to give fractions XI-I, XI-2 and X-3. Rechromatography of XI-2 gave **8**, colorless plates from Me_2CO/*n*-hexane, mp 212–214°; ir (KBr) ν max 3497, 3535, 3062, 1763, 1754, 1444, 1345, 1250, 1188, 1121, 1054 cm^{-1}; hrms found 292.0940, calcd 292.0947.

Acutotrinol [**9**].—Fraction XI-3 was rechromatographed on Si gel eluted with 50% EtOAc in *n*-

hexane to afford **9**, colorless plates from Me_2CO/*n*-hexane, mp 232–234°; ir (KBr) ν max 3450, 1775, 1720, 1650, 1550 cm^{-1}; eims *m/z* (rel abundance) $[M]^+$ 334 (11.3), 292 (22.8), 274 (base peak), 246 (29.8), 228 (30.5), 133 (4.60), 105 (34.8), 91 (57.1); hrms found 334.1052, calcd 334.1053.

AIR-OXIDATION OF (+)-LINDERADINE [**2**].—The oxidation followed the procedures described by Wu and Li (2). (+)-Linderadine (1.8 g) was dissolved in $CHCl_3$ (200 ml) to which a few drops of glacial HOAc was added. The mixture was left for 2 weeks at room temperature. After removal of solvent under reduced pressure, the residue (2.4 g) was chromatographed over Si gel (150 g) using *n*-hexane–EtOAc (1:1) as eluting solvent system to fractionate into 5 fractions (I–V). Compounds **5** and **8** and villosine (3) were separated from fractions III, V, and IV, respectively. These oxidation products were identical in mp and ^1H nmr to those isolated previously.

ACKNOWLEDGMENTS

The author gratefully acknowledges the National Science Council, Republic of China, for financial support of this research under grant NSC-79-0409-B020-13.

LITERATURE CITED

1. W.S. Li and J.D. McChesney, *J. Nat. Prod.*, **53**, 1581 (1990).
2. S.L. Wu and W.S. Li, *Phytochemistry*, **30**, 4160 (1991).
3. W.S. Li, *Phytochemistry*, **31**, in press.
4. K. Takeda, *Pure Appl. Chem.*, **21**, 181 (1970).
5. K. Takeda, I. Horibe, M. Teraoka, and H. Minato, *Chem. Commun.*, 637 (1968).
6. K. Takeda, I. Horibe, M. Teraoka, and H. Minato, *J. Chem. Soc. C*, 1491 (1969).
7. K. Tori, M. Ueyama, I. Horibe, Y. Tamura, and K. Takeda, *Tetrahedron Lett.*, 4583 (1975).
8. K. Takeda, H. Minato, and I. Horibe, *Tetrahedron*, 2307 (1963).
9. Y. Tomita, A. Uomari, and H. Minato, *Phytochemistry*, **8**, 2249 (1969).
10. K. Taketa, I. Horibe, M. Teraoka, and H. Minato, *J. Chem. Soc.*, 973 (1970).
11. B.S. Joshi, V.N. Kamat, and T.R. Govindachari, *Tetrahedron*, **23**, 261 (1966).
12. K. Taketa, I. Horibe, and H. Minato, *Chem. Commun.*, 1168 (1968).
13. K. Tori, M. Ohtsuru, I. Horibe, and K. Takeda, *Chem. Commun.*, 943 (1968).
14. B.S. Joshi, V.N. Kamat, and T.R. Govindachari, *Tetrahedron*, **23**, 273 (1967).
15. N. Goren, A. Ulubelen, J. Jakupovic, F. Bohlmann, and M. Grenz, *Phytochemistry*, **23**, 2281 (1984).
16. A. Ulubelen, N. Goren, F. Bohlmann, J. Jakupovic, M. Grenz, and N. Tanker, *Phytochemistry*, **24**, 1305 (1985).

Received 23 April 1992