

New Eudesmane Sesquiterpenes from the Root of *Lindera strychnifolia*

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Received March 29, 2000

Strychnistenolide (**1**) and its acetate **2** were isolated from the root of *Lindera strychnifolia*, along with a novel rearranged type of secoeudesmane, strychnilactone (**3**). Their structures were elucidated by extensive analysis of their NMR spectra, including 2D NMR techniques, together with an X-ray analysis for **3**. Strychnistenolide exists as a single stereoisomer in CHCl_3 , but in pyridine is epimerized.

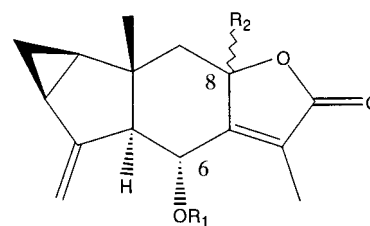
The root of *Lindera strychnifolia* (Sieb. & Zucc.) f. Villars (Lauraceae) has a strong fragrance and is used in Chinese folk medicine as a palliative and an antispasmodic. In previous studies on the constituents from this plant, about 20 eudesmanes and elemanes were isolated and structurally elucidated by Takeda et al.^{1–3} Further studies on the chemical constituents of this species led to the isolation of bisesquiterpene⁴ and rearranged secoeudesmanolide.⁵ This paper describes the isolation and structure determination of two new eudesmane sesquiterpenes, **1** and **2**, and a rearranged secoeudesmanolide, **3**.

Results and Discussion

Compound **1** displayed a molecular ion peak at m/z 262 in MS and 15 carbon signals in its ^{13}C NMR spectrum. These, together with elemental analysis, established the molecular formula of $\text{C}_{15}\text{H}_{18}\text{O}_4$.

The ^1H NMR spectrum in CDCl_3 (see Experimental Section) of compound **1** indicated the presence of a vinylidene group at δ_{H} 5.14 and 5.11, an olefinic methyl group at δ_{H} 1.92, a tertiary methyl group at δ_{H} 0.52, and a methine group having an oxygen function at δ_{H} 4.42. However, the ^1H and ^{13}C NMR spectra including 2D NMR spectra, when taken in $\text{C}_5\text{D}_5\text{N}$ (Table 1), displayed duplicate signals. These spectra revealed that in $\text{C}_5\text{D}_5\text{N}$ solution **1** is an isomeric mixture of **1A** and **1B** in a ratio of approximately 6:7. Therefore, each of the signals of the two isomers was assigned individually by interpretation of the 2D NMR spectra (COSY, HSQC, and HMBC). The ^1H and ^{13}C NMR spectra along with the interpretation of COSY, HSQC, and HMBC spectra indicated the presence of a cyclopropane ring moiety and a vinylidene group adjacent to the cyclopropane ring. The HMBC spectrum showed connectivities between the C-14 methyl carbon signal and H-1 proton signal of the cyclopropane ring.

The ^{13}C NMR spectrum showed two lactone carbonyl carbon signals at δ_{C} 171.7 (**1A**) and δ_{C} 171.1 (**1B**) and two acetal carbon signals at δ_{C} 105.7 (**1A**) and δ_{C} 105.9 (**1B**). In the HMBC spectrum, the signals at δ_{H} 4.82 (d, $J = 10.5$ Hz, H-6, **1A**) and δ_{H} 5.19 (d, $J = 1.3, 11.7$ Hz, H-6', **1B**) show long-range correlations with the carbon signals of C-4 (δ_{C} 150.3 (**1A**), δ_{C} 147.4 (**1B**)) and C-11 (δ_{C} 36.9 (**1A**), δ_{C} 38.4 (**1B**)), respectively. The downfield shifts of position 6 (δ_{H} 4.82, **1A**; δ_{H} 5.19, **1B**) revealed the presence of a hydroxyl group at C-6. When compared with previously isolated sesquiterpenoids from this plant, these data indicated that **1** is very similar to linderene or hydroxyl-



1A; R_1 : H, R_2 : α -OH; **2A** R_1 : COCH_3 , R_2 : α -OH

1B; R_1 : H, R_2 : β -OH; **2B** R_1 : COCH_3 , R_2 : β -OH

Figure 1. Structure of compounds **1A**, **1B**, **2A**, and **2B**.

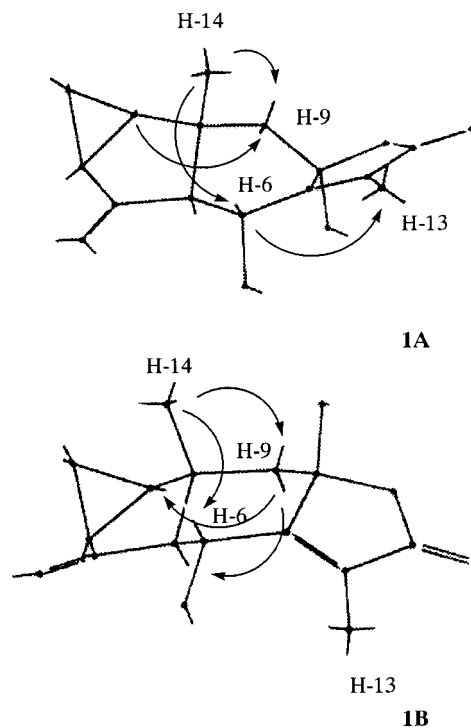


Figure 2. Stereostructure and NOE correlation of compound(s) **1A** and **1B** generated by the CAChe system.

indestenolide,⁶ which were previously isolated from *L. strychnifolia*.

The H–H coupling constants between H-5 and H-6 were $J = 10.5$ Hz (**1A**) and $J = 11.7$ Hz (**1B**), respectively, showing the *trans* axial–axial relationship of these protons. The H-6 proton signal showed a cross-peak with H-14 (δ_{H} 0.61, **1A**; δ_{H} 1.23, **1B**) in the NOESY spectrum (Figure 2), indicating the same orientation of H-6 and the C-14 methyl group. Furthermore, as can be seen in Figure 2, NOE

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Table 1. ^{13}C and ^1H NMR Spectral Data of Compounds **1A** and **1B** ($\text{C}_5\text{D}_5\text{N}$) and **2A** and **2B** (CDCl_3) (^{13}C , 125 MHz; ^1H , 500 MHz)

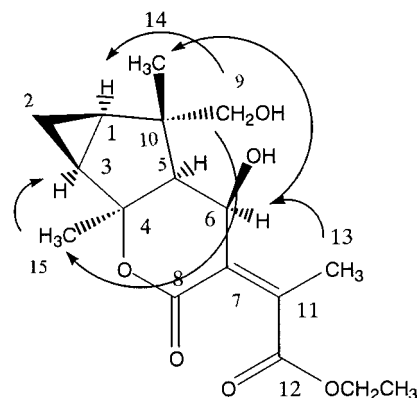
position	1A		2A		1B		2B	
	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H
1	28.8	1.33 (dt, $J = 3.5$, 7.5 Hz)	29.2	1.41 (dt, $J = 3.5$, 7.5 Hz)	27.4	1.30 (dt, $J = 3.5$, 7.5 Hz)	27.9	1.31 (dt, $J = 3.5$, 7.5 Hz)
2a	15.9	0.78 (m)	16.8	0.83 (m)	15.7	1.08 (dt, $J = 3.5$, 7.5 Hz)	16.7	0.82 (m)
2b				0.77 (dt, $J = 3.5$, 5.4 Hz)		0.79 (m)		0.77 (dt, $J = 3.5$, 5.4 Hz)
3	23.1	2.01 (m)	23.6	2.03 (m)	22.7	2.01 (m)	23.5	1.96 (m)
4	150.3		148.6		148.6		147.4	
5	61.9	4.12 (d, $J = 10.5$ Hz)	59.6	3.68 (t, $J = 2.5$, 10.9 Hz)	69.9	3.00 (dt, $J = 2.0$, 11.7 Hz)	67.2	2.86 (d, $J = 12.4$ Hz)
6	61.7	4.82 (d, $J = 10.5$ Hz)	63.4	5.70 (d, $J = 10.9$ Hz)	67.3	5.19 (dd, $J = 1.3$, 11.7 Hz)	69.7	5.61 (d, $J = 12.4$ Hz)
7	159.7		154.0		163.3		157.6	
8	105.7		105.4		105.7		105.9	
9a	49.9	2.43 (d, $J = 14.0$ Hz)	49.9	2.56 (d, $J = 14.2$ Hz)	47.3	2.74 (d, $J = 13.0$ Hz)	47.5	2.60 (d, $J = 13.9$ Hz)
9b		2.83 (d, $J = 14.0$ Hz)		2.28 (d, $J = 14.2$ Hz)		1.97 (d, $J = 13.0$ Hz)		1.85 (d, $J = 13.9$ Hz)
10	36.9		37.7		37.2		38.4	
11	125.5		130.6		121.2		122.5	
12	171.7		170.7		171.9		171.1	
13	7.3	1.94 (s)	9.2	1.94 (s)	8.4	2.38 (d, $J = 1.3$ Hz)	8.3	1.84 (s)
14	20.7	0.61 (s)	21.5	0.58 (s)	17.4	1.23 (d, $J = 1.8$ Hz)	17.9	0.99 (s)
15a	106.7	5.66 (d, $J = 1.8$ Hz)	107.5	5.04 (br s)	108.4	5.81 (d, $J = 1.8$ Hz)	108.9	5.07 (d, $J = 1.6$ Hz)
15b		5.27 (d, $J = 1.8$ Hz)		4.77 (br s)		5.28 (d, $J = 1.8$ Hz)		4.80 (d, $J = 1.6$ Hz)
-CO			171.9				171.9	
-OAc			21.0	2.10 (s)			20.8	2.20 (s)

correlations between H-9a and H-14, H-9b and H-5, and H-1 and H-9b were observed, suggesting the cyclopropane ring is in the same orientation as the C-14 methyl group. From these data, it was concluded that, in $\text{C}_5\text{D}_5\text{N}$ solution, **1** is a mixture of the 8α -OH and 8β -OH epimers at the C-8 hemiketal position of eudesmanolide.

A NOE correlation was found between H-6 and the H₃-13 methyl signals only in the case of **1A** in the NOESY of **1**. The stereostructure generated by the CAChe system⁷ and the Dreiding model showed a proximity between H-6 and the H₃-13 methyl group only in **1A**, as shown in Figure 2. In addition, the H₃-14 methyl signal at δ_{H} 1.23 of **1B** was considerably deshielded relative to that of **1A** at δ_{H} 0.61 due to the anisotropic effect of the 8β -OH oxygen atom, supporting the *anti*-relationship of the C-14 methyl group and the 8-OH in the case of **1A** and the *syn*-structure for **1B**.

On the basis of these data, it was concluded that compound **1** in CDCl_3 should be the *anti*-structure with respect to the C-14 methyl group and the 8-OH, since the chemical shift of H₃-14 at δ_{H} 0.52 was more similar to that of **1A** (δ_{H} 0.61) than that of **1B** (δ_{H} 1.23). Compound **1** was given the trivial name strychnistenolide. **1A** was named strychnistenolide A, and **1B** was named strychnistenolide B.

Compound **2** was obtained as colorless powder. EIMS showed a molecular ion peak at m/z 304 [M^+], 42 mu more than that of **1**. The ^1H and ^{13}C NMR spectra (Table 1) in CDCl_3 strongly resembled those of **1A** and **1B** in $\text{C}_5\text{D}_5\text{N}$, except for the acetyl methyl signals at δ_{H} 2.10 (3H, s) (**2A**) and δ_{H} 2.20 (3H, s) (**2B**). The position of the acetate at C-6 was supported by the downfield shifts of the H-6 proton signal at δ_{H} 5.70 (**2A**) and δ_{H} 5.61 (**2B**) in the ^1H NMR spectrum of **2**. Thus, **2** was determined to be the 8-OH epimeric mixture of 6-*O*-acetyl strychnistenolide, as shown in Figure 1. All signals in the ^1H and ^{13}C NMR spectra of a mixture of **2** (**2A** and **2B**) were assigned by analysis of the individual ^1H - ^1H COSY, HSQC, and HMBC spectra. The assignments are shown in Table 1. As was the case of **1**, the most downfield H₃-14 methyl signal at δ_{H} 0.99 was assigned to **2B** and the H₃-14 methyl signal at δ_{H} 0.58 to **2A**. The strong similarity of the ^1H and ^{13}C NMR spectra

**Figure 3.** Structure of compound **3** and NOE correlations.

for **2** and **1** led us to determine the stereochemistry of **2A** and **2B** as shown in Figure 1.

Compound **3** was obtained as colorless rods, mp 181–182 °C. EIMS showed a molecular ion peak at m/z 324 [M^+], and the ^{13}C NMR spectrum showed 17 carbon signals. These data, together with elemental analysis, established the molecular formula to be $\text{C}_{17}\text{H}_{24}\text{O}_6$. The ^1H NMR spectrum of **3** shows three CH_3 signals at δ_{H} 1.31, 1.66, and 2.02 and signals for an ethoxy group at δ_{H} 1.26 (3H), 4.22 (1H), and 4.17 (1H). The ^1H - ^1H COSY confirmed the presence of an oxygenated methylene group (H₂-9) and a cyclopropane ring (H-1, -2, and -3). The presence of an ester and lactone groups was supported by ^{13}C NMR signals at δ_{C} 167.1 and 169.5 and the absorptions at ν_{max} 1751 and 1641 cm^{-1} in the IR spectrum.

Extensive analyses of ^1H - ^1H COSY, HSQC, and HMBC spectra of **3** established the structure shown in Figure 3. In the HMBC spectrum, no connectivity was observed between the H-9 methylene group and the C-8 carbonyl carbon, indicating that C-4 and C-8 are joined by a lactone ring. This was confirmed by the proton chemical shifts of the H-9 methylene group at δ_{H} 3.51 and 3.48. The NOE correlations in the NOESY spectrum of **3**, H-1 to H₂-9, H-6 to H₃-14, H₃-13 to H-6, H₂-9 to H₃-15, and H₃-15 to H-3 as shown in Figure 3, confirmed the stereostructure, except for the configuration at C-4 and C-6.

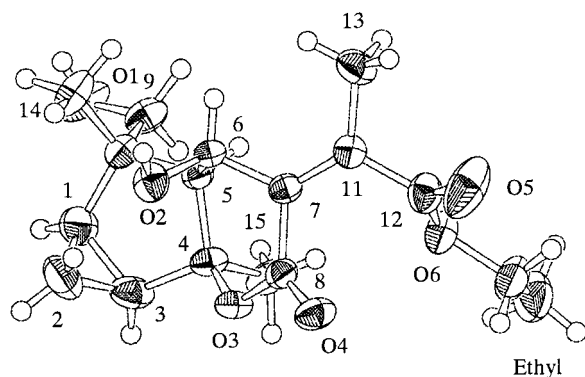


Figure 4. ORTEP drawing of compound **3**.

The structure of **3** was established by a single-crystal X-ray analysis, which also revealed the configuration of C-4. A single crystal suitable for X-ray analysis with a space group $P2_12_12_1$ ($Z = 4$) was obtained by recrystallization from CHCl_3 . After the data collection was performed on a RIGAKU AFC 7S diffractometer, the structure was solved by direct methods (SIR92),⁸ then refined by full-matrix least-squares based 1952 observed reflections ($I > 3.00\sigma(I)$). Hydrogen atoms were refined isotropically, and nonhydrogen atoms were treated anisotropically. The final refinement converged at $R = 0.032$. This led to the suggested structure (Figure 3) including the configuration of 6β -OH. The ORTEP drawing of **3**, named strychnilactone, is shown in Figure 4.

More than 10 secoeudesmane-type sesquiterpenes have been identified so far, but **3** is unique and represents the first example of a secoeudesmanolide in which the C-8, C-9 bond has been cleaved with rearrangement to form a C-8, C-4 δ -lactone.

Experimental Section

General Experimental Procedures. Optical rotation was recorded with a JASCO DIP-370 polarimeter. ^1H and ^{13}C NMR spectra were measured on a Varian Gemini 300 spectrometer operating at 300 MHz for ^1H and 75 MHz for ^{13}C for compound **1** in CDCl_3 and a Varian UNITY plus 500 spectrometer for other compounds, operating at 500 MHz for ^1H and 125 MHz for ^{13}C , including NOESY, and ^1H - ^1H COSY, HSQC, and HMBC with field gradient technique in CDCl_3 or $\text{C}_5\text{D}_5\text{N}$. Chemical shifts were given on a δ (ppm) scale with TMS as an internal standard. EIMS were measured on a JEOL JMS-DMX-303 mass spectrometer. Column chromatography was carried out by using silica gel 60 (Merck, Art. 7734 and Art. 9385). MPLC was performed on Kusano C.I.G. prepacked Si-5 column (Kusano Kagakukikai Co., 22 mm i.d. \times 100 mm).

Plant Material. The roots of *L. strychnifolia* were collected in the autumn of 1995 at the Botanical Garden, Nagasaki University, and identified by Dr. T. Ikenaga (former Assoc. Prof. of Botanical Garden). A voucher specimen (identification number NAP 20-06-95/2) was deposited at the Laboratory of Botanical Garden belonging to the Faculty of Pharmaceutical Sciences, Nagasaki University.

Extraction and Isolation. The roots (2.9 kg) of *L. strychnifolia* were extracted three times with Et_2O , followed by three times with MeOH. The Et_2O extract was evaporated in vacuo to give a residue (53.7 g). The Et_2O -soluble (33.3 g) and EtOAc-soluble (0.37 g) portions of the MeOH extracts were combined with the Et_2O extract. The combined residual part was suspended in C_6H_6 to give a supernatant, which was evaporated in vacuo to give a residue (42.2 g). This residue was chromatographed on silica gel, and the fractions eluted with C_6H_6 -MeOH (19:1) were further separated by silica gel with *n*-hexanes-EtOAc (19:1) and MPLC with CHCl_3 -*n*-hexane (3:2) successively to afford compounds **1** (416.6 mg), **2** (100.5 mg), and **3** (17.1 mg), along with known sesquiterpenes that were

identified by comparison of their spectroscopic data with those in the literature.

Strychnistenolide (1): colorless needles (*n*-hexanes-EtOAc), mp 185–186 °C; $[\alpha]_D^{20} +36.3^\circ$ (c 0.3, MeOH); IR (CHCl_3) ν_{max} 3394, 1752, 1600 cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz) δ 0.52 (s, H-14), 0.76 (m, H-2a), 0.83 (m, H-2b), 1.40 (m, H-1), 1.92 (s, H-13), 2.05 (m, H-3), 2.21 (d, $J = 14.0$ Hz, H-9a), 2.50 (d, $J = 14.0$ Hz, H-9b), 3.41 (d, $J = 10.6$ Hz, H-5), 4.42 (d, $J = 10.6$ Hz, H-6), 5.11 (br s, H-15a), and 5.14 (br s, H-15b); ^{13}C NMR (CDCl_3 , 75 MHz) δ 8.5 (C-13), 16.8 (C-2), 21.6 (C-14), 23.8 (C-3), 29.2 (C-1), 37.8 (C-10), 49.5 (C-9), 62.7 (C-6), 62.8 (C-5), 105.9 (C-8), 107.7 (C-15), 128.5 (C-11), 149.6 (C-4), 157.9 (C-7), 172.8 (C-12); EIMS m/z 262 [M^+], 244 [$\text{M}-\text{H}_2\text{O}^+$]; anal. C 68.82%, H 6.86%, calcd for $\text{C}_{15}\text{H}_{18}\text{O}_4$, C 68.68%, H 6.92%.

Strychnistenolide 6-O-acetate (2): colorless amorphous powder (*n*-hexanes-EtOAc); ^1H and ^{13}C NMR (CDCl_3 , 500 MHz), (see Table 1); EIMS m/z 304 [M^+], 286 [$\text{M}-\text{H}_2\text{O}^+$].

Strychnilactone (3): colorless rods (CHCl_3 -MeOH); mp 181–182 °C; $[\alpha]_D^{20} -267.6^\circ$ (c 0.2, MeOH); IR (KBr) ν_{max} 3357, 1751, 1641 cm^{-1} ; ^1H NMR (CDCl_3 , 500 MHz) δ 0.51 (dt, $J = 5.5, 8.4$ Hz, H-2a), 1.25 (m, H-1), 1.26 (t, $J = 7.1$ Hz, $-\text{COOCH}_2\text{CH}_3$), 1.31 (s, H-14), 1.49 (ddd, $J = 3.7, 6.1, 8.4$ Hz, H-3), 1.58 (m, H-2b), 1.66 (s, H-15), 2.02 (s, H-13), 2.10 (d, H-5), 3.48 (d, $J = 10.3$ Hz, H-9a), 3.51 (d, $J = 10.3$ Hz, H-9b), 4.17 (dq, $J = 7.1, 10.8$ Hz, $-\text{COOCH}_2\text{CH}_3$), 4.22 (dq, $J = 7.1, 10.8$ Hz, $-\text{COOCH}_2\text{CH}_3$), 4.68 (d, $J = 4.1$ Hz, H-6); ^{13}C NMR (CDCl_3 , 125 MHz) δ 5.1 (C-2), 13.6 ($-\text{COOCH}_2\text{CH}_3$), 15.9 (C-13), 18.3 (C-14), 27.1 (C-3), 27.2 (C-1), 32.9 (C-15), 49.3 (C-10), 53.6 (C-5), 61.8 ($-\text{COOCH}_2\text{CH}_3$), 65.4 (C-6), 73.4 (C-9), 92.7 (C-4), 133.4 (C-11), 134.8 (C-7), 167.1 (C-8), 169.5 (C-12); EIMS m/z 324 [M^+]; anal. C 63.29%, H 7.29%, calcd for $\text{C}_{17}\text{H}_{24}\text{O}_6$, C 62.95%, H 7.46%.

X-ray Crystal Structure Analysis of Strychnilactone (3). Crystal data: $\text{C}_{17}\text{H}_{24}\text{O}_6$, MW = 324.37; orthorhombic space group $P2_12_12_1$; $a = 14.912(2)$ Å, $b = 15.524(2)$ Å, $c = 7.368(2)$ Å, $V = 1705.5(6)$ Å³, $Z = 4$, $D_{\text{calc}} = 1.263$ g/cm³, Mo K α ($\lambda = 0.71069$ Å). The reflection data were collected on a Rigaku RASA 7S autodiffractometer using graphite-monochromated Mo K α radiation with the ω - 2θ scan technique to a maximum θ of 70° at room temperature (23 °C), independent reflections 2275, observed number of reflections 1925 [$F > 3.0\sigma(F)$]. The structure was solved by the direct method using SIR92. All atomic parameters, with anisotropic temperature factors for non-hydrogen atoms and isotropic ones for hydrogen atoms, were refined by the full-matrix least-squares method.

Acknowledgment. We express our appreciation to Mr. K. Inada, Mr. N. Yamaguchi, and Mr. M. Ohwatari of the Center for Instrumental Analysis of Nagasaki University for performing NMR, EIMS, and elemental analysis experiments, respectively, and Ms. J. Nagaoka of Nagasaki University for X-ray measurements.

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