Constituents of *Persea japonica*

Chao-Chiun Wang

Department of Medical Technology, China Junior College of Medical Technology, Tainan Hsien, Taiwan, Republic of China

Chang-Sheng Kuoh[†] and Tian-Shung Wu*[,‡]

Department of Biology and Department of Chemistry, National Cheng Kung University, Tainan, Taiwan, Republic of China

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A new sesquiterpene, machikusanol (**1**), together with γ-eudesmol, carissone, γ-selinene, isoboldine, corytuberine, (+)-*epi*-syringaresinol, β-sitosterol, stigmasterol, β-sitosteryl glucoside, and stigmasteryl glucoside, was isolated from the xylem of *Persea japonica*. The structure of **1** was elucidated by spectral analysis.

Persea japonica Sieb. (*Machilus kusanoi* Hayata) (Lauraceae) is a large evergreen tree that grows in Japan, South Korea, and Taiwan. In Taiwan, the plant occurs in primary forests up to 2300 m in altitude.[1] It is apparently not used in traditional medicine. Lu et al. reported the isolation of two alkaloids, L-(−)-*N*-norarmepavine and *dl*-coclaurine from the wood of this plant.[2,3] We describe here the isolation and structure elucidation of a new sesquiterpene (**1**) and eight known compounds from the xylem of *P. japonica*.

Separation of the MeOH extract of the air-dried xylem of *P. japonica* was carried out as described in the Experimental Section to afford nine compounds. The known compounds isolated in this study were the sesquiterpenes γ-eudesmol (**2**),[4,5] carissone (**3**),[6,7] and γ-selinene (**4**),[8,9] the alkaloids isoboldine[10] and corytuberine,[10] the lignan (+)-*epi*-syringaresinol,[11,12] and a mixture of the sterols stigmasterol and β-sitosterol, and of the sterol glucosides stigmasteryl glucoside and β-sitosteryl glucoside. These known compounds were identified and characterized from their spectroscopic data.

Machikusanol (**1**) was isolated as optically active, colorless crystals and was assigned as a sesquiterpenoid from its HREIMS [M^+, *m/z* 238.1935 ($C_{15}H_{26}O_2$)] and the occurrence of 15 carbon signals in the ^{13}C-NMR spectrum (Table 1). The presence of one secondary and one tertiary hydroxyl group and a double bond in the molecule was inferred from the IR absorption bands at 3400 and 1620 cm^{-1}, coupled with the observation of signals at δ 7.86 (1H, s, exchangeable D_2O) and δ 4.22 (1H, br s) in the ^{1}H-NMR spectrum. Two oxygen-bearing signals at δ 84.0 (d) and 72.7 (s) and two singlet olefinic carbons at δ 120.5 and 145.3 in the ^{13}C-NMR spectrum, together with the MS fragmentation peaks at *m/z* 238 $[M]^+$, 220 $[M - H_2O]^+$, 218 $[M - H_2O - H_2]^+$, and 203 $[M - H_2O - OH]^+$, also supported the presence of these functional groups. Two methyl signals at δ 26.7 (q) and 27.3 (q) and a singlet at δ 72.7 in the ^{13}C-NMR spectrum and two methyl signals at δ 1.20 and 1.21 (each 3H, s) in the ^{1}H-NMR spectrum, as well as fragment peaks at *m/z* 179 $[M - C_3H_7O]^+$, 177 $[M - C_3H_7O - H_2]^+$, and 59 $[C_3H_7O]^+$ (base peak) in the EIMS, suggested the presence of a 2-propanol moiety in the molecule of **1**. In addition, the ^{1}H-NMR spectrum of **1** showed an olefinic methyl and a tertiary methyl signal at δ 1.78 (3H, s) and 1.01 (3H, s), respectively. A combination of ^{1}H−^{1}H, ^{13}C−^{1}H COSY, and HMBC (Figure 1) NMR experiments indicated the presence of two partial structures, namely, >C(CH_3)CH_2CH_2CH(OH)C(CH_3)=C< and >C(CH_3)CH_2CH_2CH[C(CH_3)$_2$OH]-CH_2− in **1**. The stereochemistry of machikusanol (**1**) was established by a NOESY NMR experiment, in which the major interactions are shown in Figure 2. Compound **1** was oxidized with pyridinium chlorochromate to give **3**, which was identified by HPLC and by its ^{1}H- and ^{13}C-NMR spectral data. Therefore, the configuration of the C-10 methyl group and the 2-propanol moiety at C-7 of **1** were both inferred as being in the β configuration according to the known stereochemistry of **3**.[7] The configuration of the hydroxyl group at

Table 1. ^{13}C NMR Data of Compounds **1**−**4**[a]

	compound			
carbon	**1**	**2**	**3**	**4**
1	34.0 t	40.0 t	37.3 t	40.2 t
2	22.2 t	18.9 t	33.8 t	19.1 t
3	84.0 d	33.0 t	199.2 s	33.1 t
4	120.5 s	124.0 s	128.8 s	150.7 s
5	145.3 s	134.7 s	162.7 s	46.8 d
6	26.7 t	26.2 t	28.7 t	27.6 t
7	50.1 d	50.2 d	49.6 d	134.8 s
8	23.0 t	23.1 t	22.6 t	30.7 t
9	42.1 t	42.1 t	41.9 t	42.2 t
10	35.0 s	34.2 s	35.8 s	34.4 s
11	72.7 s	72.5 s	72.5 s	124.5 s
12	26.7 q[a]	26.5 q[a]	26.7 q[a]	19.2 q[a]
13	27.3 q[a]	26.7 q[a]	27.5 q[a]	20.8 q[a]
14	17.6 q	19.0 q	10.9 q	108.0 t
15	23.0 q	24.4 q	22.4 q	24.6 q

[a] Values in the same column with the same superscript can be interchanged; spectra were run in $CDCl_3$.

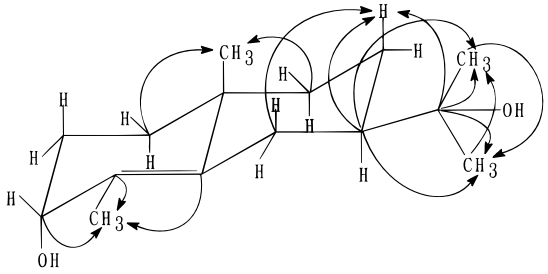

Figure 1. ^{13}C-^{1}H long-range correlations from an HMBC experiment for **1**.

[†] Department of Biology.
[‡] Department of Chemistry.
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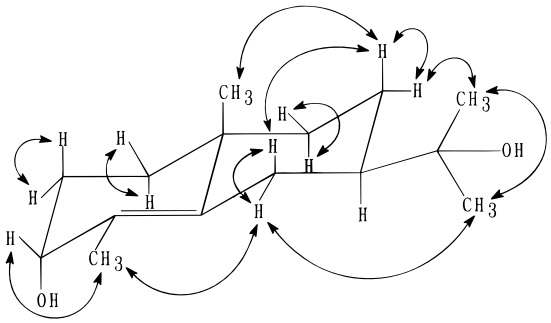
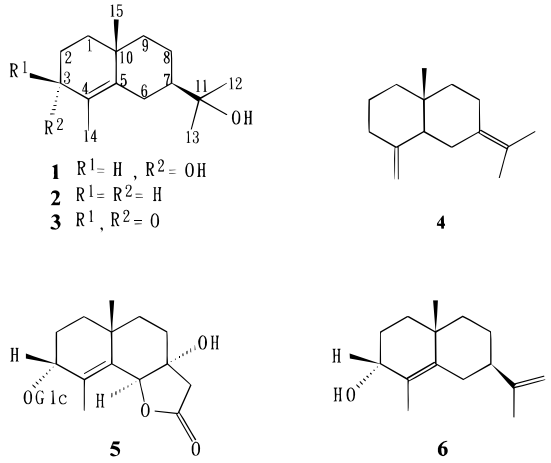


Figure 2. NOESY correlations for **1**.

C-3 was determined as α by observation of the cross-peak between the methyl group (δ 1.78) at C-4 and H-3 (δ 4.22) and between the methyl group at C-4 and H-6eq (δ 2.65). Comparisons were made between the coupling constant of H-3 of **1** at δ 4.22 (br s) with those of the model compounds sphaeranthanolide (**5**)[13] at δ 4.15 (J = 2.2 Hz) and cyperol (**6**)[14] at δ 3.74 (br s). The configuration of H-3 was confirmed as being in the β orientation. On the basis of these results, structure **1** was assigned for machikusanol. This is the first report of the occurrence of **1** as a natural product, despite the fact that its stereoisomer has been synthesized.[15,16]



1 R^1 = H, R^2 = OH
2 R^1 = R^2 = H
3 R^1, R^2 = O

4

5

6

Experimental Section

General Experimental Procedures. Melting points are reported uncorrected. UV spectra were run in MeOH and IR spectra on KBr disks, except where noted. ^{1}H-NMR spectra were measured at 200 or 400 MHz in $CDCl_3$ using TMS as an internal standard. MS were obtained at 70 eV using a direct inlet system.

Plant Material. *P. japonica* was collected from Pinlin, Tainan Hsien, Taiwan, in May 1991, and verified by Prof. C. S. Kuoh. A voucher specimen (NCKU 91050288) is deposited in the Herbarium of Cheng Kung University, Taiwan, Republic of China.

Extraction and Isolation. The dried and powdered xylem of the stems (9.3 kg) was exhaustively extracted (three times) with hot MeOH. After filtration and evaporation of the solvent, the greenish-brown residue (410 g) obtained was partitioned between $CHCl_3$ and H_2O to produce a $CHCl_3$ extract (160 g), an H_2O extract (80 g), and an insoluble residue (160 g). The $CHCl_3$ layer was extracted with 5% HCl solution. The acidic layer was neutralized with NH_4OH and again extracted with $CHCl_3$. After concentration and column chromatography over Sephadex LH-20 using a gradient of H_2O and MeOH as eluent, two alkaloids, isoboldine (12 mg) and corytuberine (15 mg), were obtained. After removal of the basic portion, the $CHCl_3$ layer was chromatographed on Si gel and eluted with CH_2Cl_2 to give three fractions (fractions 1−3). Fraction 2 was rechromatographed over a Si gel column using *n*-hexane−EtOAc (10:1) as eluent to afford γ-selinene (**4**) (0.6 g), an oily substance, and a mixture of stigmasterol and β-sitosterol (650 mg). The oily substance was separated by fractional distillation under reduced pressure to obtain a mixture of sesquiterpenoids (95−105 °C) and γ-eudesmol (**2**) (9.5 g). The mixture of sesquiterpenoids was purified by HPLC on a RP-18 column and eluted with MeOH−H_2O (80:20) to give an unknown compound (5 mg), carissone (**3**) (10 mg), and machikusanol (**1**) (20 mg), successively. A crystalline mixture was obtained from fraction 3 by filtration. This crystalline mixture was subjected to chromatography on a Si gel column and eluted with a gradient of $CHCl_3$−MeOH to afford a terpenoid mixture and a mixture of stigmasteryl glucoside and β-sitosteryl glucoside (450 mg). The filtrate was chromatographed on Sephadex LH-20 using H_2O−MeOH as eluent to give *epi*-syringaresinol (105 mg) and two unknown compounds.

Machikusanol (**1**) was obtained as colorless needles (Me_2CO): mp 126−129 °C; $[\alpha]^{25}_D$ + 45° (*c* 0.2; MeOH); EIMS (70 eV) *m/z* $[M]^+$ 238 (8), 237 (12), 220 (11), 218 (68), 203 (100), 179 (23), 177 (75), 59 (100); IR (KBr) ν max 3400, 2850, 1620 cm^{-1}; HRMS *m/z* found 238.1935 $[M]^+$ ($C_{15}H_{26}O_2$ requires 238.1933); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.01 (3H, s, H_3-15), 1.20, 1.21 (3H each, s, H_3-12, H_3-13), 1.25 (1H, m, H-8ax), 1.30 (1H, dt, J = 10.7, 3.2 Hz, H-1eq), 1.42 (1H, dt, J = 2.5, 12.1 Hz, H-7ax), 1.58 (1H, m, H-1ax), 1.61 (1H, m, H-2ax), 1.62 (1H, m, H-9ax), 1.63 (2H, m, H-8eq, H-9eq), 1.66 (1H, dd, J = 13.6, 12.1 Hz, H-6ax), 1.78 (3H, s, H-14), 2.14 (1H, dt, J = 12.0, 3.2 Hz, H-2eq), 2.65 (1H, dt, J = 13.6, 2.5 Hz, H-6eq), 4.22 (1H, br s, H-3), 7.86 (1H, s, 3-OH); ^{13}C NMR data, see Table 1.

Oxidation of Machikusanol (1). A solution of **1** (3 mg) in Me_2CO was treated with pyridinium chlorochromate solution (1 mL) and allowed to stand for 1 h at room temperature. The oxidized product was purified by HPLC to afford **3** (1.26 mg) as a colorless oil, $[\alpha]^{25}_D$ +71°, (*c* 0.063, MeOH). It was identified by comparison of spectral data (^{1}H NMR, ^{13}C NMR, IR) and HPLC on carrisone (**3**).[7]

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References and Notes

(1) Chang, C. E. *Flora of Taiwan*; Epoch Publishing: Taipei, 1976; Vol. 2, pp 458−459.
(2) Tomita, M.; Yang, T. H.; Lu, S. T. *Yakugaku Zasshi*, **1963**, *83*, 15−18.
(3) Lu, S. T. *Yakugaku Zasshi*, **1963**, *83*, 19−21.
(4) Yoshida, T.; Endo, K.; Ito, S.; Nozoe, T. *Yakugaku Zasshi* **1967**, *87*, 434−439.
(5) Van Beek, T. A.; Kleis, R.; Posthumus, M. A.; Van Veldhuizen, Z. *Phytochemistry* **1989**, *28*, 1909−1911.
(6) Joshi, D. V.; Boyce, S. F. *J. Org. Chem*. **1957**, *22*, 95−96.

(7) Hans, A.; Reiner, W.; Ivan, A. M. *Phytochemistry* **1985**, *24*, 2325−2328.
(8) Bohlmann, F.; Zdero, C.; Pickard, J.; Robinson, H.; King, R. M. *Phytochemistry* **1981**, *20*, 1323−1333.
(9) Posner, G. H.; Loomis, G. L.; Sawaya, H. S. *Tetrahedron Lett.* **1975**, 1373−1376.
(10) Lee, S. S.; Yang, H. C. *J. Chin. Chem. Soc.* **1992**, *39*, 189−194.
(11) Kunitomo, J. I.; Ju-Ichi, M.; Ando, Y.; Yoshikawa, Y.; Nakamura, S.; Shingu, T. *Yakugaku Zasshi* **1975**, *95*, 445.
(12) Dickey, E. E. *J. Org. Chem.* **1958**, *23*, 179−184.
(13) Shekhani, M. S.; Ahah, P. M.; Yasmin, A.; Siddiqui, R.; Perveen, S.; Khan, K. M.; Kazmi, S. U.; Rahman, A. U. *Phytochemistry* **1990**, *29*, 2573−2576.
(14) Hikino, H.; Aota, K.; Takemoto, T. *Chem. Pharm. Bull.* **1967**, *15*, 1929−1933.
(15) Pinder, A. R.; Williams, R. A. *J. Chem. Soc.* **1963**, 2773−2778.
(16) Marshall, J. A.; Pike, M. T. *J. Org. Chem.* **1968**, *33*, 435−437.

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