Structures of New Monoterpenes from Thai Herbal Medicine *Curcuma comosa*

Seikou Nakamura,†,‡ Yang Qu,§,∥ Fengming Xu,⊥ Hisashi Matsuda,⊥ and Masayuki Yoshikawa* ,†

† Kyoto Pharmaceutical University; Misasagi, Yamashina-ku, Kyoto 607-8412, Japan; and ‡ School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University; Shenyang 110016, China.

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Three new monoterpenes, comosoxide A (1), comosoxide B (2), and comososide (3), were isolated from the methanolic extract of the rhizomes of *Curcuma comosa* cultivated in Thailand. Their structures were elucidated on the basis of chemical and physicochemical evidence.

Key words *Curcuma comosa*, Zingiberaceae; traditional Thai medicine; monoterpenes; comosoxide; comososide

A Zingiberaceae plant, *Curcuma comosa*, is widely distributed in tropical and subtropical regions of the world, especially Thailand, Indonesia, and Malaysia. The rhizome of *C. comosa* has been used extensively as an indigenous medicine called “Waan chak mot luuk” in Thailand for the treatment of postpartum uterine bleeding. This medicine has also been called “Waan chak mot luuk” in Thailand for the treatment of comosoxide has been used extensively as an indigenous medicine especially Thailand, Indonesia, and Malaysia. The rhizome of *C. comosa* was partitioned into a mixture of ethyl acetate (EtOAc) and water to furnish the EtOAc-soluble fraction and an aqueous layer as previously described. As a continuing study on this natural medicine, we additionally isolated three new monoterpenes, comosoxide A (1), comosoxide B (2), and comososide (3), together with 7 known compounds. This paper deals with the isolation and structure elucidation of three new constituents (1—3).

The methanolic extract from the dried rhizomes of *C. comosa* (cultivated in Thailand) was partitioned into a mixture of ethyl acetate (EtOAc) and water to furnish the EtOAc-soluble fraction and an aqueous layer as previously described. The EtOAc- and n-BuOH-soluble fractions were subjected to silica gel and octadecyl silica (ODS) column chromatography and finally HPLC to furnish three new monoterpenes, comosoxide A (1, 0.00055%), comosoxide B (2, 0.00046%), and comososide (3, 0.00028%), three known monoterpenes, 1-hydroxy-α,β,4-trimethyl-3-cyclohexene-1-methanol (4, 0.00211%), 6-hydroxy-3-(1-hydroxy-1-methyl)ethyl)-6-methyl-2-cyclohexen-1-one (5, 0.00037%), (1S,2S,4R)-2-hydroxy-1,8-cineole β-D-glucopyranoside (6, 0.00020%), and four phenolic compounds, (+)-rhododendrol (7, 0.0086%), 4-(4-hydroxyphenyl)butan-2-one (8, 0.0019%), 4-hydroxybenzaldehyde (9, 0.00049%), 4-hydroxy-3-methoxybenzaldehyde (10, 0.00022%).

Comosoxide A (1) was obtained as a colorless oil with positive optical rotation ([α]<sup>25</sup> +16.0). In the electron impact (EI)-MS of 1, a molecular ion peak was observed at m/z 168 (M<sup>+</sup>), and high-resolution EI-MS (HR-EI-MS) analysis revealed the molecular formula of 1 to be C<sub>10</sub>H<sub>16</sub>O<sub>2</sub>. Its IR spectrum showed absorption bands at 3400 and 1650 cm<sup>-1</sup> assignable to hydroxyl and olefin functions. The 1H- and 13C-NMR (CD<sub>3</sub>OD, Table 1) spectra of 1 showed signals assignable to three tertiary methyls ([δ 1.16, 1.22, 1.27 (3H each, all s, 9, 10, 7-H<sub>3</sub>)], two methylenes [δ 1.56 (1H, ddd, J = 3.4, 7.6, 17.2 Hz, 5α-H), 2.00 (1H, ddd, J = 2.8, 13.1, 17.2 Hz, 5β-H), 1.68 (1H, ddd, J = 2.8, 7.6, 16.5 Hz, 6β-H), 1.88 (1H, ddd, J = 3.4, 13.1, 16.5 Hz, 6α-H)], two methines [δ 5.73, 5.89 (1H each, both d, J = 10.3 Hz, 2, 3-H)], the planar structure of 1 was confirmed by 1H-1H COSY and HMBC experiments. As shown in Fig. 1, the former indicated the presence of two partial structures written in the bold lines, while long-range correlations in the HMBC experiment on 1 were observed between the following proton and carbon pairs (H-2—C-1, C-10, and C-12).
and C-4; H-3 and C-1; H-5 and C-6; H-6 and C-5; H2-7 and C-1, 2, 6; H3-9 and C-4, 8, 10; H-10 and C-4, 8, 9). The relative stereostructures of the 1 and 4-positions in one methine bearing an oxygen function was identified by HPLC analysis using an optical rotation detector. The 1H- and 13C-NMR (CD3OD, Table 1) spectra of the compound was determined by a NOESY experiment, in which the NOE correlations were observed between the following proton pairs (H-2 and H2-7, H-3 and H-9; H-5α and H-6α, H-7; H-5β and H-6β, H-10; H-6α and H-7). Those findings led us to formulate the structure of comosoxide A (1) to be as shown.25)

Comosoxide B (2) was isolated as a colorless oil with positive optical rotation ([α]21D +18.1). The EI-MS spectrum of 2 showed a molecular ion peak at m/z 168 (M+) and the molecular formula of 2 was established as C18H18O5 by HR-EI-MS measurement. Its IR spectrum showed absorption bands at 3365, 1681, and 1554 cm−1 ascribable to hydroxyl functions and olefinic aromatic ring. The UV spectrum of 2 showed absorption maxima at 210 nm (log ε 4.54) and 277 nm (3.84), suggesting the presence of aromatic ring. The acid hydrolysis of 3 with 1 M HCl in 1,4-dioxane (1:1, v/v) liberated d-glucose, which was identified by HPLC analysis using an optical rotation detector.10) The 1H- and 13C-NMR (CD3OD, Table 1) spectra of 3 showed signals assignable to three methyls [δ 1.50, 1.50, 2.24 (3H each, all s, δ 9, 10, 11-H3)], three ortho- and meta-coupled aromatic protons [δ 7.01 (1H, d, J = 7.8, 1.1 Hz, 5-H)], 7.25 (1H, d, J = 1.8 Hz, 3-H), 7.25 (1H, d, J = 7.8 Hz, 6-H)] together with a glucopyranosyl moiety (δ 4.89 (1H, d, J = 7.7 Hz, 1′-H), 3.44, 3.46, 3.46, 3.49 (1H each, all m, 4′, 3′, 5′, 2′-H).Those proton signals due to the aglycon part in the 1H-NMR data of 3 were superimposable on those of 3-hydroxy-α,α,4-trimethyl benzyl alcohol.26) The position of the glycoside linkage was clarified by the HMBC experiment, which showed long-range correlation between the anomeric proton and the 2-carbon (Fig. 1). On the basis of these findings, the structure of 3 was determined to be as shown.

**Experimental**

The following instruments were used to obtain physical data: specific rotation, Horiba SEPA-300 digital polarimeter (5°=5 cm); UV spectra, Shimadzu UV-1600 spectrometer; IR spectra, Shimadzu FTIR-8100 spectrometer; 13C-NMR and high-resolution EI-MS, JEOL JNM-ECL600 (150 MHz) spectrometers with tetramethylsilane as an internal standard; and HPLC detector, Shimadzu RID-6A refractive index and SPD-10AvP UV−VIS detectors. HPLC column, COSMOSIL 5C18-PAQ (250×4.6 mm i.d.) and (250×20 mm i.d.) columns were used for analytical and preparative purposes, respectively.

The following experimental conditions were used for chromatography: ordinary-phase silica gel column chromatography, Silica gel GW-200 (Fuji Silysia Chemical, Ltd., Aichi, Japan, 150—350 mesh); reversed-phase silica gel column chromatography, Chromatorex ODS DM10020T (Fuji Silysia Chemical, Ltd., Aichi, Japan, 100—200 mesh); TLC, precoated TLC plates with Silica gel 60F254 (Merck, 0.25 mm) (ordinary phase) and Silica gel RP-18 F254s (Merck, 0.25 mm) (reversed phase); reversed-phase HPTLC, precoated TLC plates with Silica gel RP-18 60F254s (Merck, 0.25 mm); and detection was achieved by spraying with 1% Ce(SO4)2·10H2O solution following by heating.

**Plant Material**

*C. comosa* was cultivated in Nakhon Si Thammarat of Thailand in July 2006, and identified by Dr. Yutana Pongsriyadach. A voucher specimen (No. T-32) is on file in our laboratory (Kyoto Pharmaceutical University, Department of Pharmacognosy).

**Extraction and Isolation**

Fractions 5-2 (34 mg), 5-3 (79 mg), 5-6 (23 mg), 6 (19.3 mg), 6-2 (274 mg), 8 (22.4 mg), and 9 (4.5 mg) were obtained from the EtOAc-soluble fraction of the MeOH extract from the rhizomes of *C. comosa* of Thailand in July 2006, and identified by Dr. Yutana Pongsriyadach. A voucher specimen (No. T-32) is on file in our laboratory (Kyoto Pharmaceutical University, Department of Pharmacognosy).
(542 mg), and Fr. 9-5 (2294 mg). Fr. 9-1 (130 mg) was purified by HPLC [CH$_3$OH-H$_2$O (15:85, v/v)] to give comosoxide B (2, 18.0 mg, 0.00046%). Fr. 9-2 (48 mg) was isolated on HPLC to give (1S,2S,4R)-2-hydroxy-1,8-cineole β-D-glucopyranoside (6, 7.8 mg, 0.00020%).

The MeOH-eluted fraction (7.0 g) was subjected to reversed-phase silica gel column chromatography [210 g, MeOH–H$_2$O (5:95→10:90→85→20:80, v/v)+MeOH] to give seven fractions [Fr. 12-1 (561 mg), Fr. 12-2 (66 mg), Fr. 12-3 (136 mg), Fr. 12-4 (136 mg), Fr. 12-5 (238 mg), Fr. 12-6 (200 mg), and Fr. 12-7 (405 mg)]. Fr. 12-7 (405 mg) was further isolated by HPLC [CH$_3$OH-H$_2$O (20:80, v/v)] to give comosoxide (3, 11.5 mg, 0.00028%).

The known compounds [4→7, 4-(4-hydroxyphenyl)butan-2-one, 4-hydroxybenzaldehyde, and 4-hydroxy-3-methoxybenzaldehyde] were identified by comparison of their physical data ([$\delta$$_{H}$, IR, 1H-NMR, 13C-NMR, MS] with reported values$^{19-22}$ or those of commercial samples.$^{22}$

**Comosoxide A (1):** Colorless oil, [α]$_{D}^{25}$ +16.0 ($c$=0.6, MeOH). HR-EL-MS: Calcd for C$_{13}$H$_{18}$O$_{5}$ (M+) 268.1042; Found 268.1041. 1H-NMR (600 MHz, CD$_3$OD) δ: 1.15, 1.29, 1.29 (J 6.0, MeOH). IR (film, cm$^{-1}$): 3370, 1458, 1026. 1H-NMR (150 MHz, CD$_3$OD) δ: given in Table 1. EI-MS (m/z (%): 168 (M$_{+}$), 7, 100 (100).

**Comosoxide B (2):** Colorless oil, [α]$_{D}^{25}$ +16.0 ($c$=0.1, MeOH). HR-EL-MS: Calcd for C$_{13}$H$_{18}$O$_{5}$ (M+) 268.1042; Found 268.1041. 3370, 1458, 1026. 1H-NMR (600 MHz, CD$_3$OD) δ: 1.15, 1.29, 1.29 (J 6.0, MeOH). UV [MeOH, nm, (log ε)]: 210 (4.54), 277 (3.84). IR (film, cm$^{-1}$): 3365, 2932, 1541, 1246, 1035, 771. 1H-NMR (600 MHz, CD$_3$OD) δ: 1.50, 1.50, 2.24 (J 3.0, MeOH). 1H-NMR (600 MHz, CD$_3$OD) δ: 1.50, 1.50, 2.24 (J 3.0, MeOH). 1H-NMR (600 MHz, CD$_3$OD) δ: 1.50, 1.50, 2.24 (J 3.0, MeOH).

**Acid Hydrolysis of Comosside (3):** A solution of 3 (2.0 mg) in 1 M HCl in 1,4-dioxane (1:1, v/v, 0.5 ml) was heated under reflux for 2 h. After cooling, the reaction mixture was poured into ice-water and neutralized with Amberlite IRA-400 (OH$^{-}$ form), and the resin was removed by filtration. Then, the filtrate was extracted with EtOAc. The aqueous layer was subjected to HPLC analysis under the following conditions: HPLC column, Kusaior LC NH$_2$-60-5, 4.6 mm i.d.×250 mm (Tokyo Kasei Co., Ltd., Tokyo, Japan); detection, optical rotation [Shodex OR-2 (Showa Denko Co., Ltd., Tokyo, Japan)]; mobile phase, CH$_3$CN–H$_2$O (85:15, v/v); flow rate 0.8 ml/min; column temperature, room temperature. Identification of α-glucose from comoside B was carried out by comparison of its retention time and optical rotation with those of authentic sample. $t_{R}$: 12.7 min (positive optical rotation).

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**References and Notes**

24) 4-Hydroxybenzaldehyde and vanillin were identified by comparison of their physical data with those of commercial samples. The absolute configurations of 1 and 2 have not been characterized yet.