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Phenylpropanoid glycosides from *Lamiophlomis rotata*

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Abstract

Two new phenypropanoid glycosides were isolated from the roots of *Lamiophlomis rotata*, together with a known compound, cistanoside C. On the basis of spectral and chemical evidence, the structures of two new compounds were identified as 6′-β-D-apiofuranosyl cistanoside C and *cis*-lamiophlomiside A. © 1999 Elsevier Science Ltd. All rights reserved.

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1. Introduction

The genus *Lamiophlomis* kudo (Labiatae) is represented by only one species, which is widely spread over high mountainous regions in Tibet (Wu, 1985). *Lamiophlomis rotata* (Benth.) kudo, a Chinese folk medicine, is used to promote blood circulation, remove blood stasis, subdue swelling and alleviate pain (Jiangsu New Medical College, 1977). In previous studies (Yi, Zhong, Luo, Wu, & Zheng, 1990; Yi, Zhong, Luo, & Xiao, 1991; Yi, Chen, Luo, & Yan, 1995; Yi, Yan, Luo, & Zhong, 1995) two phenylpropanoid glycosides and seven iridoids from *L. rotata* have been isolated. In this study, two new phenylpropanoid glycosides, 6′-β-D-apiofuranosyl cistanoside C (**2**) and *cis*-lamiophlomiside A (**3**) as well as a known compound, cistanoside C (**1**) were isolated from roots of the same plant. The structures of **1**–**3** were characterized by spectral and chemical evidence.

2. Results and discussion

Compound **2** was obtained as an off-white amorphous powder, whose molecular formula $C_{35}H_{46}O_{19}$ was determined by FABMS (*m*/*z* 809 $[M+K]^+$, 793 $[M+Na]^+$). It gave a positive visualization with ferric chloride and Molish reagent (Jin, Liu, & Wang, 1992), indicating that **2** is a glycoside with a phenolic hydroxyl group. The UV absorption at λ_{max} 333 and 220 nm confirmed the presence of hydroxycinnamic acid derivatives. The IR spectrum suggested the hydroxyl groups (br, 3410 cm^{-1}), an α,β-unsaturated ester (1700 and 1630 cm^{-1}) and aromatic rings (1600 and 1510 cm^{-1}). On exhaustive hydrolysis with 5 M hydrochloric acid, **2** afforded 2-(4-hydroxy-3-methoxyphenyl)ethanol, caffeic acid, glucose, rhamnose and apiose, identified by TLC. The ^1H NMR spectrum of **2** exhibited similar signals to lamiophlomiside A (**4**) (Yi et al., 1995), alyssonoside and forsythoside B (Calis, Hosny, Khalifa, & Ruedi, 1992), indicating its trisaccharide structure. Three signals for anomeric protons appearing at 4.37 (d, $J=8.0$ Hz), 4.90 (d, $J=2.4$ Hz) and 5.18 (d, $J=1.0$ Hz) provided the following configuration of C-1 in the sugar: β for D-glucose, β for D-apiose and α for L-rhamnose, respectively (Calis et al., 1992). Moreover, characteristic signals belonging to (*E*)-caffeic acid and 2-(4-hydroxy-3-methoxyphenyl)ethanol moieties (six aromatic protons as two ABX systems, and olefinic protons, AB system), as well as a benzylic methylene, two non-equivalent protons and a methoxyl group were observed. This spectrum also exhibits a well-resolved triplet at 4.94 ($J=9.3$ Hz),

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which could be assigned to the ester bearing a methine proton as observed for lamiophlomiside A (**4**) (Yi et al., 1995), alyssonoside, forsythoside B (Calis et al., 1992) and cistanoside C (Kobayashi, Karasawa, Miyase, & Fukushima, 1984). This determined the acylation position. In the ^1H and ^{13}C NMR spectra of **2**, the signals due to the sugar moiety were also superimposable on those of lamiophlomiside A (Yi et al., 1995), alyssonoside and forsythoside B (Calis et al., 1992). Permethylation of **2** and **4** by dimethyl sulphate in acetone gave the same compound (**5**). Thus, compound **2** was identified as 6′-β-D-apiofuranosyl cistanoside C (Fig. 1).

Compound **3** was isolated as an off-white amorphous powder, with molecular formula $C_{36}H_{48}O_{19}$ as determined by FABMS (*m/z* 823 $[M+K]^+$, 807 $[M+Na]^+$). Its UV, IR, FABMS, ^1H and ^{13}C NMR were very similar to those of lamiophomiside A (**4**), except that the α′ and β′ protons of their feruloyl moiety had different chemical shifts and coupling constants. In the ^1H NMR spectrum of **3**, a pair of signals for olefinic protons appeared at δ 5.79 and 6.93 (each 1H, d, $J=13.0$ Hz) as observed for (*Z*)-leucosecptoside A and (*Z*)-martynoside (Jia, Gao, & Liu, 1994), whereas the corresponding proton signals of **4** appeared at δ 6.37 and 7.64 (each 1H, d, $J=16.0$ Hz). The feruloyl moiety of **3** was further examined by NOE difference experiments, which showed enhancement (5%) of H-2″ (δ 7.87, d, $J=2.0$ Hz) on irradiation of the protons of OCH_3 (δ 3.89, s). On the basis of the above spectral data, compound **3** was identified as *cis*-lamiophlomiside A.

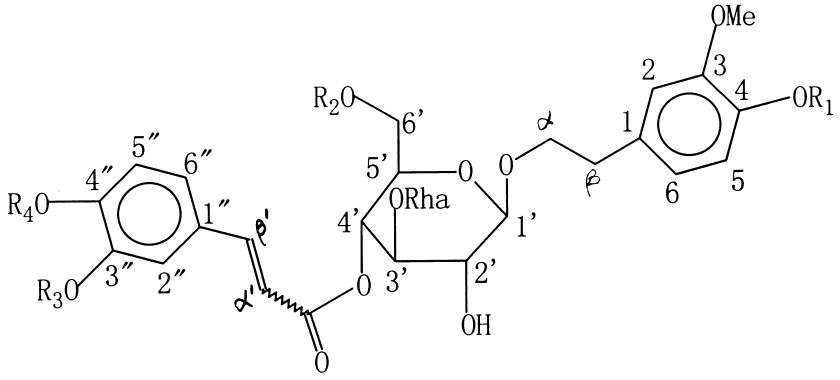
3. Experimental

3.1. General

UV: in MeOH; IR: KBr discs; NMR: 400 MHz for ^1H and 100 MHz for ^{13}C, TMS as int. standard; CC: silica gel. HPLC was carried out in reverse-phase mode using a C-18 column (18 × 350 mm i.d.). Silica gel $60F_{254}$ TLC plates were used. Phenylpropanoid glycosides were detected by UV after spraying with vanillin–H_2SO_4 followed by heating at 100°C for 5 min.

3.2. Plant material

Lamiophlomis rotata (Benth.) Kudo was collected at Ganzhi, Sichuan Province, China, in October 1994 and identified by Professor J.H. Chen (Sichuan Institute of Chinese Materia Medica), where a voucher specimen is kept at Chengdu Institute of Biology.



	R_1	R_2	R_3	R_4	Acid moiety
1.	H	H	H	H	E
2.	H	Api	H	H	E
3.	H	Api	Me	H	Z
4.	H	Api	Me	H	E
5.	Me	Api	Me	Me	E

Fig. 1. Structures of compounds **1**–**5**.

3.3. Extraction and separation

The dried and powdered roots (2.5 kg) were extracted with 80% EtOH under reflux (10 l × 3, each 2 h). After the removal of solvent, the residue was successively fractionated with petroleum ether (b.p. 60–90°C), EtOAc and *n*-BuOH. The *n*-BuOH extract was chromatographed on silica gel with $CHCl_3$:MeOH:H_2O (3:6:2 lower layer) to give frs A–F. Fr. D was subjected to HPLC gradient-eluted with H_2O/MeOH (40–60%) at a flow rate of 5 ml/min to yield compounds **1** (30 mg), **2** (65 mg) and **3** (28 mg).

3.4. Compound *1*

Off-white amorphous powder. UV ν_{max} (nm): 332, 290, 220; IR ν_{max} cm^{-1}: 3450 (OH), 1700 (C=O), 1630 (C=C), 1602 and 1510 (aromatic rings), 1445, 1265, 1155, 1035, 810; ^1H, ^{13}C NMR (CD_3OD) and FABMS were identical to those reported for cistanoside C (Kobayashi et al., 1984).

3.5. Compound *2*

Off-white amorphous powder. UV λ_{max} (nm): 333, 219; IR ν_{max} cm^{-1}: 3420 (OH), 1700 (C=O), 1630 (C=C), 1600 and 1510 (aromatic rings), 1445, 1265, 1155, 1035, 810; ^1H NMR(CD_3OD) δ ppm: 6.85 (d, J=2.0 Hz, 1H, H-2), 6.70 (d, J=8.0 Hz, 1H, H-5), 6.67 (dd, J=8.0/2.0 Hz, 1H, H-6), 4.04 (m, 1H, H-α), 3.72 (m, 1H, H-α), 2.85 (m, 2H, H-β), 3.84 (s, 3H, OMe), 7.05 (d, 1H, J=2.0 Hz, H-2″), 6.77 (d, 1H, J=8.3 Hz, H-5″), 6.95 (dd, 1H, J=8.3/2.0 Hz, H-6″), 6.27 (d, 1H, J=16.0 Hz, H-α′), 7.59 (d, 1H, J=16.0 Hz, H-β′), 4.37 (d, 1H, J=8.0 Hz, H-1′), 3.38 (dd, 1H, J=9.3/8.0 Hz, H-2′), 3.80 (t, 1H, J=9.3 Hz, H-3′), 4.94 (t, 1H, J=9.3 Hz, H-4′), 5.18 (d, 1H, J=1.0 Hz, H-1 of Rha), 3.29 (t, 1H, J=9.5 Hz, H-4 of Rha), 1.09 (d, 3H, J=6.0 Hz, H-6 of Rha), 4.90 (d, 1H, J=2.4 Hz, H-1 of Api), 3.86 (d, 1H, J=2.4 Hz, H-2 of Api), 3.53 (s, 3H, H-3′ of Api). ^{13}C NMR (CD_3OD) δ ppm: 132.4 (C-1), 114.5 (C-2), 149.6 (C-3), 146.7 (C-4), 116.9 (C-5), 123.2 (C-6), 73.1 (C-α), 37.6 (C-β), 57.2 (OMe), 128.4 (C-1″), 115.5 (C-2″), 150.6 (C-3″), 148.8 (C-4″), 117.3 (C-5″), 124.0 (C-6″), 116.0 (C-α′), 147.6 (C-β′), 169.0 (C=O), 105.0 (C-1′), 76.9 (C-2′), 82.4 (C-3′), 71.7 (C-4′), 75.4 (C-5′), 69.3 (C-6′), 103.8 (C-1 of Rha), 73.1 (C-2 of Rha), 72.8 (C-3 of Rha), 74.6 (C-4 of Rha), 71.2 (C-5 of Rha), 19.2 (C-6 of Rha), 111.9 (C-1 of Api), 78.9 (C-2 of Api), 81.4 (C-3 of Api), 75.9 (C-4 of Api), 66.4 (C-3′ of Api). FABMS (m/z): 809 $[M+K]^+$, 793 $[M+Na]^+$.

3.6. Hydrolysis of compound *2*

Compound **2** (10 mg) was dissolved in 5 M HCl (2 ml) and heated at 90°C for 2.5 h. After cooling, the reaction mixture was extracted with EtOAc. The organic layer was concentrated to dryness. 2-(4-Hydroxy-3-methoxyphenyl)ethanol and caffeic acid were identified in the EtOAc layer by TLC on Kieselgel 60 F_{254} ($CHCl_3$:EtOH, 9:1). Glucose, rhamnose and apiose were detected in the aqueous layer by TLC on silica gel G (lower phase of $CHCl_3$:MeOH:H_2O 15:6:2–HOAc (9:1)) and PC (*n*-BuOH–HOAc–H_2O (4:1:5), upper phase).

3.7. Permethylation of compound *2* and lamiophlomisde A (*4*)

Compound **2** and lamiophlomiside A (ca. 10 mg), Me_2SO_4 (0.2 ml) and K_2CO_3 (30 mg) in Me_2CO (6 ml) were stirred for 24 h. After filtration, the filtrate was concentrated and analysed by TLC. They both gave the same permethyl (tetramethyl) compound (**5**), which was obtained as an off-white amorphous powder by preparative TLC ($CHCl_3$:MeOH:H_2O, 13:5:2 lower layer). ^1H NMR (CD_3OD) was identical to those reported (Yi et al., 1995).

3.8. Compound *3*

Off-white amorphous powder. UV λ_{max} (nm): 331, 289, 220; IR ν_{max} cm^{-1}: 3440 (OH), 1670 (C=O), 1630 (C=C), 1595 and 1510 (aromatic rings), 1445, 1425, 1265, 1155, 1030, 810. ^1H NMR (CD_3OD) dppm: 6.85 (d, 1H, J=2.0 Hz, H-2), 6.70 (d, 1H, J=8.0 Hz, H-5), 6.67 (dd, 1H, J=8.0/2.0 Hz, H-6), 4.04 (m, 1H, H-α0), 3.72 (m, 1H, H-α) 2.85 (m, 2H, H-β), 3.83 (s, 3H, OMe), 7.87 (d, 1H, J=2.0 Hz, H-2″), 6.77 (d, 1H, J=8.3 Hz, H-5″), 7.15 (dd, 1H, J=8.3/2.0 Hz, H-6″), 5.79 (d, 1H, J=13.0 Hz, H-α′), 6.93 (d, 1H, J=13.0 Hz, H-β′), 3.89 (s, 3H, OMe), 4.35 (d, 1H, J=8.0 Hz, H-1′), 3.38 (dd, 1H, J=9.3/8.0 Hz, H-2′), 3.80 (t, 1H, J=9.3 Hz, H-3′), 4.94 (t, 1H, J=9.3 Hz, H-4′), 5.18 (d, 1H, J=1.0 Hz, H-1 of Rha), 3.29 (t, 1H, J=9.5 Hz, H-4 of Rha), 1.08 (d, 3H, J=6.0 Hz, H-6 of Rha), 4.90 (d, 1H, J=2.4 Hz, H-1 of Api), 3.87 (d, 1H, J=2.4 Hz, H-2 of Api), 3.53 (s, 3H, H-3′ of Api). ^{13}C NMR (CD_3OD) δ ppm: 132.3 (C-1), 114.5 (C-2), 149.6 (C-3), 146.7 (C-4), 116.9 (C-5), 123.2 (C-6), 73.1 (C-α), 37.6 (C-β), 57.2 (OMe), 128.7 (C-1″), 116.5 (C-2″), 150.6 (C-3″), 149.1 (C-4″), 116.4 (C-5″), 128.2 (C-6″), 116.2 (C-α′), 148.6 (C-β′), 167.6 (C=O), 57.3 (OMe), 105.0 (C-1′), 76.9 (C-2′), 82.7 (C-3′), 71.6 (C-4′), 75.4 (C-5′), 69.2 (C-6′), 104.0 (C-1 of Rha), 73.1 (C-2 of Rha), 72.9 (C-3 of Rha), 74.6 (C-4 of Rha), 71.2 (C-5 of Rha), 19.2 (C-6 of Rha), 111.7 (C-1 of Api), 78.9 (C-2 of Api), 81.4 (C-3 of Api), 75.8 (C-4 of Api), 66.4 (C-3′ of Api). FABMS (m/z) 823 $[M+K]^+$, 807 $[M+Na]^+$.

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