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Note

The structure of an iridoid glycoside, 8-deoxyshanzhiside, from *Lamiophlomis rotata*

Maoxing Li,[a,b] Zhengping Jia,[b] Ruxue Zhang,[b] Zhide Hu[a,*] and Xuan Tian[a]

[a]*Department of Chemistry, Lanzhou University, Lanzhou 730000, China*
[b]*Department of Pharmacy, Lanzhou General Hospital of PLA, Lanzhou 730050, China*

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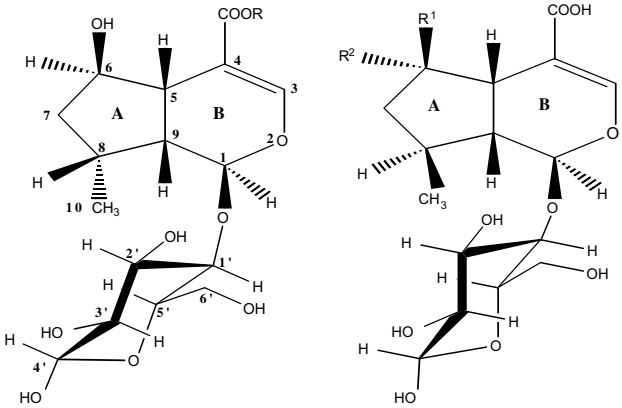
Abstract—8-Deoxyshanzhiside was extracted from *Lamiophlomis rotata* (Benth.) Kudo. Extensive NMR spectroscopy techniques were used to fully assign the ^{1}H and ^{13}C spectra. X-ray investigation was used to identify its conformation, and absolute configuration.

Keywords: *Lamiophlomis rotata*; Iridoid glycoside; 8-Deoxyshanzhiside; NMR; X-ray

Lamiophlomis rotata (Benth.) Kudo is a perennial herb (from the family Labiatae), which grows wild in Qinghai-Tibet Plateau in northwest of China. For thousands of years, the aerial parts and the root of *L. rotata* have been used as traditional drug by the Tibetan, Mongolian, and Na-Xi nations with beneficial effects on hemostasis and alleviating pain.[1] Iridoid glycosides and flavanoids are two main components of this plant. 8-*O*-Acetylshanzhiside methylester, 6-*O*-acetylshanzhiside methylester, sesamoside, penstemoside, shanzhiside methylester, loganin, phlomiol, 7,8-dehydro-enstemoside, phloyoside I, phloyoside II, lamiophlomiside, phlorigidoside C, and seven other iridoid diglycosides have been isolated and reported by many researchers.[2–6] Luteolin, luteolin-7-*O*-glycoside, quercetin, quercetin-3-*O*-arabinoside, apigenin-7-*O*-neo-esperidoside, luteolin-7-*O*-[β-D-apiose-(6→1)]-β-D-glycoside have been reported as the main flavanoids in the plant.[6,7] Our initial searches[8,9] showed for the first time that the Total Iridoid Glycoside (name of one extract, which was obtained from an aqueous extract of this plant using macroporous adsorptive resins and was enriched with iridoid glycosides) was responsible for the hemostatic bioactivity. Study of the chemical constituents of the Total Iridoid Glycoside caused the isolation of a novel iridoid glycoside, 8-deoxyshanzhiside (**1**) (Chart 1).

MS and NMR spectroscopy have frequently been used to elucidate the structure of iridoid glycosides.[10] Iridoid glycosides contain cyclopentane and pyrane



1 R=H
2 R=CH_3

3 R^1=H R^2=OH
4 R^1=OH R^2=H

Chart 1. Structures of 8-deoxyshanzhiside (**1**), penstemonoside (**2**), 6α-dihydrocornic acids (**3**) and 6β-dihydrocornic acids (**4**).

* Corresponding author. Tel.: +86 931 891 2540; fax: +86 931 891 2582; e-mail: huzhidelz@yahoo.com.cn

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rings. As fine differences exist in the categories and positions of substituting groups on cyclopentane, some mistakes about structure elucidation have been reported.[11,12] Recently, X-ray crystallography has been used more and more frequently to define the molecular structure, conformation, and absolute configuration of natural organic compounds, especially carbohydrates.[13,14] But to our knowledge, very few papers on crystal and molecular structures have been published for iridoid glycosides, which are important secondary metabolites of many plants. Here, we report the complete NMR characterization and crystal structures of a new iridoid glycoside, 8-deoxyshanzhiside.

The ^{1}H and ^{13}C NMR spectra (Figs. 1 and 2) of 8-deoxyshanzhiside were almost identical to those of iridoid glycosides obtained from *L. rotata*,[2–5] except for the disappearance of the signal of the carboxyl methyl ester group (COOMe) at C-4. Compared with penstemonoside (**2**),[15] 6α-dihydrocornic acid (**3**), and 6β-dihydrocornic acid (**4**)[16] (Chart 1 and Table 1), the configuration of the hydroxy group at C-6 of compound **1** was supposed to be β, similar to compound **4**, considering the chemical shifts (δ 77.1 and δ 78.8) of C-6 in ^{13}C NMR and the chemical shifts (δ 4.12 and δ 4.05) of H-6 in ^{1}H NMR; the configuration of the methyl group at C-8 of compound **1** was supposed to be α, similar to compound **2** and in agreement with the chemical shifts of C-7 (δ 40.8 and δ 41.7), C-8 (δ 32.5 and δ 33.8), C-9 (δ 40.5 and δ 42.5), and C-10 (δ 15.7 and δ 16.7) in the ^{13}C NMR spectrum. HMBC gave more correlation information about H-1/C-8 (3), H-1/C-9 (2); and H-10/C-7 (3), H-10/C-8 (2), H-10/C-9 (3). In NOE analysis, when irradiating the proton signal of H-10 (δ 0.87), it caused intense enhancement of H-1 (δ 5.44), and weak enhancement of H-6 (δ 4.11), H-7a (δ 1.38), and H-8 (δ 2.43); when irradiating the proton signal of H-8 (δ 2.43), it caused enhancement of H-1′ (δ 4.63), H-5 (δ 2.70), H-7b (δ 1.65), H-10 (δ 0.87). The results suggest that H-5, H-8 should be on the same face with H-7b (δ 1.65), and H-1, H-3, H-10, H-6 on another face with H-7a (δ 1.38). As H-8 and H-9 have very close chemical shift (δ 2.43 and δ 2.56, respectively) the interaction between these two protons in NOE is difficult to observe. At the same time, the NMR spectra, including the 2D NMR spectra and NOE, could not provide sufficient information to elucidate the anomery of the glycosidic bond and connecting pattern of rings A and B. Therefore, a single crystal X-ray diffraction analysis

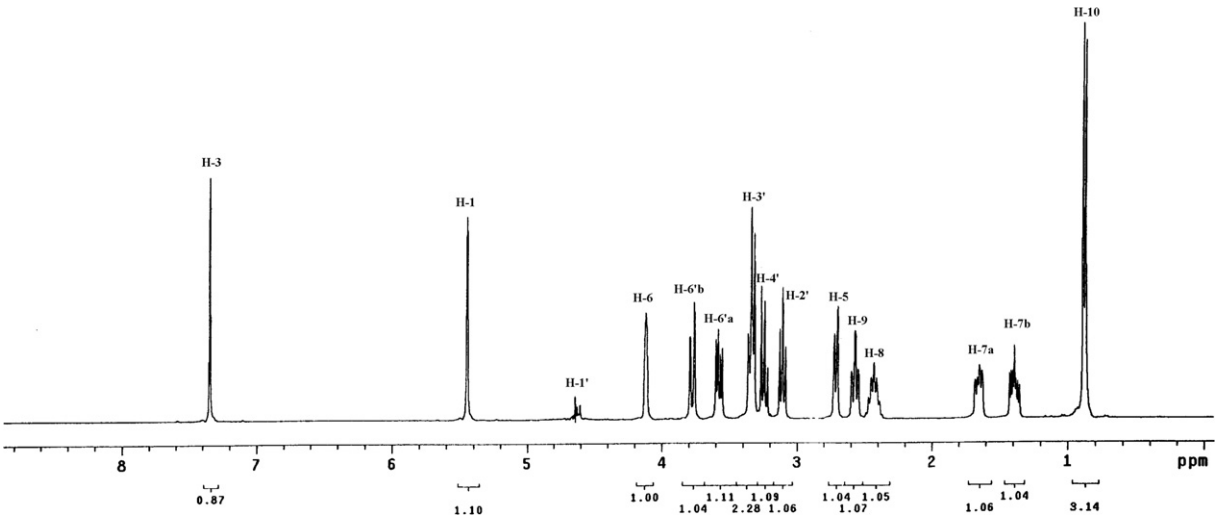

Figure 1. ^{1}H NMR spectrum of 8-deoxyshanzhiside.

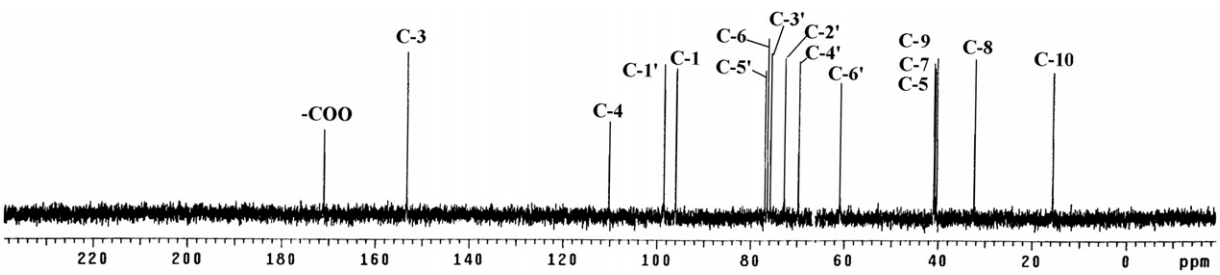

Figure 2. ^{13}C NMR spectrum of 8-deoxyshanzhiside.

Table 1. NMR spectral data of 8-dihydroshanzhiside,[a] penstemonoside,[b] 6α-dihydrocornic acids,[b] and 6β-dihydrocornic acids[b]

	Compound **1**[a]		Compound **2**[b]		Compound **3**[b]		Compound **4**[b]	
	δ_H Multiplicity (J in Hz)	δ_C	δ_H Multiplicity (J in Hz)	δ_C	δ_H Multiplicity (J in Hz)	δ_C	δ_H Multiplicity (J in Hz)	δ_C
1	5.44 (d, 2)	96.0	6.0	96.1	5.21 (d, 9)	101.2	5.25 (d, 5)	97.5
3	7.34 (s)	153.3	7.90 (d, 1)	153.7	7.62 (s)	155.9	7.41 (s)	153.6
4	—	110.3		111.0	—	107.4	—	110.8
5	**2.70 (d, 9)**	**41.1**		43.0	2.82 (dd, 4, 9)	43.5	**2.79 (t, 6)**	**43.7**
6	**4.11 (t, 2)**	**77.1**		77.8	4.47 (t, 4)	75.1	**4.05 (m)**	**78.8**
7a	1.38 (ddd, 4, 10, 13)	**40.8**		**41.7**	1.38 (ddd, 4, 10, 13)	43.2	1.25 (m)	42.7
7b	1.65 (dd, 8, 13)				1.92 (dd, 8, 13)		2.17 (m)	
8	2.43 (m)	**32.5**		**33.8**	2.30 (m)	35.2	1.96 (q, 7)	34.3
9	2.56 (dt, 2, 9.2, 9.2)	**40.5**		**42.5**	1.70 (dt, 4, 8)	47.0	2.03 (dt, 5, 6, 7)	47.9
10	0.87 (d, 7)	**15.7**	**1.46 (d, 7)**	**16.7**	1.12 (d, 8)	21.9	1.15 (d, 7)	21.1
COO		171.1		169.5		171.1		171.0
CH_3			4.18 (s)	51.82				
Glucoside								
1′	4.63 (d, 8)	98.6	5.18 (d, 7)		4.70 (d, 8)	100.4	4.65 (d, 8)	100.2
2′	3.10 (t, 9)	72.9			3.24 (dd, 8, 9)	74.9	3.20 (t, 8)	74.8
3′	3.33 (t, 9)	75.9			3.40 (t, 9)	78.1	3.37 (m)	78.1
4′	3.23 (t, 9)	69.9			3.31 (m)	71.7	3.37 (m)	71.7
5′	3.33 (t, 9)	76.5			3.29 (m)	78.5	3.30 (m)	78.4
6′a	3.62 (dd, 6, 12)	61.0			3.67 (dd, 6, 12)	63.0	3.67 (dd, 6, 12)	62.8
6′b	3.77 (d, 12)				3.86 (dd, 2, 12)		3.89 (dd, 2, 12)	

[a] Acquired in D_2O at 400 MHz (^1H) and 100 MHz (^{13}C), respectively.
[b] Acquired in CD_3OD at 500 MHz (^1H) and 125 MHz (^{13}C), respectively.

was adopted to solve this problem (HMOC, HMBC, and NOE see Supplementary data).

Suitable crystals of the compound were obtained by slow evaporation of a solution (1:8 MeOH–EtOAc) at room temperature. Crystals of 8-deoxyshanzhiside ($C_{16}H_{24}O_{10}$) are orthorhombic, space group $P2_12_12_1$, with cell dimensions $a = 7.6883(5)$, $b = 12.7944(8)$, $c = 21.3759(14)$, $a = 90.00(1)$, $b = 90.00(1)$, $c = 90.00(1)$, $V = 2102.7(2)$ Å^3. A crystal of approximate dimensions $0.20 \times 0.25 \times 0.38$ mm^3 was chosen for data collection. Diffraction data were collected at 294 K using a Nonius Kappa CCD diffractometer with graphite-monochromated Cu Kα radiation ($\lambda = 0.71073$ Å). Table 2 shows the crystal data and the parameters used for the structure determination. The data were analyzed using a structure determination package SDP[17] from the Enraf-Nonius Company on a microvax computing system. A total of 11,161 reflections had their intensities integrated and scaled, of which 2939 were considered significant. The structure was solved by direct methods and refined by full-matrix least squares on F^2 with anisotropic displacement parameters for the nonhydrogen atoms using Bruker, SHELXT[18] version 6.10. Hydrogen atoms were located from a different Fourier map and were refined isotropically. The structure was refined to a goodness of fit (GOF) of 1.051 and final residuals of $R_1 = 0.0818\%$ ($I > 2\sigma r(I)$) for the correct conformation. The other conformation had an R value of 0.06434. A total of 3872 reflections were employed for 252 parameter determinations, with three restraining parameters. The final fractional coordinates; equivalent isotropic displacement parameters [U(eq)] of the atoms in the structure; bond lengths and angles are deposited (see Supplementary data).

Table 2. Crystal data and structure refinement for 8-dihydroshanzhiside

Empirical formula	$C_{16}H_{18}O_{10}$
Formula weight	376.35
Temperature (K)	294(2)
Wavelength (Å)	0.71073
Crystal system, space group	Orthorhombic, $P2_12_12_1$
Unit cell dimensions	$A = 7.6883(5)$ Å $\alpha = 90.00$ $B = 12.7944(8)$ Å $\beta = 90.00$ $C = 21.3759(14)$ Å $\gamma = 90.00$
Volume (Å^3)	2102.7(2)
Z, Calculated density (mg/m^3)	4, 1.239
Absorption coefficient (mm^{-1})	0.106
$F(000)$	832
Crystal size (mm)	$0.20 \times 0.25 \times 0.38$
Range for data collection (°)	1.85–25.50
Index ranges	$-8 \leqslant h \leqslant 9$, $-15 \leqslant k \leqslant 11$, $-22 \leqslant l \leqslant 25$
Reflections collected/unique	11161/2939 [R(int) = 0.0415]
Completeness to $\theta = 25.50°$	98.7%
Max. and min. transmission	0.9609 and 0.9791
Data/restraints/parameters	3872/0/252
Goodness-of-fit on F^2	1.051
Final R indices [$I > 2\sigma r(I)$]	$R_1 = 0.0818$, $wR_2 = 0.2188$
R indices (all data)	$R_1 = 0.1004$, $wR_2 = 0.2412$
Largest diff. peak and hole (e Å^{-3})	0.885 and −0.396

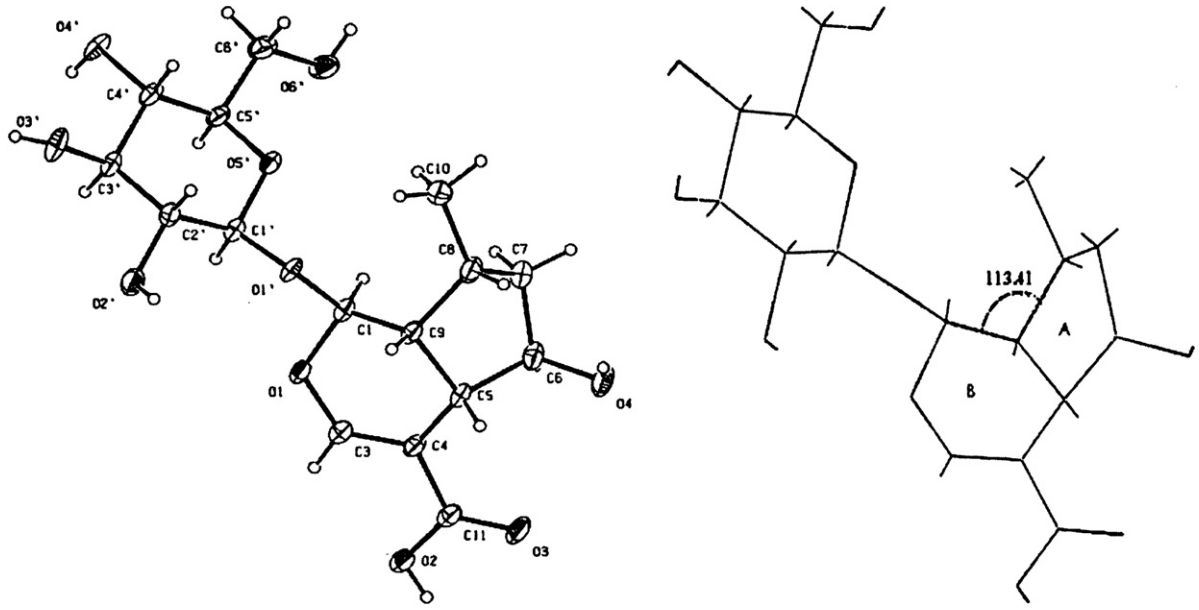

Figure 3. X-ray structure of 8-deoxyshanzhiside.

In general, the determination of the absolute configuration of iridoid glycosides by chemical methods is rather difficult. Our present study on the absolute configuration of 8-deoxyshanzhiside not only confirmed the chemical structure, but also gave very valuable justification to the positions of C-5, C-8, and C-9 in the cyclopentane ring. The *R* values for *R* (+) and *R* (−) are 0.0818 and 0.0643, respectively, and the ratio is 1.176. The absolute structure of 8-deoxyshanzhiside could be obtained by the *Flack* parameter 0.2(19) and the *Flack* parameter of its mirror image 1.4(17).[19] Results also showed that the cyclopentane and pyran rings were not on the same plane. The angle of rings A and B was 113.41°. The absolute configurations of C-1, C-5, C-6, C-8, and C-9 were (*S*), (*S*), (*R*), (*R*), and (*R*), respectively. The Glc moiety was found to adopt a chair-like conformation, the absolute configurations at its five stereogenic C-atoms being (*R*), (*R*), (*S*), (*R*), and (*R*) from C-1′ to C-5′, respectively (Fig. 3). The absolute configuration, as found from X-ray investigation, is in good agreement with those deduced from chemical studies.

1. Experimental

1.1. General methods

The melting point was determined on a Kofler melting point apparatus and was uncorrected. Optical rotation was determined at 25 °C with a Perkin–Elmer 341 polarimeter in 1-dm tube at the D line of sodium for a solution in water. The UV spectra were obtained on a HP-8453 spectrophotometer and the IR spectrum was recorded with a Nicolet 170 SX spectrometer. NMR spectra were recorded at room temperature on a Bruker FT 400 MHz spectrometer. ^{1}H NMR spectra were measured at 400 MHz and ^{13}C spectra at 100 MHz in D_2O solns, using the standard pulse sequence and procedures. The elemental analysis was obtained using a CEST MOD 110 elemental analyzer. HRESIMS were obtained on a Bruker Daltonics APEX II 47e spectrometer.

1.2. Extraction and characterization of 8-deoxyshanzhiside

The powder of aerial parts of *L. rotata* (250 g) was extracted at 100 °C with 1500 mL water three times. After being cooled and filtered, the aq extract was passed on a XDA-16 macroporous resin chromatographic column (250 g, ∅ 8 cm × 50 cm) at a flow rate of 2.0 mL/min. Eight hundred mL of 50% alcohol was used to elute the column after it had been eluted with distilled water till the Molish reaction became negative. Total Iridoid Glycoside (22.5 g) was obtained from the alcohol elution after being vacuum-dried at 60 °C. An aliquot (4 g) was applied to a silica gel column and eluted with a 10:1→1:2 $CHCl_3$–MeOH gradient. The 4:1 $CHCl_3$–MeOH eluted fraction was again purified by chromatography using the same solvent and recrystallized from 1:8 MeOH–EtOAc yielding compound **1** (315 mg); mp: 213–214 °C; $[\alpha]_D^{25}$: −155.73 (*c* 0.18, water); UV (MeOH) λ_{max} (log ε): 237 nm (6.33); $IR_{\nu max}^{KBr}$ cm^{-1}: 3613, 3467, 3382, 2943, 2884, 1676, 1641 cm^{-1}; ^{1}H and ^{13}C NMR (see Table 1). HRFABMS: $[M+Na]^+$ found 399.3400, calcd 399.3380, and $[M+K]^+$ found 415.2600, calcd 415.2689. Anal. Calcd for $C_{16}H_{24}O_{10}$:

C, 51.06; H, 6.43; O, 42.51. Found: C, 52.35; H, 6.40; O, 41.25.

1.3. Crystal structure determination

Crystal structure was determined on a Nonius Kappa CCD diffractometer using monochromatic Mo Kα radiation ($\lambda = 0.71073$ Å) at 294(2) K. The structures of all complexes were solved by the SHELX-97 program and refined using full-matrix least squares and the F^2 method. The direct method was used for primary solution and difference Fourier maps for secondary solution. Crystallographic data for the structure have been deposited at the Cambridge Crystallographic Data Center (deposition number: CCDC 624696). Copies of these data can be obtained, free of charge, by application to the CCDC via http://www.ccdc.cam.ac.uk/deposit (or 12 Union Road, Cambridge CB2 1EZ, UK, fax: + 44 1223 336033, e-mail: deposit@ccdc.cam.ac.uk).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2007.11.020.

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