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Two new terpenoid glucosides from *Clerodendrum serratum*

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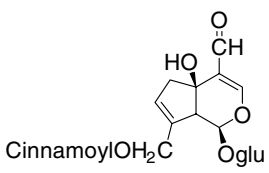
Clerodendrum serratum (Verbenaceae), named "san dui lie" in China, a small shrub with fragrant flowers found widely in forests in the south of China, has been used as a folk medicine in Yunna for the treatment of many diseases, such as hepatitis, malaria [1]. Several works had been done with *Clerodendrum serratum* [2–4]. In our research, we isolated two new compounds from the CH_2Cl_2 and EtOAc extracts, 5-hydroxyl-10-*O*-cinnamoyloxy-tarennoside (**1**) and 17-aldehedeyloxy-19-β-D-glucopranosyloxy-lab- 8,13(E)-dien-15-iol (**2**), which, to the best of our knowledge, are described as natural products for the first time.

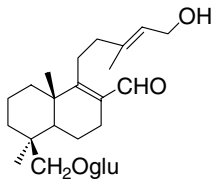
Compound **1** has the molecular formular $C_{25}H_{28}O_{11}$ based on FAB-MS data (*m/z* 511$[M + Li]^+$; 525$[M + Na]^+$) and on counting its ^1H and ^{13}C NMR DEPT spectra. The IR spectrum of compound **1** shows that the typical absorption of the iridoid enol ether system at 1630 cm^{-1} [5], of an aldehyde function at 1661 cm^{-1} and of an ester function at 1710 cm^{-1}. The UV maximum at 301 nm revealed the presence of an α, β unsaturated ester (β-aromatic substituted) [6]; a shoulder at 241 nm was attributed to a conjugated ether system [5]. The ^1H NMR spectrum of **1** shows the presence of a cinnamoyl moiety with one *trans*-olefinic system (δ 6.44 and δ 7.61 J = 16), and shows the signals of one glucose moiety, which revealed sugar protons H-1' at δ 4.60 (d J = 8) and H-6′ at δ 3.56 and δ 3.70 (brd). Compared with the ^1H NMR of tarennoside, theveside and theviridoside [7–8], the singlet at δ 7.36 could be attributed to H-3 in an iridoid substituted at C-5, the signals at δ 5.76 and δ 2.78 (brd) could be assigned to H-1 and H-6. The other signlet signals δ 5.73 (m) and δ 9.19 (s) should be assigned to H-7 and H-11 (the proton of a carbon aldehyde group). These were in good agreement with the document. The only difference lies in the signals of H-10. Comparing the chemical shifts of H-10 and C-10 of **1** with similar compounds like isoscrophularioside, melittoside and 10-*O*-cinnamoylmelittoside [9–11], we concluded that the cinnamoyl was attached to C-10, and this was identified by the HMBC NMR (O=C related to H-10). The ^{13}C NMR spectrum of **1** shows the presence of 25 carbon atoms. The DEPT spectrum of **1** shows 16 methines, 3 methylenes and 9 quarter carbons. Nine of them could be ascribed to the cinnamoyl unit and six carbons assigned to the glucose moiety (Table 1). Two absorptions at δ 164.1 and δ 125.84 could be assigned to C-3 and C-4. The typical signals of a C-4 substituted iridoid glucoside with a carbonaldehyde function at C-4 and a hydroxyl function at C-5. This conclusion was supported by the signals at δ 192.6, δ 75.30 and δ 9.19 (s). Two signals at δ 100.28 and δ 97.36 were attributed to C-1′ and C-1, respectively, on the basic of published values [5]. The signals at δ 136.55 and δ 131.58 were assigned to C-8 and C-7. Compound **1** yielded cinnamaric acid by alkaline hydrolysis, which ascertained further the presence of a cinnamoyl group in **1**.

Compound **2** has molecular formular $C_{26}H_{42}O_8$ by FAB-MS data (*m/z* 469$[M + Li]^+$; 485$[M + Na]^+$) and on counting its ^1H and ^{13}C NMR DEPT spectra. The ^{13}C NMR spectra (DEPT, see Table 1) shows signals for 3 CH_3, 10 CH_2, 8 CH and 5 quartcarbons. Comparison of the chemical shifts with literature data [4, 12, 13] confirmed the presence of a Labdane with a Δ 13, 14 unsaturated side-chain, a free hydroxyl at C-15, an aldehyde function at C-17 and a glucose attached to C-19. The double bond at C-13 was shown to be *E*-configurated by comparison of the chemical shifts of C-12 and C-16 (δ 43.9 and 16.3) with similar compounds [12, 13]. And if the double bond would be *Z*-configurated, the chemical shift of C-12 would be shifted upfield to about δ 35.6, as well as C-16 be shifted downfield to about δ 23.7 [14]. The 2D-$^1H/^1H$ COSY, HMQC and HMBC spectra allowed unambiguous assignment of all proton signals in the ^1H NMR spectrum (Table 2). The absolute stereochemistry of **2** was established by NOSY NMR. It shows that the pro-

Table 1: ^{13}C NMR (400 MHz) of compounds 1 and 2 (TMS as int. standard)



1



2

C	1*	2**	C	1*	2**
1	97.3	36.6	14(3″)	78.3	126.3
2		18.6	15(4″)	71.4	59.0
3	164.1	36.1	16(5″)	77.4	16.3
4	125.8	38.8	17(6″)	62.2	193.1
5	75.3	52.5	18(α)	118.5	27.9
6	45.8	19.1	19(β)	146.1	73.0
7	131.5	25.7	20(O=C)	166.2	20.8
8	136.5	132.3	1′	135.5	104.2
9	56.7	167.5	2′	129.9	74.7
10	62.9	41.5	3′	129.2	76.9
11	192.6	25.4	4′	130.7	71.6
12(1″)	100.2	43.9	5′	129.2	77.9
13(2″)	74.4	136.9	6′	129.9	62.8

* Assigned by $^1H/^1H$COSY and HMBC NMR; Solvent CD_3OD
** Assigned by HMBC, HMQC and NOSY NMR; In C_3D_6O

Table 2: ^1H NMR (400 MHz) of compounds 1 and 2 (TMS as int. standard)

H	1*	2**	H	1*	2**
1α(1)	5.76 s	0.93 m!	15		4.06 d 6.4
1β(3)	7.36 s	1.85 m!	16		1.68 s
2α(6)	2.78 brd	1.35 m!	17		10.05 s
2β(7)	5.73 m	1.73 m!	18		1.09 s
3α(9)	3.06 s	0.97 m!	19		3.99 d 9.6
3β(10)	4.72 m	2.01 m!			3.34 d 9.2
5(11)	9.19 s	1.26 m!	20		1.03 s
6α(α)	6.44 d 16	2.03 m!	1′	4.60 d 8.0	4.23 d 7.6
6β(β)	7.61 d 16	1.54 m!	2′	3.16 t 8.4	3.21 t 7.6
7α(2″)	7.31 d 7.2	1.53 m!	3′	3.24 m	3.29 m
7β(3″)	7.50 m!	2.03 m!	4′	3.19 t 4.8	3.12 m
11α(4″)	7.50 m!	1.90 m!	5′	3.32 m	3.40 m
11β		1.47 m!	6′	3.70 dd 12.0	3.81 d 10.4
12α		2.28 ddd		3.56 dd	3.69 brd
12β		2.1o ddd		12, 6.4	
14		5.42 t 6.4			

* Assigned by $^1H/^1H$COSY and HMBC NMR; Solvent CD_3OD.
** Assigned by HMBC, HMQC and NOSY NMR; In C_{3D6O}.
! Overlapping signals

tons of CH_3-20 have the NOE information with CH_2-19, not with CH_3-18. Thus, the structure of **2** was determined as 17-aldehedeyloxy-19-β-D-glucopranosyloxy-lab-8,13(E)-dien-15-iol.

Experimental

1. Equipment

M.p.s.: X4-microp (The fourth instrument of Beijing) uncorr. Optical rotation: polarimeter 241 (Perkin Elmer) solvent MeOH. IR-spectra were recorded on Nicolet-5DX. IR spectrometer. ^1H, ^{13}C and 2D NMR spectra were recorded at 400 Mhz, solvent CD_3OD and C_3D_6O, using TMS as int. standard. FAB-MS was determined on a ZAB-HS mass spectrometer.

2. Plant material

The plant material was collected from Longling county Yunna province of P.R. China and identified by Prof. Ru-Neng Zhao, Faculty of Pharmacy, Lanzhou Medical colleage of P.R. China. A voucher specimen (no. cler1) has been deposited at the Lab. of Natural Products, Chemistry Department, Lanzhou University, Lanzhou, P.R. China.

3. Extraction and isolation

Air-dried and powdered leaves of *C. serratum* (1 kg) were exhaustively extracted with EtOH at RT. The extract was concentrated under reduced pressure. The residue was supported in H_2O, extracted with petroleum, CH_2Cl_2 and EtOAc, respectively. The extract of CH_2Cl_2 and EtOAc (30 g) was obtained and chromatographed on a silica gel column (200–300 mesh 350 g) with a $CHCl_3$–MeOH gradient as the developing solvent. Combination of the appropriate fractions (monitored by TLC analysis) led to four fractions. From fr.1 ($CHCl_3$–MeOH 20 : 1) a crude oily material was received and purified by rechromatography on a silica gel column (300–400 mesh) with C_6H_6–C_3H_6O–H_2O (4 : 1 : 0.05) twice, to give compounds **1** (30 mg) and **2** (50 mg). The other fractions have earlier been studied [4].

4. Alkaline hydrolysis

Compounds **1** and **2** (10 mg each) were refluxed for 12 h in 5% KOH–MeOH (3 ml). After extraction with EtOAc, the aq. of **1** and **2** were examined for glucose by PC. Then 1% HCl was added to **1** up to pH 2 and the solution was further extracted with $CHCl_3$. The $CHCl_3$ solution was evaporated to dryness and the residue was identified as cinnamaric acid by directed comparison with an authentic sample. The sugar gained from the aq. (**1** and **2** were silylated in pyridine with hexamethyldisilazane and trimethylchlorosilane for 2 min. A GC of the trimethylsilyl derivatives showed that they had the same R_f as an authentic sample.

5. 5-Hydroxyl-10-O-cinnamoyltarennoside (1)

White crystals ($CHCl_3$/MeOH) 10 : 1). m.p. 113–115 °C UV_λ^{MeOH}: 301, 241. IR (KBr, cm^{-1}): 3357(OH); 1710, 1661 (O=C–C=C); 1630 (C=C-O); 1604, 1515 (C=C); 1076, 1023 (glu); 857; 834. FAB-MS m/z: 511 $[M + Li]^+$; 527 $[M + Na]^+$; 342 $[M\text{-glu}]^+$. ^1H and ^{13}C NMR: Tables.

6. 17-Aldehydlyloxy-19-β-D-glucopyranosyloxy-lab-8,13(E)-dien-15-iol (2)

White gum. UV_λ^{MeOH}: 243. IR (KBr, cm^{-1}): 3420, 3318 (OH); 1654 (O=C–C=C); 1435; 1376; 1158; 1073, 1026 (glu); 926. FAB-MS m/z: 469 $[M + Li]^+$; 485 $[M + Na]^+$; 320 $[M\text{-glu}]^+$. ^1H and ^{13}C NMR: Table.

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