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Two new terpenoid glucosides from *Clerodendrum serratum*

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Two new compounds, 7-*o*-*m*-coumaroyloxyugandoside and 19-β-D-glucopyranosyloxy-lab-13(*E*)-en-8α,15-diol, were isolated from the *Clerodendrum serratum*. Their structures were elucidated, mainly by interpretation of their spectroscopic data (UV, IR, MASS, ^{1}H, ^{13}C, ^{1}H/^{1}H-COSY, HMQC and NOSY NMR). This is the first report on labdane diterpene glucosides from *Clerodendrum* species.

1. Introduction

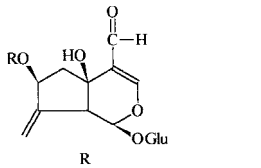
The genus *Clerodendrum* belongs to the subfamily Viticoideae, and is the largest genus of the verbenaceae. Several plants of the genus *Clerodendrum* are well known as folk medicine in China for their antibacterial properties. In southern China, leave extracts are used against hermorrhage, to eliminate inflammation and as a pesticide [1]. *Clerodendrum serratum*, named "san dui jie" in China, a small shrub with fragrant flowers found widely in the forests in the south of China, has been used as a folk medicine in Yunnan for the treatment of many diseases, e.g. hepatitis and malaria [1]. Several works had been done with *C. serratum* [2–3]. From the n-BuOH and EtOAc extracts two compounds, 7-*o*-*p*-coumaroyloxy- and 7-*o*-cinnamoyloxyugandoside, were gained [18]. Now, we isolated another two new compounds and wish to report their structural elucidation as 7-*o*-*m*-coumaroylugandoside and 19-β-D-glucopyranosyloxy-lab-13(*E*)-en-8α,15-diol.

2. Investigations, results and discussion

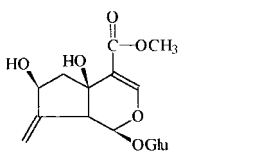
An alcoholic extract of the leaves of *C. serratum* was fractionated as described in the experimental section. From the n-BuOh extract, we afforded compounds **1** and **2**, which, to the best of our knowledge, are described as natural products for the first time. Their structures were elucidated by spectroscopic data and chemical methods. This is the first report on labdane diterpene glucosides from *Clerodendrum* species.

Compound **1** has a molecular formular $C_{25}H_{28}O_{12}$ based on FAB-MS data (*m/z* 527 $[M+Li]^+$; 543 $[M+Na]^+$) and on counting carbons and hydrogens from the data of its ^{1}H and ^{13}C NMR DEPT spectra. The IR of the compound **1** showed the typical absorption of the enol ether system of iridoid at 1625 cm^{-1} [4], of an aldehyde function at 1687 cm^{-1} and of an ester function at 1710 cm^{-1}. The UV maximum at 315 nm revealed the presence of an α, β unsaturated ester (β-aromatic substituted) [5]; a shoulder at 246 was attributable to a conjugated ether system [4]. The ^{1}H NMR spectrum of **1** showed the presence of a *m*-coumaroyl moiety with one trans-olefinic system (δ 6.37 and δ 7.53, J = 16), 3″-OH at δ 10.03 and showed the signals of one glucose moiety, which revealed sugar protons H-1′ at 4.43 (J = 8), the H-6 at 3.63 and 3.69 (*br d*). Compared with the ^{1}H NMR of the ugandoside [6], the singlet at δ 7.54 could be attributed to H-3 in an iridoid substituted at C-5, the signals at δ 5.93 (d J = 1.5) could be assigned to H-1, the other singlet signals δ 5.18 (2 H *s*) and 9.27 (*s*) should be assigned to H-10 and H-11 (the proton of a carbon aldehyde group). These were in good agreement with ugandoside. The only difference lies in the signals of H-6 and H-7. The ^{13}C NMR spectrum of **1** showed the presence of 25 carbon atoms. The DEPT spectrum of **1** showed the presence of 16 methines, 3 methylenes and 9 quarter carbons. Nine of them could be ascribed to the *m*-coumaroyl unit (see Table 1), two absorptions at δ 163.26 and δ 122.16 could be assigned to C-3 and C-4. The typical signal of a C-4 substituted iridoid glucoside with a carbonaldehyde function at C-4 was identified by the signals of δ 190.63 and 9.27 (*s*). Two signals at δ 98.67 and 95.02 were attributed to C-1′ and C-1, respectively, on the basic of published values [4]. The signals at δ 145.21 and 113.51 were assigned to C-8 and C-10. The compound **1** yielded *m*-coumaric acid by alkaline hydrolysis, which ascertained further the presence of a *m*-coumaroyloxy group in **1**. A comparison of the ^{13}C NMR data of the similar known compounds penstemoside, β-dihydrohastatoside with 5-hydroxy-8-epi-loganin, tecomoside, acyl-tecomoside [7–11] resulted in the conclusion that the two former compounds were different from compound **1** (C-7 at δ 72.7, C-6 at δ 40.5). Comparing **1** with **6**, the most data of **1** (Tables 1 and 2) were in good agreement with **6** [18]. However, compound **1** had a *m*-coumaroyl instead of a *p*-coumaroyl moiety.

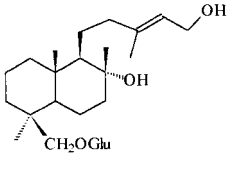
Compound **2** was determined as $C_{26}H_{46}O_8$ by FAB-MS data (*m/z* 509 $[M+Na]^+$; 493 $[M+Li]^+$) and by counting carbons and hydrogens from its ^{13}C NMR DEPT. The ^{13}C NMR spectra (DEPT see Table 1) shows signals for 4 CH_3, 10 CH_2, 8 CH and 3 quartcarbons. It has not been possible to evidence the shielding of C-10 owing to the solvent (DMSO-*d6*) signal pattern, which is superimposed by this signal. Comparison of the chemical shifts with literature data [12–14] confirmed the presence of a labdane with a Δ 13, 14 unsaturated sidechain, two free hydroxyls at C-8 and C-15 and a glucose attached to C-19. The stereochemistry at C-8 (β-CH_3, α-OH) follows from the



1 m-coumaroyl
6 p-coumaroyl



3 strictomoside



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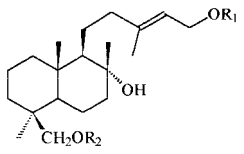

	R_1	R_2
4	OAc	H
5	OAc	OAc

Table 1: ^{13}C NMR (400 MHz) of compounds 1 and 2 (in DMSO-*d6*, TMS as int. standard)

C	1	2**	6*	4	C	1	2**	6*	4
1	95.0	38.4	95.0	39.8	14(3″)	159.9	124.3	114.9	123.6
2		17.7		18.1	15(4″)	115.1	57.6	159.9	59.2
3	163.2	36.2	163.3	36.4	16(5″)	130.4	16.2	114.9	16.4
4	122.1	37.4	122.2	37.0	17(6″)	132.5	23.6	130.5	23.9
5	68.0	56.4	68.0	56.8	18(α)	115.8	27.7	113.7	27.4
6	40.5	20.4	40.6	20.6	19(β)	145.2	71.8	145.2	67.1
7	72.8	44.5	72.7	42.8	20(O=C)	166.4	15.7	166.4	15.9
8	145.6	72.1	145.6	73.8	1′	98.6	103.5	98.7	
9	51.6	61.0	51.6	61.3	2′	71.8	73.5	71.8	
10	113.5		113.5	39.1	3′	75.8	76.6	75.6	
11	190.6	23.8	190.7	23.8	4′	70.1	70.1	70.1	
12(1″)	125.0	42.7	125.0	44.9	5′	77.4	76.7	77.4	
13(2″)	114.9	137.0	130.5	140.5	6′	61.2	61.1	61.2	

* Assigned by HMBC and HMQC NMR
** Assigned by ^{1}H/^{1}H COSY and HMQC NMR

Table 2: ^{1}H NMR (400 MHz) of compounds 1 and 2 (in DMSO-*d6*, TMS as int. standard)

H	1	6*	2**	H	1	6*	2**	5
1α(1)	5.93 *d* 1.5	5.93 *d* 1.1	0.81 *m****	14(6″)	6.76 *d* 8.6	6.76 *d* 8.6	5.21 *t* 6.4	5.25 *t* 7.0
1β(3)	7.54 *s*	7.54 *s*	1.56 *m****	15(OH)	10.03 *s*	10.06 *s*	3.91 *d* 6.4	4.58 *d* 7.6
2α(6α)	3.03 *d* 9.3	3.03 *d* 9.3	1.13 *m****	16			1.56 *s*	1.71 *s*
2β(6β)	1.86 *dd*	1.87 *dd*	1.53 *m****	17			0.96 *s*	1.13 *s*
3α	12.8, 9.6	12.4, 9.6	0.85 *m****	18			0.92 *s*	0.96 *s*
3β(7)	5.18 *m*	5.21 *m*	1.64 *m****	19			3.17 *d* 9.6	3.98 *d* 11.0
5(9)	2.93–2.95	2.95–2.99	0.94 *m****				3.79 *d* 9.6	4.16 *d* 10.9
6α(10)	5.30 *s*	5.32 *s*	1.64 *m****	20			0.73 *s*	0.82 *s*
6β(11)	9.27 *s*	9.26 *s*	1.33 *m****	1′	4.42 *d* 8.0	4.43 *d* 8.0	4.08 *d* 8.0	
7α(α)	6.37 *d* 16	6.37 *d* 16	1.32 *m****	2′	2.93–2.99	2.95–2.99	2.90 *t* 8.2	
7β(β)	7.53 *d* 15.2	7.53 *d* 16	1.67 *m****	3′, 5′	3.14–3.21	3.14–3.19	3.00–3.06	
11α(2″)	7.52 *s*	6.76 *d* 8.4	1.46 *m****	4′	2.93–2.95	2.95–2.99	3.10 *t* 8.4	
11β(3″)		7.54 *d* 8.8	1.12 *m****	6′	3.63 *brd*	3.66 *brd*	3.77 *d* 9.6	
12α(4″)	7.56 *d* 7.2		2.10 *ddd*		3.69 *brd*	3.71 *brd*	3.40 *d* 9.6	
12β(5″)	6.73 *d* 8.8	7.54 *d* 8.8	1.91 *ddd*					

* Assigned by HMBC and HMQC NMR
** Assigned by ^{1}H/^{1}H COSY, HMQC and NOSY NMR
*** Overlapping signals

chemical shifts of C-6 (δ 20.5) which should be shifted upfield to about δ 17 as a consequence of a γ-gauche-effect in the case of a β-axially orientated −OH function [15]. The double bond was shown to be *E*-configurated by comparison of the chemical shifts of C-12 and C-16 (δ 42.7 and 16.2) with **4**, **5** (δ 44.9, 45.0 and 16.4, 16.6) [14] and with the corresponding carbons in nerol(*Z*) and geraniol(*E*) [16] and with Labd-13(*Z*)-8α,15-diol [12] (δ 35.6 and 23.7) and with Labd-13(*E*)-8α,15-diol [17] (δ 44.6 and 16.5). Comparing the ^{13}C NMR data of **2** with the compounds **4** and **5**, we found the most data in good agreement with the document. The only difference lies in the data of C-12 and C-7 changed each other and the C-12 and C-13, their chemical shifts were upfield (see Table 1). The 2D-^{1}H/^{1}H COSY and HMQC spectra allowed unambiguous assignment of all proton signals in the ^{1}H NMR spectrum (Table 1). Interestingly, the spin system of H-15 *a*/*b* in **2**, unlike that of the *E*-isomer of **5** [14] (*dd* δ 4.16 and 4.06), displays one distinct *d* signal at δ 3.91, which indicates that free rotation of the sidechain of **2** is not hindered. Possibly, there is not a hydrogen bond between the free hydroxyls. The absolute stereochemistry of **2** was established by NOSY NMR. It showed that the protons of CH_3-20 had the NOE information with CH_2-19, CH_2-11 and CH_3-17 and the protons of the CH_3-18 had not. Thus, the structure of **2** was determined as 19-β-D-glucopyranosyloxy-Lab-13(*E*)-en-8α,15-diol.

3. Experimental

3.1. Equipment

M.p.s.: X4-micrope (The fourth instrument of Beijing) uncorr. Optical rotation: polarimeter 241 (Perkin Elmer) solvent MeOH. IR-spectra were recorded on Nicolet-5DX. IR spectrometer. ^{1}H, ^{13}C and 2 DNMR spectra were recorded at 400 Mhz, solvent DMSO-*d6*, using TMS as int. standard. EIMS and FAB-MS were determined on a MS50 (A. E. I. Brunner) and a ZAB-HS mass spectrometer.

3.2. Plant material

The plant material was collected from Longling country Yunnan province of P. R. China and was 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.

3.3. Extraction and isolation

Air-dried and powdered leaves of *C. serratum* (Hand-Mass 500 g) 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, EtOAC and BuOH, respectively. The BuOH extract (15 g) was obtained and chromatographed on a silica gel column (200–300 mesh 150 g) with a $CHCl_3$–MeOH–H_2O gradient as the developing solvent. Combination of the appropriate fractions (monitored by TCL analysis) led to three fractions. From fr. 1 ($CHCl_3$–MeOH–H_2O 15:1:0.3) a crude crystalline material was obtained and purified by rechromatography on a silica gel column (300–400 mesh) with C_6H_6–C_3H_6O–H_2O (2:1:0.05), to give compounds **1** (40 mg) and **2** (50 mg).

3.4. Alkaline hydrolysis

Compounds **1** and **2** (10 mg each) in 5% KOH−MeOH (3 ml) were refluxed for 12 h. 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 further extracted with $CHCl_3$. The $CHCl_3$ solution was evaporated to dryness and identified as *m*-coumaric acid by directed comparison with an authentic sample. The sugar gained from 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_t as authentic samples.

3.5. 7-O-m-Coumaroyloxy-ugandoside (1)

White crystals ($CHCl_3$/MeOH 10:1), m.p. 164−165 °C UV_λ^{MeOH}: 315, 246. IR (KBr, cm^{-1}): 3338 (OH); 1687, 1710 (O=C=C); 1625 (C=C−O); 1604, 1515 (C=C); 1073, 1025 (glu); 855; 833. FAB-MS m/z: 543 $[M + Na]^+$; 527 $[M + Li]^+$; 358 $[M\text{-glu}]^+$. ^1H and ^{13}C NMR: Table.

3.6. 19-β-D-Glucopyranosyloxy-lab-13(E)-en-8α,15-diol (2)

White crystals (acetone/$CHCl_3$ 2:1), m.p. 125−126 °C IR (KBr, cm^{-1}): 3444, 3369 (OH); 1455; 1388; 1238; 1166; 1077, 1032 (glu); 934. FAB-Ms m/z: 509 $[M + Na]^+$; 493 $[M + Na]^+$; 324 $[M\text{-glu}]^+$. ^1H and ^{13}C NMR: Table.

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