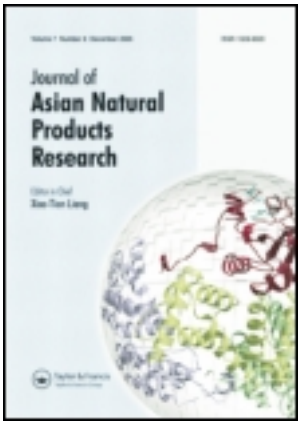

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Two new sesquiterpene glycosides from *Pogostemon cablin*

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Two new rearranged patchoulane sesquiterpene glycosides, 3α-hydroxypatchoulol 3-*O*-β-D-glucopyranoside (**1**) and 15-hydroxypatchoulol 15-*O*-β-D-glucopyranoside (**2**) together with rubusoside, a known diterpene glycoside, were isolated from the ethanol extract of the whole plant of *Pogostemon cablin* (Blanco) Benth. The structures of **1** and **2** were elucidated by spectroscopic analysis.

Keywords: Lamiaceae; *Pogostemon cablin*; sesquiterpene glycoside; 3α-hydroxypatchoulol 3-*O*-β-D-glucopyranoside; 15-hydroxypatchoulol 15-*O*-β-D-glucopyranoside

1. Introduction

Pogostemon cablin (Blanco) Benth (Lamiaceae), a bushy herb known as patchouli, is native to tropical Asia and is now cultivated in South China and other tropical areas worldwide. It is used in traditional Chinese medicine for the treatment of upset stomach, vomiting and diarrhea, headache, and fever [1]. Several medicaments prepared from this plant have been used in clinic in China [2]. Previous phytochemical studies have focused on constituents in the essential oil, which showed the presence of a number of various sesquiterpenes. Patchoulol, a rearranged patchoulane sesquiterpene, was found to be the most abundant constituent and to be the primary component responsible for the typical patchouli aroma [3–6]. Patchoulol was also reported to possess antifungal [7,8] and Ca^{2+} antagonist [9] activities. In continuation of our phytochemical studies on the medicinal plants growing in South China, the hypophilic constituents of this herb were investigated, and two new rearranged patchoulane sesquiterpene glycosides, trivially named 3α-hydroxypatchoulol 3-*O*-β-D-glucopyranoside (**1**) and 15-hydroxypatchoulol 15-*O*-β-D-glucopyranoside (**2**), and the known diterpene glycoside rubusoside (**3**) [10] were isolated and characterized (Figure 1). Herein, we report the isolation and structure elucidation of these new compounds.

2. Results and discussion

Compound **1** was obtained as needle crystal (methanol). The molecular formula was determined as $C_{21}H_{36}O_7$ from ESI-MS ions at *m/z* 423 $[M + Na]^+$, 399 $[M - H]^-$, and 435 $[M + Cl]^-$ as well as the HR-ESI-MS ion at *m/z* 423.2368 $[M + Na]^+$. The ^1H and ^{13}C NMR spectra (Table 1) showed the presence of a β-D-glucopyranosyl moiety [δ_H 5.01 (1H, d, $J = 7.8$); δ_c 102.5, 75.2, 78.5, 71.9, 78.7, 63.0]. Acid hydrolysis of **1** with 1 mol/l HCl afforded β-D-glucose ($[\alpha]_D^{20}$ +46.6, $c = 0.02$, H_2O) that was

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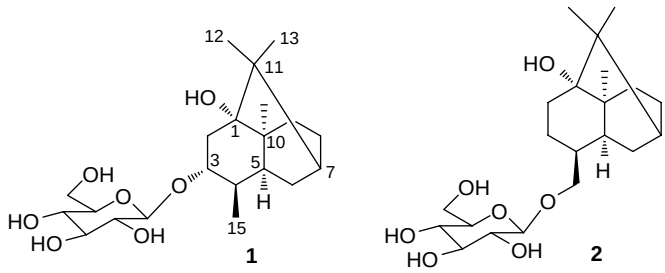

Figure 1. Structures of **1** and **2**.

identified by direct comparison with an authentic sample. Besides, the ^{1}H and ^{13}C NMR spectra and DEPT experiment indicated the presence of four methyl [δ_H 1.02, 1.14, 1.31 (each 3H, s), 1.21 (3H, d, $J = 6.5$ Hz)], four methylene, four methine, and three quaternary carbons, of which a methine (δ_C 78.5) and a quaternary (δ_C 75.9) carbon were oxygenated. Analysis of the $^{1}H-^{1}H$ COSY in combination with HSQC showed connectivities from C-2 to C-9 and of C-4 with C-15 as shown by bold lines in Figure 2. In the HMBC spectrum (Figure 2), long-range correlations were observed from both H_3-12 and H_3-13 to C-11, C-1, and 7, and from H_3-14 to C-1, C-5, C-9, and C-10, indicating that C-12 and C-13 were connected to both C-1 and

Table 1. ^{1}H and ^{13}C NMR spectroscopic data of compounds **1** and **2** (δ in ppm).

	1		**2**	
Position	δ_C	δ_H	δ_C	δ_H
1	75.9		75.0	
2	39.6	α-2.81 (dd, $J = 13.6$, 6.0 Hz) β-2.16 (m)	32.5	1.81 (m), 1.88 (m)
3	78.5	3.93 (td, $J = 10.7$, 10.6, 6.0 Hz)	24.2	1.36 (m), 1.61 (m)
4	35.4	2.10 (m)	34.7	2.33 (m)
5	43.8	1.44 (m)	39.2	1.86 (m)
6	26.4	1.24 (m), 1.45 (m)	25.1	1.24 (m), 1.45 (m)
7	39.1	1.07 (m)	39.5	1.08 (m)
8	24.8	1.18 (m), 1.90 (m)	24.8	1.13 (m), 1.88 (m)
9	28.8	1.06 (m), 2.22 (m)	29.4	0.99 (m), 2.21 (m)
10	37.9		37.6	
11	40.5		40.8	
12	25.4	1.14 (s)	24.8	1.08 (s)
13	27.5	1.31 (s)	28.0	1.28 (s)
14	21.6	1.02 (s)	21.4	1.01 (s)
15	15.5	1.21 (d, $J = 6.5$ Hz)	72.7	3.74 (dd, $J = 9.2$, 6.4 Hz) 4.01 (m)
1′	102.5	5.01 (d, $J = 7.8$ Hz)	105.1	4.87 (d, $J = 7.8$ Hz)
2′	75.2	4.03 (t, $J = 7.8$ Hz)	75.3	4.08 (dd, $J = 8.4$, 7.8 Hz)
3′	78.5	4.16 (m)	78.6	4.29 (dd, $J = 9.3$, 8.4 Hz)
4′	71.9	4.28 (m)	71.8	4.26 (dd, $J = 9.3$, 8.4 Hz)
5′	78.7	4.26 (m)	78.7	4.02 (m)
6′	63.0	4.41(dd, $J = 11.5$, 4.9 Hz) 4.55 (br dd, $J = 11.5$, 1.8 Hz)	63.0	4.43 (dd, $J = 11.6$, 5.2 Hz) 4.61 (dd, $J = 11.6$, 2.0 Hz)

Note: ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR in pyridine-d_5.

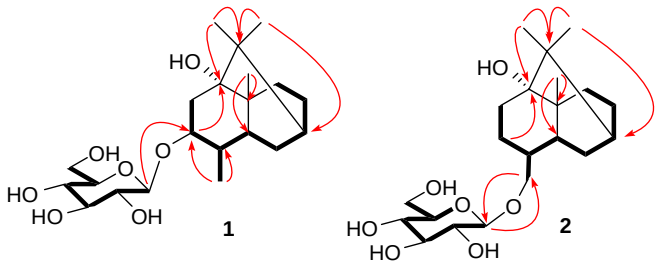

Figure 2. $^{1}H-^{1}H$ COSY (bold line) and main HMBC (arrow) correlations of **1** and **2**.

C-7 via C-11, C-5 was connected with C-10, and C-14 was bound to C-10. On the basis of the above evidence, the aglycone of **1** was deduced to be a rearranged patchoulane sesquiterpene [8,11] with a basic structure closely similar to that of patchoulol [11], except that C-3 was oxygenated in **1**. The glucose moiety was attached to C-3 via a glycosidic linkage, as deduced from the HMBC correlations of H-1′ with C-3 and H-3 with C-1′. The relative configuration of this compound was determined by the NOESY experiment and analysis of the proton coupling constants. In the NOESY spectrum (Figure 3), cross peaks were observed between H-3/H_3-13, H-3/H_3-15, H-2α/H-4, H_3-14/H-2α, and H_3-14/H-4. This in combination with the axial–axial coupling constants between H-3 and H-2 ($J = 6.0$ Hz) and between H-3 and H-4 ($J = 10.6$ Hz) showed a chair form ($_2C^5$)

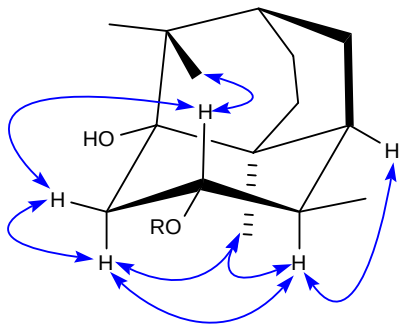

Figure 3. Key NOESY correlations of **1**.

for the six-membered ring consisting of C-1 ~ C-5 and C-10, with H-3 and 10-Me in axial positions, whereas H-5, 4-Me, and 1-OH in equatorial positions. Therefore, the structure of **1** was established as 3α-hydroxypatchoulol 3-*O*-β-D-glucopyranoside (Figure 1).

Compound **2** was obtained as colorless oil. It had the same molecular formula as that of **1**, as determined from the ESI-MS and HR-ESI-MS data. The ^{1}H and ^{13}C NMR spectra (Table 1) were closely similar to those of **1** except for the absence of the resonances for 4-Me and the oxymethine C-3. Instead, the spectra indicated the presence of an oxymethylene [δ_c 72.7, δ_H 3.74 (dd, $J = 9.2$, 6.4) and 4.01]. On the basis of the above evidence, the aglycone of **2** was determined as patchoulan-1, 15-diol [12,13]. Acid hydrolysis of **2** afforded β-D-glucose and patchoulan-1, 15-diol, which were identified by comparison of ^{1}H NMR spectral data with those values in the literature [12,13]. HMBC correlations (Figure 2) were observed from H-15 to C-1′, and from H-1′ to C-15, indicating the attachment of β-glucopyranosyl moiety to C-15. Therefore, the structure of compound **2** was determined as 15-hydroxypatchoulol 15-*O*-β-D-glucopyranoside (Figure 1).

As patchoulol and its derivatives were reported to possess antifungal activity [6,7], the antifungal properties of compounds **1** and **2** against *Botrytis cinerea* were tested, and the result showed that both of them were inactive at 100 ppm. At present, patchouli plants are the only commercial

source of patchoulol, and cost-effective synthetic routes for enantiomeric pure patchoulol have yet to be developed [4]. In our research, compounds **1** and **2** were first obtained from the strong polarity part of the patchouli plant extraction as the precursors of the patchoulol, which cannot be obtained by traditional steam distillation [6]. These findings are valuable for making the most use of the patchouli plants.

3. Experimental

3.1 General experimental procedures

Melting points were determined on a Yanagimoto Seisakusho MD-S2 micro-melting point apparatus and are uncorrected. Optical rotations were obtained on a Perkin-Elmer 341 polarimeter with MeOH as solvent. The IR spectra were measured in KBr on a WQF-410 FT-IR Spectrophotometer. The ^{1}H (400 MHz), ^{13}C (100 MHz), and 2D NMR spectra were recorded on a Bruker DRX-400 instrument using TMS as an internal standard. HR-ESI-MS data were obtained on an API QSTAR mass spectrometer in positive ion mode. ESI-MS data were collected on MDS XCIEX API 2000 LC/GC/MS instrument in positive-ion mode. For column chromatography (CC), silica gel 60 (100–200 mesh, Qingdao Marine Chemical Ltd, Qingdao, China) and Sephadex LH-20 (GE healthcare, Uppsala, Sweden) were used. TLC was performed on precoated plates (Kieselgel 60GF254, Merck, Darmstdt, Germany).

3.2 Plant material

The whole plants of *P. cablin* were purchased from Guangzhou Qingping Professional Traditional Chinese Medicine Market, Guangdong, China, in August 2007, and identified by Prof. Binhui Chen, South China Botanical Garden, Chinese Academy of Sciences, China. An authenticated voucher specimen (no. 299295) has been deposited at the herbarium of South China Institute of Botany, Chinese Academy of Sciences, Guangzhou, China.

3.3 Extraction and isolation

The powdered air-dried whole plants of *P. cablin* (19.5 kg) were extracted with 85% ethanol thrice, each for 48 h at room temperature, and the extract was concentrated under reduced pressure to give 5000 ml of residue. The residue was suspended in water and then sequentially extracted with petroleum ether, EtOAc, and n-BuOH. Then the n-BuOH layer was evaporated under vacuum to yield a BuOH-soluble fraction (480 g). The n-BuOH-soluble fraction was subjected to macroreticular absorbing resin D101 column and eluted with MeOH–H_2O (0, 30%, 70%, 97%) to give four fractions (fraction, weight (g): Fr. 1 > 100; Fr. 2, 78.4; Fr. 3, 33.3; Fr. 4, 21.7). Fraction 2 (78.4 g) was fractionated by silica gel CC with chloroform–methanol (9:1–1:1) to give eight fractions (fr.A–fr.H). Fraction B (4.0 g) was further chromatographed on an ODS column eluted with MeOH–H_2O mixtures of decreasing polarities (3:7 to 9:1) to obtain seven subfractions (B-1–B-7). Subfraction B-3 (180 mg) was further separated by HPLC using 50% MeOH to afford **2** (29 mg). Fraction 3 (33.3 g) was fractionated by silica gel CC with chloroform–methanol (9:1 to 6:4) and methanol to obtain six fractions. Fr.3-3 (1.7 g) was subjected to silica gel CC with petroleum ether–acetone (4:6) to obtain four fractions. The second fraction (78 mg) followed by purification on a Sephadex LH-20 column with MeOH as eluent was separated by HPLC using 60% MeOH to afford **1** (10 mg). Fr.3-4 (2.8 g) was subjected to silica gel CC with petroleum ether–acetone (2:8) to give four fractions. The second fraction (800 mg) was subjected to purification on a Sephadex LH-20 column with MeOH as eluent, then separated by HPLC using 70% MeOH to give compound **3** (5 mg).

*3.3.1 3α-Hydroxypatchoulol 3-O-β-D-glucopyranoside (**1**)*

Colorless needle crystal; mp 188–190°C, $[\alpha]_D^{20}$ −139.5 ($c = 1.0$, MeOH). IR (KBr) ν_{max} 3388, 2933, 1635, 1463, 1382, 1307, 1234 cm^{-1}; ^1H NMR (400 MHz, pyridine-d_5) and ^{13}C NMR (100 MHz, pyridine-d_5) spectral data see Table 1; ESI-MS: *m/z* 423 $[M + Na]^+$ on positive mode, 399 $[M - H]^-$ and 435 $[M + Cl]^-$ on negative mode; HR-ESI-MS: *m/z* 423.2368 $[M + Na]^+$ (calcd for $C_{21}H_{36}O_7Na$, 423.2353).

*3.3.2 15-Hydroxypatchoulol 15-O-β-D-glucopyranoside (**2**)*

Colorless oil; $[\alpha]_D^{20}$ −67.1 ($c = 1.0$, MeOH). IR (KBr) ν_{max} 3394, 2935, 2362, 2134, 1650, 1436 cm^{-1}; ^1H NMR (400 MHz, pyridine-d_5) and ^{13}C NMR (100 MHz, pyridine-d_5) spectral data, see Table 1; ESI-MS: *m/z* 423 $[M + Na]^+$ on positive mode, 399 $[M - H]^-$ and 435 $[M + Cl]^-$ on negative mode; HR-ESI-MS: *m/z* 423.2329 $[M + Na]^+$ (calcd for $C_{21}H_{36}O_7Na$, 423.2353).

3.4 Acid hydrolysis of 1 and 2

Compound **1** (1.8 mg) in 1 mol/l HCl–MeOH was heated at 80°C for 8 h. After cooling, the mixture was extracted with $CHCl_3$. The water layer was neutralized with 8% NaOH and concentrated to afford a pure sugar (0.24 mg). The sugar was confirmed as D-glucose by comparing with an authentic sample on TLC [silica-gel, EtOAc–MeOH–H_2O–AcOH (6.5:2.0:1.5:1.5), $R_f = 0.40$] and by measuring its optical rotation ($[\alpha]_D^{20}$ +46.6, $c = 0.02$, H_2O). Compound **2** was hydrolyzed to give D-glucose (0.40 mg) and patchoulan-1, 15-diol (0.28 mg) by the same method. Patchoulan-1, 15-diol: white solid; mp 80–82°C; $[\alpha]_D^{20}$ −38 ($c = 0.54$, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 0.87 (3H, s), 1.08 (6H, s), 3.47 (2H, $J = 7.3$ Hz); ^{13}C NMR δ 75.9 (C-1), 65.3 (C-15), 40.1 (C-11), 38.8 (C-7), 38.6 (C-5), 37.1 (C-10), 36.6 (C-4), 31.8 (C-2), 28.7 (C-9), 26.7 (C-13), 24.7 (C-6), 24.2 (C-8), 24.1 (C-12), 23.3 (C-3), 20.5 (C-14). ESI-MS: *m/z* 237 $[M - H]^-$, 273 $[M + Cl]^-$.

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