New Patchoulol-Type Sesquiterpenoids from Pogostemon cablin

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Four new patchoulol derivatives, 8α , 9α -dihydroxypatchoulol (1), 3α , 8α -dihydroxypatchoulol (2), 6α -hydroxypatchoulol (3), and 2β ,12-dihydroxypatchoulol (4), were isolated from the aerial part of *Pogostemon cablin* (Labiatae), together with nine known compounds, sesquiterpenoids **5–8** and flavonoids **9–13**. Their structures were elucidated by detailed spectroscopic analysis, using 1D- and 2D-NMR techniques.

Introduction. - Pogostemon cablin (BLANCO) BENTH (Labiatae) is a well-known traditional medicinal plant, called 'Guang-Huo-Xiang' in China. As a native species of the Philippines, Malaysia, and India, it has been introduced into China and cultivated widely in Guangdong, Hainan, Guangxi, Taiwan, and Yunnan. The plant has been used against common colds and as an antifungal agent in some southeastern Asian countries [1]. In China, the aerial part of this herb has been recorded in the Chinese Pharmacopoeia and used to remove dampness, relieve summer-heat and exterior syndrome, stop vomiting, and stimulate the appetite. It was also used as an ingredient of some proprietary Chinese medicines against indigestion, headache, and fever [2]. A number of mono- and sesquiterpenoids [3-5], sesquiterpene hydroperoxides [6], flavonoids [7-9], and alkaloids [10] were reported from the title plant. As part of our continuing research on chemical constituents of Traditional Chinese Medicine (TCM), a detailed phytochemical investigation on the involatile fraction of P. cablin was carried out, which led to the isolation of four new patchoulol-type sesquiterpenoids, $8\alpha,9\alpha$ dihydroxypatchoulol (1), 3α , 8α -dihydroxypathoulol (2), 6α -hydroxypatchoulol (3), and $2\beta_1$ 2-dihydroxypathoulol (4), in addition to nine known compounds, 5–13. Here, we describe the isolation and structural elucidation of the new compounds based on detailed spectroscopic analyses.

Results and Discussion. – The MeOH extract of the dried aerial parts of *P. cablin* was suspended in H₂O and extracted with petroleum ether. The H₂O layer was concentrated and then subjected to *Diaion HP20SS*, *MCI-gel CHP 20P*, *Chromatorex ODS*, *Rp-8*, and silica-gel column chromatographies, to afford 13 compounds, 1–13 (*Fig. 1*). Of them, compounds 5-8 were identified as known sesquiterpenoids, *i.e.*, (5R)-5-hydroxypathoulol (5) [11], (9R)-9-hydroxypathoulol (6) [11], (8S)-8-hydroxypathoulol (7) [11], and (3R)-3-hydroxypathoulol (8) [11], and 9-13 as known

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flavonoids, *i.e.*, 5-hydroxy-7,3',4'-trimethoxyflavanone (9) [12], 5-hydroxy-7,4'-dimethoxyflavanone (10) [12], 5,4'-dihydroxy-7,3'-dimethoxyflavanone (11) [13], 3,5-dihydroxy-7,4'-dimethoxyflavone (12) [14], and 5,4'-dihydroxy-3,7,3'-trimethoxyflavone (13) [12], by comparison of their spectroscopic data with those reported in the literature.



Fig. 1. Structures of compounds 1-13

Compound **1** was obtained as a white amorphous powder. The molecular formula was determined as $C_{15}H_{26}O_3$ by the HR-ESI-MS (m/z 277.1779 ([M + Na]⁺); calc. 277.1779) and ¹³C-NMR (DEPT) spectra. On the basis of 1D- and 2D-NMR spectral data, compound **1** was determined as $8\alpha,9\alpha$ -dihydroxypatchoulol.

The IR spectrum of **1** showed the presence of OH groups (3420 cm^{-1}). The ¹H-NMR spectrum of **1** (*Table*) displayed three Me *singlets* at $\delta(H)$ 1.10 (*s*), 1.19 (*s*), and 1.10 (*s*), one Me *doublet* at $\delta(H)$ 0.83 (*d*, J = 6.6), as well as some aliphatic CH₂ *multiplets*. In the ¹³C-NMR and DEPT spectra of **1** (*Table*), 15 C-atom signals were observed, including those of four Me groups ($\delta(C)$ 17.9, 19.1, 25.6, and 26.7), three CH₂ groups ($\delta(C)$ 32.3, 28.8, and 16.4), five CH groups including two O-bearing ones ($\delta(C)$ 28.7, 41.8, 46.2, 74.9, and 85.1), and three quaternary C-atoms including one O-bearing C-atom ($\delta(C)$ 42.1, 43.0, and 77.1). These NMR features were similar to those of (8*S*)-8-hydroxypatchoulol (**7**) [11], a known patchoulol-type sesquiterpenoid with tricyclic skeleton, which was also isolated in the current study. The main differences between **1** and (8*S*)-8-hydroxypatchoulol (**7**) are the molecular weight, and the chemical shifts of the H- and C-atoms associated with C(8), C(9), and C(10). The molecular weight of **1** was 16 Da higher than that of (8*S*)-8-hydroxypatchoulol (**7**). In the ¹³C-NMR spectrum of **1**, the chemical shifts of C(8), C(9), and C(10) were downshifted to $\delta(C)$ 74.9, 85.1,

Position	1 ^a)		2 ^a)		3 ^a)		4 ^b)	
	$\delta(C)$	$\delta(H)$	$\delta(C)$	$\delta(H)$	$\delta(C)$	$\delta(H)$	$\delta(C)$	$\delta(H)$
1	77.1		76.9		76.5		77.2	
2	32.3	1.68 - 1.69(m),	42.7	2.06 - 2.07 (m),	32.9	1.68 - 1.69(m),	76.6	3.61 (dd,
		1.63 - 1.64 (m)		1.51 - 1.52 (m)		1.51 - 1.52 (m)		J = 3.0, 2.8)
3	28.8	1.49 - 1.50 (m),	72.9	3.57 - 3.58(m)	31.0	1.55 - 1.56 (m),	35.1	2.09 - 2.10(m),
		1.37 - 1.38(m)				1.28 - 1.29 (m)		1.75 - 1.76(m)
4	28.7	2.05 - 2.06(m)	37.7	1.74 - 1.75(m)	28.5	2.03 - 2.04(m)	38.4	1.31 - 1.32 (m)
5	41.8	1.24 - 1.25(m)	44.3	1.49 - 1.51 (m)	48.5	1.21 - 1.22 (m)	36.3	1.45 - 1.46 (m)
6	16.4	1.21 - 1.22 (m),	17.8	1.88 - 1.89(m),	66.8	3.86 (br. s)	22.5	1.56 - 1.57(m),
		1.66 - 1.68 (m)		1.11 - 1.12 (m)				1.30 - 1.31 (m)
7	46.2	1.40 (<i>m</i>)	46.3	1.48 - 1.49 (m)	54.7	1.15 - 1.16 (m)	35.9	2.10 - 2.12 (m)
8	74.9	4.23 (br. d,	66.4	4.15 (dd,	16.3	1.87 - 1.88 (m),	23.8	1.46 - 1.48 (m),
		J = 3.8)		J = 9.0, 4.3)		1.58 - 1.59 (m)		1.23 - 1.24 (m)
9	85.1	3.15 (d, J = 3.8)	39.6	2.27 (dd,	29.3	1.89 - 1.90 (m),	31.4	1.73 - 1.74(m),
				J = 15.4, 9.0),		1.02 - 1.04 (m)		1.56 - 1.57 (m)
				0.93 - 0.94(m)				
10	43.0		39.7		39.8		40.3	
11	42.1		41.4		39.9		40.2	
12	19.1	0.83 (d, J = 6.6)	15.4	0.99 (d, J = 6.6)	19.1	0.94 (d, J = 6.7)	65.4	3.47 (d, J = 6.7)
13	17.9	1.10(s)	21.3	0.87(s)	21.4	0.84(s)	17.4	0.93(s)
14	25.6	1.19(s)	25.6	1.10(s)	24.4	1.09(s)	24.5	1.12(s)
		1 10 ()	265	1.02 (s)	27.8	1.08(s)	267	1.17(s)

Table. ¹³C- and ¹H-NMR Data of Compounds 1-4 (500 MHz and 125 MHz for ¹H- and ¹³C-NMR, respectively, δ in ppm, J values in Hz)

and 43.0, respectively, instead of $\delta(C)$ 66.7, 40.6, and 39.6 for (8*S*)-8-hydroxypatchoulol. These data indicated the presence of one additional OH group at C(9) or C(10) in **1**. The additional OH group was located at C(9), on the basis of the HMBCs of H–C(9) ($\delta(H)$ 3.15) with C(10), C(13), C(8), and C(7), and the ¹H,¹H-COSY correlations of –CH(O)–CH(O)–CH– (*Fig.* 2). The relative configuration of compound **1** was determined on the basis of the coupling constant data and the key ROESY correlations (*Fig.* 3). A small coupling constant (3.5 Hz) of two O-bearing CH groups, *i.e.*, H–C(9) ($\delta(H)$ 3.15) and H–C(10) ($\delta(H)$ 4.23), was observed, indicating that the two OH groups at C(9) and C(10) had the same orientation. In addition, the ROESY correlations of H–C(8) and H–C(9) with Me(15) ($\delta(H)$ 1.10 (*s*)) indicated that the two



Fig. 2. Selected ¹H, ¹H-COSY (—) and HMBC (\rightarrow) correlations for 1–4



Fig. 3. Selected ROESY (\leftrightarrow) correlations for 1-4

OH groups at C(8) and C(9) were α -oriented (*Fig. 3*). On the basis of the above data, the relative configuration of **1** was established as shown in *Fig. 1*.

Compound **2** was obtained as an off-white powder. The HR-ESI-MS (m/z 277.1787 ($[M + Na]^+$)) and ¹³C-NMR (DEPT) data indicated the molecular formula $C_{15}H_{26}O_3$ for **2**, the same as for compound **1**. According to 1D- and 2D-NMR spectral data, **2** was established to be 3α , 8α -dihydroxypatchoulol.

In the IR spectrum of **2**, the absorption band at 3424 cm⁻¹ indicated the presence of OH groups. The ¹H- and ¹³C-NMR data of **2** suggested, as in the case of **1**, compound **2** also had one more OH group compared to compound **7**. The location of the additional OH group was assigned to be C(3) in **2**, based on the HMBCs and ¹H,¹H-COSY correlations (*Fig.* 2). The ROESY correlations between H–C(3) and Me(12), Me(14), H–C(8) and Me(15), Me(13) established the relative configuration of both HO–C(3) and HO–C(8) as α (*Fig.* 3).

Compound **3** was obtained as jasmine oil. Its molecular formula, with three degrees of unsaturation, was established as $C_{15}H_{26}O_2$ by the HR-ESI-MS (m/z 239.1068 [M + H]⁺; calc. 239.1072) and ¹³C-NMR (DEPT) spectra. The structure of **3** was established as 6α -hydroxypatchoulol on the basis of 1D- and 2D-NMR analyses.

The IR absorption at 3442 cm⁻¹ revealed the presence of OH groups. In the ¹H-NMR spectrum, four Me signals at $\delta(H) 1.08(s)$, 1.09(s), 0.84(s), and 0.94(d, J = 6.5) were observed. The ¹³C-NMR and DEPT spectra revealed the presence of 15 C-atoms including three quaternary C-atoms ($\delta(C)$ 76.5, 39.8, and 39.9). These characteristic data were similar to those of patchoulol [11]. Careful comparison of the two compounds suggested the molecular weight of **3** was 16 Da higher than that of patchoulol, and the signals of C(5), C(6), C(7) of **3** in the ¹³C-NMR spectrum were shifted downfield to $\delta(C)$ 48.5, 66.8, and 54.7, respectively, instead of $\delta(C)$ 43.7, 24.6, and 39.1 for patchoulol. These data indicated the presence of one additional OH group at C(6) in **3**, which was confirmed by the HMBCs of H–C(6) ($\delta(H)$ 3.86 (br. *s*)) with C(4) ($\delta(C)$ 28.5) and C(8) ($\delta(C)$ 16.3), and the ¹H,¹H-COSY correlations of –CH–CH(O)–CH–(*Fig. 2*). The relative configuration at C(6) was established as OH being *a*-oriented by the ROESY correlations of H_β–C(6) with Me(12) ($\delta(H)$ 0.94) and Me(14) ($\delta(H)$ 1.09) (*Fig. 3*).

Compound **4** was obtained as an off-white amorphous powder. The $[M + Na]^+$ ion peak at m/z 277.1781 in HR-ESI-MS and ¹³C-NMR (DEPT) spectra provided the molecular formula of C₁₅H₂₆O₃ for **4**, which is an isomer of **1** and **2**. Based on 1D- and 2D-NMR spectral data, **4** was established as 2β ,12-dihydroxypathoulol.

An IR absorption band at 3405 cm⁻¹ was observed for the OH groups. In the ¹H-NMR spectrum, three Me signals (δ (H) 0.93 (s), 1.12 (s), and 1.17 (s)), one signal for an O-bearing CH group (δ (H) 3.61 (dd, J = 3.0, 2.8)), and one signal for an O-bearing CH₂ group (δ (H) 3.5 (d, J = 6.7)) were observed. The ¹³C-NMR and DEPT spectra showed 15 C-atom signals, including those of three Me groups (δ (C) 17.4, 24.5 and 26.7) and one O-bearing quaternary C-atom (δ (C) 77.2). This NMR evidence suggested the similarity of **4** to (2*S*)-2,14-dihydroxypatchoulol, a known patchoulol-type sesquiterpenoid [11]. The major differences between **4** and (2*S*)-2,14-dihydroxypatchoulol were the chemical shifts of the H- and C-atoms associated with C(12) and C(14). Unlike (2*S*)-2,14-dihydroxypatchoulol, the oxygenation in **4** occurred at C(12), which was revealed by the absence of the Me *doublet* in the ¹H-NMR spectrum of **4**. Furthermore, the HMBCs of H–C(12) (δ (H) 3.47 (d, J = 6.7)), with C(3), C(5), and C(6), and the ¹H,¹H-COSY correlations of –CH₂(O)–CH– (*Fig. 2*) confirmed the location of the OH group at C(12) in **4**. The ROESY correlations (*Fig. 3*) of H–C(2) with Me(13) (δ (H) 0.93 (s)) indicated that the OH group linked to C(2) was β -oriented.

Experimental Part

General. Column chromatography (CC): silica gel (SiO₂; 200–300 mesh; Qingdao Marine Chemical Factory); Diaion HP20SS (Mitsubishi Chemical Industry, Ltd.); MCI gel CHP20P (75–150 µm; Mitsubishi Chemical Industry, Ltd.); and Chromatorex ODS (100–200 mesh; Fuji Silysia Chemical Co., Ltd.). TLC: silica gel G pre-coated plates (Qingdao Haiyan Chemical Co.), with CHCl₃/MeOH/H₂O 7:3:0.5; visualization by spraying with 10% H₂SO₄ reagent, followed by heating. Optical rotations: SEPA-3000 automatic digital polarimeter. IR Spectra: Bio-Rad FTS-135 spectrometer; in cm⁻¹. 1D- and 2D-NMR spectra: Bruker DRX-500 MHz instrument, with TMS as internal standard. MS: VG Autospect 3000 spectrometer; in m/z.

Plant Material. The aerial parts of *Pogostemon cablin* were collected at Guangzhou, Guangdong Province, P. R. China. A voucher specimen is deposited with the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The air-dried aerial parts of *Pogostemon cablin* (11.0 kg) were exhaustively extracted three times by MeOH at r.t. to give an extract (*ca.* 342 g), which was suspended in H₂O and then partitioned with petroleum ether (PE). After concentration *in vacuo*, the H₂O layer (*ca.* 235 g) was chromatographed over *Diaion HPSS 20* to give ten fractions, *Frs.* 1-10. *Fr.* 2 was repeatedly subjected to CC (SiO₂; CHCl₃/MeOH 100:0 to 80:20; and *RP-8*; 80% MeOH) to give compound **1** (12 mg). The same method was applied to *Fr.* 3 and gave compounds **2** (3 mg) and **4** (4 mg). Repeated CC of *Frs.* 4-6 (SiO₂; CHCl₃/MeOH 100:0 to 80:20; and *RP-8*; 70% –80% MeOH) to yield compounds **3** (5 mg), **5** (13 mg), **6** (9 mg), **7** (14 mg), and **8** (6 mg). *Frs.* 7-10 were subjected to CC (SiO₂; PE/AcOEt 9:1–7:3; and *MCI* gel *CHP20P*; 60% –80% MeOH) to yield five flavonoids, *i.e.*, **9** (43 mg), **10** (127 mg), **11** (26 mg), **12** (19 mg), and **13** (21 mg).

 8α ,9 α -Dihydroxypatchoulol (= rel-(4S,6R,7R,8S,8aR)-Octahydro-4,8a,9,9-tetramethyl-1,6-methanonaphthalene-1,7,8(2H)-triol; **1**). White amorphous powder. $[\alpha]_{25}^{25} = -56.4$ (c = 0.016, CHCl₃). IR (KBr): 3420, 2953, 2929, 2873, 1638, 1470, 1455, 1054, 757. ¹H- and ¹³C-NMR: see the *Table*. ESI-MS (pos.): 277 ($[M + Na]^+$). HR-ESI-MS (pos.): 277.1779 ($[M + Na]^+$, C₁₅H₂₆O₃Na⁺; calc. 277.1779).

3a,8a-Dihydroxypathoulol (=rel-(3R,4R,6R,7S,8aS)-Octahydro-4,8a,9,9-tetramethyl-1,6-methanonaphthalene-1,3,7(2H)-triol; **2**). Off-white powder. $[a]_{15}^{25} = -50.8$ (c = 0.012, CHCl₃). IR (KBr): 3424, 2956, 2930, 2876, 1638, 1459, 1382, 1089, 1074, 1030. ¹H- and ¹³C-NMR: see the *Table*. ESI-MS (pos.): 277 ($[M + Na]^+$). HR-ESI-MS (pos.): 277.1787 ($[M + Na]^+$, C₁₅H₂₆O₃Na⁺; calc. 277.1779).

6β-Hydroxypathoulol (= rel-(4\$,5\$,6\$,8a\$)-Octahydro-4,8a,9,9-tetramethyl-1,6-methanonaphthalene-1,5(2H)-diol; **3**). Jasmine oil. $[a]_D^{25} = -71.7$ (c = 0.0084, CHCl₃). IR (KBr): 3442, 2955, 2924, 2855, 1712, 1642, 1378, 1046. ¹H- and ¹³C-NMR: see the *Table*. ESI-MS (pos.): 239 ($[M + H]^+$). HR-ESI-MS (pos.): 239.1068 ($[M + H]^+$, C₁₅H₂₇O₂⁺; calc. 239.1072).

 $2\beta_{12}$ -Dihydroxypathoulol (=rel-(2R,4S,6R,8aS)-Octahydro-4-(hydroxymethyl)-8a,9,9-trimethyl-1,6-methanonaphthalene-1,2(2H)-diol; **4**). Off-white amorphous powder. $[\alpha]_{25}^{25} = -89.3$ (c = 0.016, CHCl₃). IR (KBr): 3405, 2936, 2876, 1630, 1455, 1384, 1045, 1029. ¹H- and ¹³C-NMR: see the *Table*. ESI-MS (pos.): 277 ($[M + Na]^+$). HR-ESI-MS (pos.): 277.1781 ($[M + Na]^+$, $C_{15}H_{26}O_3Na^+$; calc. 277.1779).

We are grateful to the members of the Analytical Group of State Key Laboratory of Phytochemistry and Plant Resource in West China, Kunming Institute of Botany, Chinese Academy of Sciences, for recording all spectra. This work was supported by the grants from *NSFC* (U0632010, 2008ZX09401, and 2011CB915503) and the *State Key Laboratory of Phytochemistry and Plant Resources in West China, Chinese Academy of Sciences* (P2008-ZZ08).

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Received April 11, 2010