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New terpenoid glycosides obtained from *Rosmarinus officinalis* L. aerial parts

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ABSTRACT

Five new terpenoid glycosides, named as officinoterpenosides A_1 (**1**), A_2 (**2**), B (**3**), C (**4**), and D (**5**), together with 11 known ones, (1*S*,4*S*,5*S*)-5-exo-hydrocamphor 5-O-β-D-glucopyranoside (**6**), isorosmanol (**7**), rosmanol (**8**), 7-methoxyrosmanol (**9**), epirosmanol (**10**), ursolic acid (**11**), micromeric acid (**12**), oleanolic acid (**13**), niga-ichigoside F_1 (**14**), glucosyl tormentate (**15**), and asteryunnanoside B (**16**), were obtained from the aerial parts of *Rosmarinus officinalis* L. Their structures were elucidated by chemical and spectroscopic methods (UV, IR, HRESI-TOF-MS, 1D and 2D NMR). Among the new ones, **1** and **2**, **3** and **4** are diterpenoid and triterpenoid glycosides, respectively; and **5** is a normonoterpenoid. For the known ones, **6** was isolated from the *Rosmarinus* genus first, and **15**, **16** were obtained from this species for the first time.

1. Introduction

Rosmarinus officinalis L. (Lamiaceae), popularly known as Rosemary in English and 迷迭香 in Chinese, is a shrub widely distributed in Europe, Asia, and Africa. And one of its elective growing areas is the Mediterranean basin where spontaneous plants are diffusely distributed. Rosemary has been traditionally used as a culinary spice, mainly to modify or to improve food flavors as well as in folk medicine, being a greatly valued medicinal herb [1]. Nowadays, it is one of the most appreciated sources of natural bioactive compounds which are of special interest in functional food industries. In fact, this plant exerts various pharmacological activities, such as hepatoprotective [2], antibacterial [3], antithrombotic [4], antiulcerogenic [5], diuretic [6], antidiabetic [7], antinociceptive [8], anti-inflammatory [9], antitumor [10], and antioxidant [11] activities.

The two types of compounds that are mainly responsible for the biological activities of this plant are the volatile fraction and the phenolic constituents. The derived essential oils are mainly used in local application for their balsamic, antispasmodic and anti-inflammatory activities [12]. The phenolic constituents are mainly constituted by three groups: phenolic diterpenes of an abietic acid related structures (carnosol, carnosic acid, rosmadial or rosmanol, etc.), and flavonoids (genkwanin, cirsimaritin) derived from two common flavones: apigenin and luteolin, and phenolic acids (rosmarinic acid) [13]. Some scientists have observed that among these constituents, carnosic acid, carnosol, and abietane diterpenes are the main antioxidant compounds present in Rosemary [14]. Are there any other active terpenoids in the plant? And then, the phytochemical research for it was developed. As a result, 16 terpenoids including five new ones, officinoterpenosides A_1 (**1**), A_2 (**2**), B (**3**), C (**4**), and D (**5**), together with 11 known isolates, (1*S*,4*S*,5*S*)-5-exo-hydrocamphor 5-*O*-β-D-glucopyranoside (**6**) [15], isorosmanol (**7**) [16], rosmanol (**8**) [17], 7-methoxyrosmanol (**9**) [17], epirosmanol (**10**) [18], ursolic acid (**11**) [19], micromeric acid (**12**) [20], oleanolic acid (**13**) [21], niga-ichigoside F_1 (**14**) [22],

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glucosyl tormentate (**15**) [23], and asteryunnanoside B (**16**) [24] were isolated and identified. Among the new ones, **1** and **2**, **3** and **4** are diterpenoid and triterpenoid glycosides, respectively; and **5** is a normonoterpenoid. This paper deals with the isolation and structure elucidation of the new compounds.

2. Experimental

2.1. General

Optical rotations were measured on a Rudolph Autopol® IV automatic polarimeter. IR spectra were recorded on a Varian 640-IR FT-IR spectrophotometer. UV spectra were obtained on a Varian Cary 50 UV–Vis spectrophotometer. NMR spectra were determined on a Bruker 500 MHz NMR spectrometer at 500 MHz for ^{1}H and 125 MHz for ^{13}C NMR, with TMS as an internal standard. Positive- and Negative-ion HRESI-TOF-MS were recorded on an Agilent Technologies 6520 Accurate-Mass Q-Tof LC/MS spectrometer.

Column chromatographies (CC) were performed on macroporous resin D101 (Haiguang Chemical Co., Ltd., Tianjin, China), Silica gel (74–149 μm, Qingdao Haiyang Chemical Co., Ltd., Qingdao, China), and ODS (50 μm, YMC Co., Ltd., Tokyo, Japan). Preparative HPLC (PHPLC) column (Cosmosil $5C_{18}$-MS-II (20 mm i.d. × 250 mm, Nakalai Tesque, Inc., Tokyo, Japan)) were used to purify the constituents. Pre-coated TLC plates with Silica gel GF_{254} (Tianjin Silida Technology Co., Ltd., Tianjin, China) were used to detect the purity of isolates achieved by spraying with 10% aqueous H_2SO_4–EtOH, followed by heating.

2.2. Plant material

The dried aerial parts of *R. officinalis* were collected from Butarie, Rwanda and identified by Dr. Li Tianxiang (The Hall of TCM Specimens, Tianjin University of TCM, China). The voucher specimen was deposited at the Academy of Traditional Chinese Medicine of Tianjin University of TCM (No. 20110910).

2.3. Extraction and isolation

The dried aerial parts of *R. officinalis* (2.5 kg) were refluxed with 95% EtOH. The solvent was evaporated under reduced pressure to yield the 95% EtOH extract (455 g). Then, the extract (379 g) was partitioned in a $CHCl_3$–H_2O mixture (1:1, v/v) to give both $CHCl_3$ (269 g) and H_2O (100 g) partitions. Then, the H_2O layer (100 g) was subjected to D101 macroporous resin column chromatography (CC) and eluted with H_2O and 95% EtOH, successively. As a result, H_2O (47 g) and 95% EtOH (45 g) eluted fractions were obtained.

The EtOH fraction (36 g) was subjected to normal phase silica gel CC [$CHCl_3$ → $CHCl_3$–MeOH (100:3 → 100:5 → 100:7, v/v) → $CHCl_3$–MeOH–H_2O (10:3:1 → 7:3:1, v/v/v) → MeOH] to yield 11 fractions (Fr. 1–11).

Fraction 7 (5.5 g) was subjected to ODS CC [MeOH–H_2O (20:80 → 30:70 → 40:60 → 50:50 → 60:40 → 70:30 → 100:0, v/v)] to yield 9 fractions (Fr. 7-1–7-9). Fraction 7-5 (1610.0 mg) was also purified by PHPLC [CH_3CN–1% CH_3COOH (18:82, v/v)], as a result, 19 fractions (Fr. 7-5-1–7-5-19) were obtained. Fraction 7-5-6 (36.8 mg) was subjected to PHPLC [CH_3CN–1% CH_3COOH (10:90, v/v)] to offer (1*S*,4*S*,5*S*)-5-exo-hydrocamphor 5-*O*-*β*-D-glucopyranoside (**6**, 3.5 mg). Fraction 8 (5480.0 mg) was subjected to PHPLC through gradient elution [MeOH–H_2O (30:70 → 50:50 → 70:30 → 100:0, v/v)] to yield 22 fractions (Fr. 8-1–8-22). Fraction 8-9 (121.6 mg) was purified by PHPLC [CH_3CN–H_2O (11:89, v/v)] to yield officinoterpenoside D (**5**, 44.0 mg). Fraction 8-21 (70.3 mg) was purified by PHPLC [CH_3CN–H_2O (28:72, v/v)] to yield glucosyl tormentate (**15**, 3.8 mg). Fraction 9 (10.0 g) was separated by ODS CC [MeOH–H_2O (20:80 → 30:70 → 40:60 → 50:50 → 60:40 → 70:30 → 100:0, v/v)] to yield 14 fractions (Fr. 9-1–9-14). Fraction 9-10 (1510.0 mg) was purified by Sephadex LH-20 CC [$CHCl_3$–MeOH (1:1, v/v)] to yield 8 fractions (Fr. 9-10-1–9-10-8). Fraction 9-10-2 (512.2 mg) was subjected to PHPLC [MeOH–1% CH_3COOH (45:55, v/v)] to obtain 14 fractions (Fr. 9-10-2-1–9-10-2-14). Fraction 9-10-2-11 (85.0 mg) was purified by PHPLC [CH_3CN–1% CH_3COOH (23:77, v/v)] to yield officinoterpenoside C (**4**, 6.1 mg) and niga-ichigoside F_1 (**14**, 34.7 mg). Fraction 10 (6.3 g) was subjected to PHPLC through gradient elution [MeOH-H_2O (25:75 → 40:60 → 60:40 → 80:20 → 100:0, v/v)] to yield 35 fractions (Fr. 10-1–10-35). Fraction 10-26 (93.7 mg) was purified by PHPLC [CH_3CN–1% CH_3COOH (16:84, v/v)] to yield officinoterpenoside A_2 (**2**, 7.1 mg). Fraction 10-27 (756.1 mg) was subjected to Sephadex LH-20 CC (MeOH) to yield 9 fractions (Fr. 10-27-1–10-27-9). Fraction 10-27-3 (217.5 mg) was purified by PHPLC [CH_3CN–1% CH_3COOH (18:82, v/v)] to obtain officinoterpenosides A_2 (**2**, 10.7 mg) and A_1 (**1**, 41.2 mg). Fraction 10-31 (198.5 mg) was separated by PHPLC [CH_3CN–1% CH_3COOH (26:74, v/v)] to give 5 fractions (Fr. 10-31-1–10-31-5). Fraction 10-31-2 (14.1 mg) was purified by Sephadex LH-20 (MeOH), and officinoterpenoside B (**3**, 8.2 mg) was obtained. Asteryunnanoside B (**16**, 6.3 mg) was isolated from fraction 10-33 (132.4 mg) by PHPLC [CH_3CN–1% CH_3COOH (28:72, v/v)].

The $CHCl_3$ partition (200 g) of the rosemary extract was subjected to silica gel CC [$CHCl_3$ → $CHCl_3$–MeOH (100:1 → 100:3 → 100:5 → 100:7, v/v) → $CHCl_3$–MeOH–H_2O (10:3:1 →

Table 1
^{1}H and ^{13}C NMR data for **1** in CD_3OD.

No.	δ_C	δ_H (J in Hz)	No.	δ_C	δ_H (J in Hz)
1	35.8	1.27 (ddd, 3.0, 13.5, 13.5)	17	22.6	1.18 (d, 6.5)
		3.31 (m, overlapped)	18	28.5	1.02 (s)
2	28.4	1.67 (m), 1.76 (m)	19	16.0	0.92 (s)
3	78.4	3.18 (dd, 4.5, 11.5)	20	17.7	1.40 (s)
4	40.1	–	1′	105.3	4.74 (d, 8.0)
5	51.3	1.69 (dd, 3.0, 14.0)	2′	82.9	3.87 (dd, 8.0, 9.0)
6	36.2	2.58 (dd, 3.0, 17.0)	3′	77.6	3.75 (dd, 9.0, 9.0)
		2.62 (dd, 14.0, 17.0)	4′	70.6	3.52 (dd, 9.0, 9.0)
7	200.9	–	5′	78.3	3.30 (m)
8	129.6	–	6′	62.0	3.75 (dd, 4.5, 12.0)
9	140.8	–			3.83 (br. d, *ca.* 12)
10	41.3	–	1″	105.4	4.86 (d, 8.0)
11	148.8	–	2″	75.6	3.43 (m, overlapped)
12	150.2	–	3″	77.5	3.43 (m, overlapped)
13	132.5	–	4″	71.1	3.42 (dd, 9.0, 9.0)
14	122.2	7.46 (s)	5″	78.4	3.34 (m)
15	41.0	2.63 (m, overlapped)	6″	62.4	3.68 (dd, 5.0, 12.0)
		3.30 (dd, 6.0, 13.0)			3.83 (br. d, *ca.* 12)
16	68.0	4.16 (m)			

Table 2
^1H and ^{13}C NMR data for **2** in CD_3OD.

No.	δ_C	δ_H (J in Hz)	No.	δ_C	δ_H (J in Hz)
1	35.8	1.42 (ddd, 4.0, 14.0, 14.0)	16	68.3	4.12 (m)
		3.43 (ddd, 4.0, 4.0, 14.0)	17	22.9	1.11 (d, 6.5)
2	27.7	1.88 (m)	18	28.3	1.13 (s)
		2.11 (m)	19	16.6	1.02 (s)
3	89.6	3.31 (dd, 4.5, 11.5)	20	17.6	1.41 (s)
4	40.4	–	1′	106.7	4.35 (d, 8.5)
5	51.6	1.79 (dd, 3.0, 14.0)	2′	75.6	3.22 (dd, 8.5, 9.0)
6	36.0	2.57 (dd, 3.0, 17.0)	3′	78.2	3.34 (dd, 9.0, 9.0)
		2.62 (dd, 14.0, 17.0)	4′	71.6	3.32 (dd, 9.0, 9.0)
7	201.2	–	5′	77.7	3.26 (m)
8	129.7	–	6′	62.8	3.67 (dd, 5.0, 12.0)
9	140.9	–			3.86 (dd, 2.0, 12.0)
10	41.2	–	1″	107.6	4.56 (d, 8.0)
11	149.3	–	2″	75.3	3.52 (dd, 8.0, 9.0)
12	150.6	–	3″	77.9	3.43 (dd, 9.0, 9.0)
13	132.6	–	4″	70.8	3.47 (dd, 9.0, 9.0)
14	121.7	7.44 (s)	5″	78.6	3.30 (m)
15	40.9	2.68 (dd, 6.5, 13.0)	6″	62.1	3.76 (dd, 4.5, 12.0)
		3.19 (dd, 6.5, 13.0)			3.83 (dd, 2.0, 12.0)

7:3:1, v/v/v) → MeOH] to yield 23 fractions (Fr. 1–23). Fraction 9 (56.3 g) was further subjected to silica gel CC [Pet. Ether (PE) → PE–EtOAc (20:1 → 15:1 → 10:1 → 5:1 → 3:1, v/v) → EtOAc] to yield 19 fractions (Fr. 9-1–9-19). Fraction 9–16 (5024.0 mg) was purified by PHPLC [MeOH–H_2O (90:10, v/v)], and 13 fractions (Fr. 9-16-1–9-16-13) were given. Fraction 9-16-2 (415.5 mg) was subjected to PHPLC [MeOH–H_2O (70:30, v/v)] to obtain 8 fractions (Fr. 9-16-2-1–9-16-2-8), out of which, isorosmanol (**7**, 68.3 mg) and rosmanol (**8**, 128.3 mg) were obtained. Fraction 9-16-2-7 (32.8 mg) was isolated by PHPLC [CH_3CN–H_2O (45:55, v/v)] to yield epirosmanol (**10**, 3.3 mg). Fraction 9-16-4 (629.8 mg) was subjected to PHPLC [MeOH–H_2O (75:25, v/v)] to give 10 fractions (Fr. 9-16-4-1–9-16-4-10). Fraction 9-16-4-9 (53.4 mg) was purified by PHPLC [CH_3CN–H_2O (41:59, v/v)] to give 7-methoxyrosmanol (**9**, 4.7 mg). Fraction 9-16-10 was isolated by PHPLC [MeOH–H_2O (88:12, v/v)] to yield micromeric acid (**12**, 23.7 mg), oleanolic acid (**13**, 28.3 mg), and ursolic acid (**11**, 16.7 mg). Fraction 9-16-12 was purified by PHPLC [MeOH–H_2O (88:12, v/v)], too, and ten, oleanolic acid (**13**, 22.0 mg) was obtained.

Table 3
^1H and ^{13}C NMR data for **3** in C_5D_5N.

No.	δ_C	δ_H (J in Hz)	No.	δ_C	δ_H (J in Hz)
1	48.0	1.23 (m, overlapped)	22	37.5	1.88 (ddd, 4.5, 13.0, 13.0)
		2.20 (dd, 4.0, 12.5)			1.98 (m, overlapped)
2	68.6	4.03 (m)	23	29.4	1.17 (s)
3	83.9	3.32 (d, 9.5)	24	17.6	0.99 (s)
4	39.8	–	25	17.4	0.96 (s)
5	56.1	0.99 (m, overlapped)	26	16.7	1.07 (s)
6	19.0	1.36 (m), 1.54 (m)	27	24.7	1.64 (s)
7	33.6	1.50 (m), 1.65 (m)	28	177.0	–
8	40.7	–	29	27.0	1.38 (s)
9	47.9	1.90 (dd, 9.5, 9.5)	30	17.0	1.06 (d, 7.0)
10	38.5	–	1′	93.7	6.15 (d, 7.5)
11	24.2	2.08 (m)	2′	79.3	4.42 (dd, 7.5, 8.5)
12	128.2	5.50 (t, 3.5)	3′	79.0	4.28 (dd, 8.5, 9.0)
13	139.5	–	4′	70.9	4.20 (dd, 9.0, 9.0)
14	42.1	–	5′	79.1	3.92 (m)
15	29.8	1.36 (m, overlapped)	6′	62.4	4.32 (dd, 6.0, 11.5)
		2.40 (ddd, 5.0, 14.5, 14.5)			4.40 (br. d, *ca.* 12)
16	26.8	1.21 (m, overlapped)	1″	104.8	5.64 (d, 7.5)
		1.97 (m, overlapped)			
17	48.6	–	2″	75.9	4.05 (dd, 7.5, 9.0)
18	54.5	2.89 (s)	3″	78.3	4.20 (dd, 9.0, 9.0)
19	72.7	–	4″	72.9	4.07 (dd, 9.0, 9.0)
20	42.2	1.44 (m)	5″	78.1	3.97 (m)
21	25.9	2.23 (m, overlapped)	6″	63.9	4.34 (dd, 6.0, 12.0)
		3.08 (ddd, 4.5, 13.0, 13.0)			4.58 (dd, 3.0, 12.0)

Table 4
^1H and ^{13}C NMR data for **4** in CD_3OD.

No.	δ_C	δ_H (J in Hz)	No.	δ_C	δ_H (J in Hz)
1	47.9	0.89 (dd, 12.0, 12.0)	19	41.4	1.10 (dd, 4.0, 13.5)
		1.94 (dd, 5.0, 12.0)			1.81 (dd, 13.5, 13.5)
2	69.6	3.78 (ddd, 5.0, 9.0, 12.0)	20	36.8	–
3	85.9	3.04 (d, 9.0)	21	28.9	1.09 (m), 1.79 (m)
4	44.4	–	22	32.4	1.65 (m), 1.75 (m)
5	57.2	0.97 (dd, 3.0, 11.5)	23	23.8	1.22 (s)
6	19.9	1.40 (m), 1.62 (m)	24	66.2	3.38 (d, 11.5)
7	34.2	1.33 (m), 1.47 (m)			4.02 (d, 11.5)
8	40.7	–	25	17.6	0.99 (s)
9	49.3	1.63 (dd, 3.0, 9.0)	26	17.6	0.78 (s)
10	39.1	–	27	26.3	1.17 (s)
11	24.9	1.92 (m)	28	178.0	–
12	123.6	5.27 (t, 3.5)	29	74.3	3.19 (s)
13	144.9	–	30	19.5	0.92 (s)
14	42.9	–	1′	95.8	5.38 (d, 8.0)
15	29.3	1.17 (m, overlapped)	2′	73.9	3.33 (dd, 8.0, 9.0)
		1.50 (m)	3′	78.7	3.35 (dd, 9.0, 9.0)
16	24.0	1.73 (m), 2.05 (m)	4′	71.1	3.35 (dd, 9.0, 9.0)
17	48.3	–	5′	78.3	3.41 (m)
18	41.8	2.89 (dd, 4.0, 13.5)	6′	62.4	3.65 (dd, 5.0, 11.5)
					3.81 (br. d, *ca.* 12)

*2.3.1. Officinoterpenoside A_1 (**1**)*

Table 5
^1H and ^{13}C NMR data for **4** in C_5D_5N.

No.	δ_C	δ_H (J in Hz)	No.	δ_C	δ_H (J in Hz)
1	47.7	1.28 (m, overlapped)	19	41.0	1.44 (m, overlapped)
		2.25 (dd, 5.0, 12.0)			2.12 (dd, 14.0, 14.0)
2	68.7	4.28 (m)	20	36.4	–
3	85.6	3.55 (d, 9.5)	21	28.9	1.28 (m, overlapped)
4	44.0	–			1.75 (m, overlapped)
5	56.5	1.09 (m, overlapped)	22	32.0	1.86 (m)
6	19.3	1.42 (m, overlapped)	23	24.1	1.55 (s)
		1.64 (m)	24	65.6	3.71 (d, 10.5)
7	33.5	1.42 (m)			4.44 (d, 11.5)
8	40.0	–	25	17.3	0.99 (s)
9	48.3	1.75 (m, overlapped)	26	17.4	1.10 (s)
10	38.3	–	27	26.1	1.22 (s)
11	24.2	1.97 (m)	28	176.6	–
12	122.7	5.45 (t, 3.0)	29	73.7	3.56 (s)
13	144.3	–	30	19.7	1.09 (s)
14	42.1	–	1′	95.8	6.32 (d, 8.0)
15	28.3	1.17 (m, overlapped)	2′	74.1	4.21 (dd, 8.0, 8.5)
		2.35 (ddd, 4.5, 14.0, 14.0)	3′	78.9	4.30 (dd, 8.5, 9.0)
16	23.5	2.00 (m), 2.18 (m)	4′	71.1	4.36 (dd, 9.0, 9.0)
17	47.5	–	5′	79.3	4.04 (m)
18	41.2	3.30 (dd, 4.0, 14.0)	6′	62.2	4.40 (dd, 5.0, 12.0)
					4.46 (dd, 2.0, 12.0)

Table 6
^{1}H and ^{13}C NMR data for **5** in CD_3OD.

No.	δ_C	δ_H (J in Hz)	No.	δ_C	δ_H (J in Hz)
1	213.4	–	8	18.8	1.02 (s)
2	130.0	5.86 (d, 1.0)	9	15.1	2.13 (s)
3	185.4	–	1′	104.3	4.26 (d, 8.0)
4	51.7	–	2′	74.8	3.13 (dd, 8.0, 9.0)
5	48.6	2.67 (q, 7.5)	3′	78.1	3.32 (dd, 9.0, 9.0)
6	10.2	1.05 (d, 7.5)	4′	71.5	3.27 (dd, 9.0, 9.0)
7	73.5	3.58 (d, 9.5)	5′	77.9	3.26 (m)
		3.92 (d, 9.5)	6′	62.7	3.66 (dd, 5.0, 11.5)
					3.87 (dd, 1.5, 11.5)

White powder. $[\alpha]_D^{25}$ +17.5° (c = 0.89, MeOH); IR ν_{max} (KBr) cm^{-1}: 3503, 2931, 2867, 1674, 1602, 1560, 1454, 1422, 1320, 1251, 1069, 1023; UV λ_{max} (MeOH) nm (log ε): 312 (3.54), 265 (3.97). ^{1}H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) spectroscopic data, see Table 1; HRESI-TOF-MS: Negative-ion mode m/z 671.2899 $[M–H]^-$ (calcd for $C_{32}H_{47}O_{15}$ 671.2920).

*2.3.2. Officinoterpenoside A_2 (**2**)*

White powder. $[\alpha]_D^{25}$ −0.5° (c = 0.85, MeOH); IR ν_{max} (KBr) cm^{-1}: 3367, 2926, 2851, 1671, 1600, 1560, 1456, 1420, 1364, 1327, 1255, 1221, 1071, 1016; UV λ_{max} (MeOH) nm (log ε): 312 (3.54), 262 (3.88). ^{1}H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) spectroscopic data, see Table 2; HRESI-TOF-MS: Negative-ion mode m/z 671.2897 $[M–H]^-$ (calcd for $C_{32}H_{47}O_{15}$ 671.2920).

*2.3.3. Officinoterpenoside B (**3**)*

White powder. $[\alpha]_D^{25}$ −6.9° (c = 0.38, MeOH); IR ν_{max} (KBr) cm^{-1}: 3367, 2919, 2851, 1733, 1641, 1457, 1384, 1077. ^{1}H NMR (500 MHz, C_5D_5N) and ^{13}C NMR (125 MHz, C_5D_5N) spectroscopic data, see Table 3; HRESI-TOF-MS: Negative-ion mode m/z 847.4210 $[M + Cl]^-$ (calcd for $C_{42}H_{68}O_{15}Cl$ 847.4252).

*2.3.4. Officinoterpenoside C (**4**)*

White powder. $[\alpha]_D^{25}$ +6.2° (c = 0.78, MeOH); IR ν_{max} (KBr) cm^{-1}: 3367, 2932, 2840, 1732, 1648, 1559, 1457, 1267, 1072. ^{1}H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) spectroscopic data, see Table 4; ^{1}H NMR (500 MHz, C_5D_5N) and ^{13}C NMR (125 MHz, C_5D_5N) spectroscopic data, see Table 5; HRESI-TOF-MS: Negative-ion mode m/z 711.3961 $[M + COOH]^-$ (calcd for $C_{37}H_{59}O_{13}$ 711.3938).

*2.3.5. Officinoterpenoside D (**5**)*

White powder. $[\alpha]_D^{25}$ −55.9° (c = 0.88, MeOH); IR ν_{max} (KBr) cm^{-1}: 3382, 2970, 2916, 2877, 1690, 1618, 1434, 1381, 1320, 1285, 1218, 1162, 1077, 1039, 860; UV λ_{max} (MeOH) nm (log ε): 224 (4.11). ^{1}H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) spectroscopic data, see Table 6; HRESI-TOF-MS: Negative-ion mode m/z 351.1225 $[M + Cl]^-$ (calcd for $C_{15}H_{24}O_7Cl$ 351.1216).

*2.4. Acid hydrolysis of **1**–**5***

A solution of officinoterpenosides A_1–D (**1**–**5**, 2.5 mg each) in 1 M HCl (1 mL) was heated under reflux for 3 h, respectively. The reaction mixture was neutralized with Amberlite IRA-400 (OH^- form) and removed by filtration. The aqueous layer was subjected to the HPLC analysis under the following conditions, respectively: HPLC column, Kaseisorb LC NH_2-60-5, 4.6 mm i.d. × 250 mm (Tokyo Kasei Co. Ltd., Tokyo, Japan); detection, optical rotation [Chiralyser (IBZ Messtechnik GMBH, Mozartstrasse 14–16 D-30173 Hannover, Germany)]; mobile phase, CH_3CN–H_2O (75:25, v/v); and flow rate 1.0 mL/min. D-Glucose from **1**–**5** presented in the aqueous was carried out by comparison of its retention

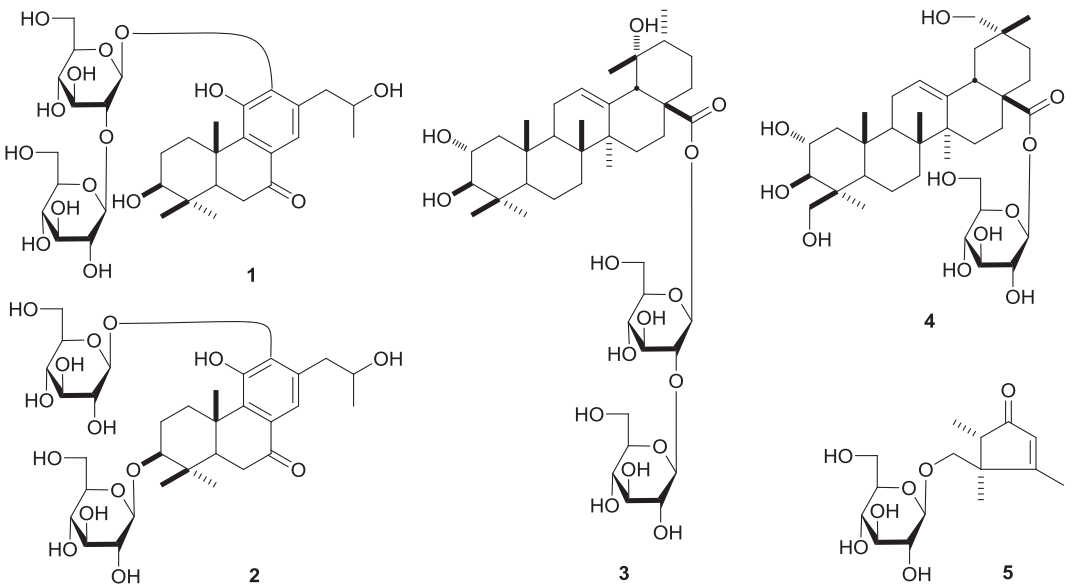

Fig. 1. The new compounds (**1**–**5**) obtained from the aerial parts of *R. officinalis*.

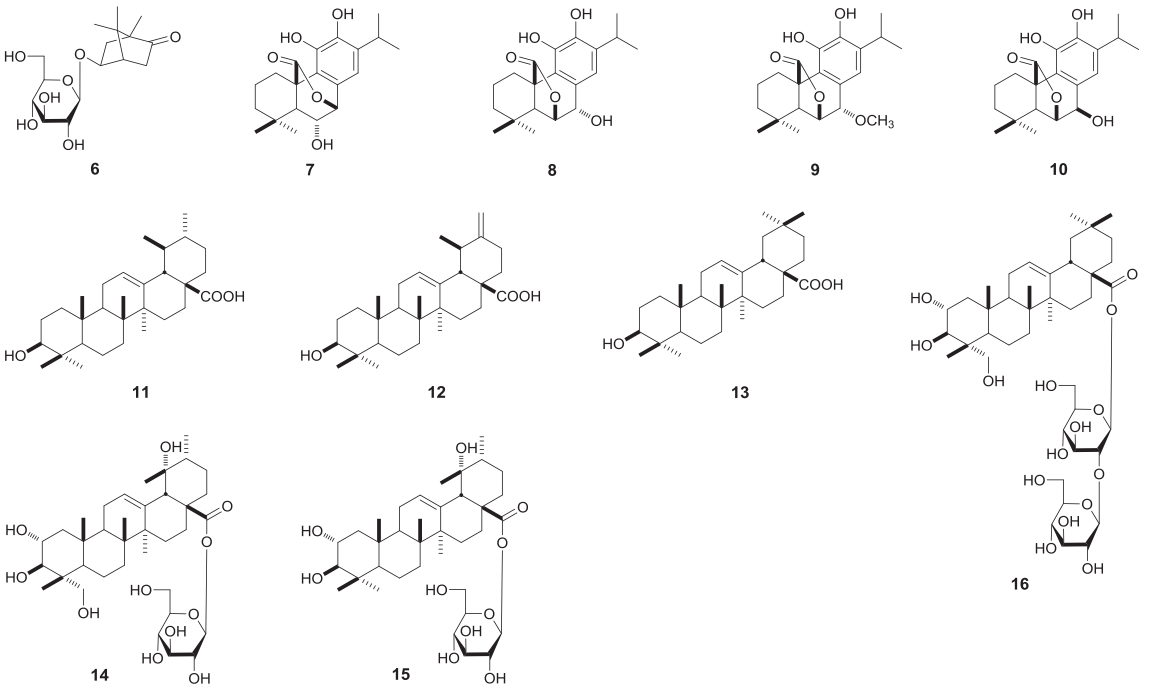

Fig. 2. The known terpenes (**6**–**16**) obtained from the aerial parts of *R. officinalis*.

time and optical rotation with that of authentic samples, t_R 17.6 min (positive).

3. Results and discussion

The dried aerial parts of *R. officinalis* were refluxed with 95% EtOH for 3 times. Evaporation of the solvent was done under reduced pressure to yield the 95% ethanol extract. The extract was partitioned in a $CHCl_3$–H_2O mixture (1:1) to give both $CHCl_3$ and H_2O partitions. Then, the H_2O layer was subjected to D101 macroporous resin CC, and eluted sequentially with H_2O and 95% EtOH. $CHCl_3$ partition and 95% EtOH eluate from D101 CC were subjected to normal- and reverse-phase CC, and finally preparative HPLC (PHPLC) to give 16 terpenoids, which included five new ones, officinoterpenosides A_1 (**1**), A_2 (**2**), B (**3**), C (**4**), and D (**5**), together with 11 known isolates, (1*S*,4*S*,5*S*)-5-exo-hydrocamphor 5-*O*-β-D-glucopyranoside (**6**), isorosmanol (**7**), rosmanol (**8**), 7-methoxyrosmanol (**9**), epirosmanol (**10**), ursolic acid (**11**), micromeric acid (**12**), oleanolic acid (**13**), niga-ichigoside F_1 (**14**), glucosyl tormentate (**15**), and asteryunnanoside B (**16**). Among the known ones, **6** was isolated from the *Rosmarinus* genus first, and **15** and **16** were obtained from this species for the first time. The structures of the new compounds **1–5** and known ones were shown in Figs. 1 and 2, respectively.

Officinoterpenoside A_1 (**1**) was obtained as a white powder with positive optical rotation ($[\alpha]_D^{25}$ +17.5° in MeOH). The molecular formula $C_{32}H_{48}O_{15}$ of it was determined by negative-ion HRESI-TOF-MS (*m/z* 671.2899 $[M-H]^-$, calcd for $C_{32}H_{47}O_{15}$ 671.2920). The IR spectrum of **1** suggested the presence of hydroxyl (3503 cm^{-1}), α,β-unsaturated ketone (1674 cm^{-1}), aromatic ring (1602, 1560, 1514, 1454 cm^{-1}), and an

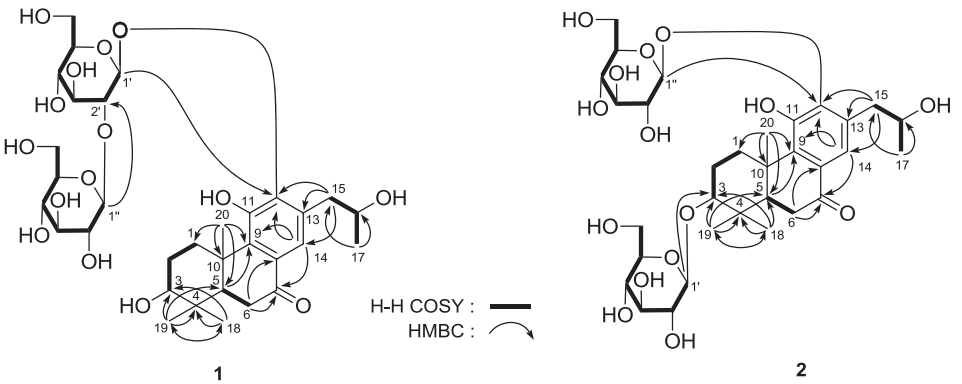

Fig. 3. The main ^1H ^1H COSY and HMBC correlations of **1** and **2**.

O-glycosidic linkage (1069 cm^{-1}). On acid hydrolysis and identification with HPLC analysis, the presence of D-glucose was determined [25]. The ^{13}C NMR (CD_3OD, Table 1) spectrum displayed 32 carbons including 20 carbons for the aglycon, and 12 carbons for two sugar units. The carbon type for 20 carbons in aglycon of **1** was determined by DEPT experiment, which sorted by 4 methyls, 4 methylenes, 4 methines, and 8 quaternary carbon signals. Among them, δ_C 122.2 (C-14), 129.6 (C-8), 132.5 (C-13), 140.8 (C-9), 148.8 (C-11), and 150.2 (C-12) revealed the presence of a penta-substituted aromatic ring. Meanwhile, in the ^1H-^1H COSY experiment, the correlations between δ_H [1.27 (1H, ddd, J = 3.0, 13.5, 13.5 Hz), 3.31 (1H, m), H_2-1] and δ_H 1.67, 1.76 (1H each, both m, H_2-2); δ_H 1.67, 1.76 (H_2-2) and δ_H 3.18 (1H, dd, J = 4.5, 11.5 Hz, H-3); δ_H 1.69 (1H, dd, J = 3.0, 14.0 Hz, H-5) and δ_H [2.58 (1H, dd, J = 3.0, 17.0), 2.62 (1H, dd, J = 14.0, 17.0), H_2-6]; δ_H [2.63 (1H, m), 3.30 (1H, dd, J = 6.0, 13.0 Hz), H_2-15] and δ_H 4.16 (1H, m, H-16); δ_H 4.16 (H-16) and δ_H 1.18 (3H, d, J = 6.5 Hz, H_3-17) were observed, which indicated the presence of partial structure written in bold lines (Fig. 3). In the HMBC experiment, the long-range correlations were observed between the following proton and carbon pairs: H-14 and C-9, 12; H_2-15 and C-12–14; H_3-17 and C-15, 16; H_3-18 and C-3–5, 19; H_3-19 and C-3–5, 18; and H_3-20 and C-1, 5, 9, 10 (Fig. 3). Furthermore, the relative configurations of rings A and B were elucidated by NOESY experiment. The NOE correlations were observed between δ_H 1.67 (H_β-2) and δ_H 0.92 (H_3-19), 1.40 (H_3-20); δ_H 3.18 (H-3) and δ_H 1.27 (H_α-1), 1.02 (H_3-18), 1.69 (H-5); δ_H 1.69 (H-5) and δ_H 1.27 (H_α-1), 2.62 (H_α-6), 1.02 (H_3-18); δ_H 2.58 (H_β-6) and δ_H 0.92 (H_3-19), 1.40 (H_3-20); δ_H 0.92 (H_3-19) and δ_H 1.40 (H_3-20), which suggested that the relative configuration of **1** was as shown in Fig. 4 with the abietane skeleton. Furthermore, the linkages of two D-glucose were determined by the observed long-range correlations between δ_H 4.74 (1H, d, J = 8.0 Hz, H-1′) and δ_C 150.2 (C-12), and δ_H 4.86 (1H, d, J = 8.0 Hz, H-1″) and δ_C 82.9 (C-2′). On the basis of the above-mentioned evidence, the structure of **1** was elucidated, and named as officinoterpenoside A_1.

Officinoterpenoside A_2 (**2**) was also isolated as a white powder. The molecular formula was the same as that of **1**, which was determined by negative-ion HRESI-TOF-MS (*m/z* 671.2897 $[M–H]^-$, calcd for $C_{32}H_{47}O_{15}$ 671.2920), too. From the acid hydrolysis of **2** with 1.0 M HCl, D-glucose was given, which was identified by HPLC analysis using an optical rotation detector [25]. The ^1H (500 MHz) and ^{13}C NMR (125 MHz) (CD_3OD, Table 2) spectra of **2**, which were assigned by various NMR experiments including ^1H ^1H COSY, HSQC, HMBC, and NOESY spectra suggested that **2** had same aglycon as **1** and two β-D-glucopyranosyl moieties [δ_H 4.35 (1H, d, J = 8.5 Hz, H-1′), 4.56 (1H, d, J = 8.0 Hz, H-1″)]. According to the long-range correlations between δ_H 4.35 (H-1′) and δ_C 89.6 (C-3), and δ_H 4.56 (H-1″) and δ_C 150.6 (C-12) observed in the HMBC experiment, the linkages of two D-glucose were determined. Consequently, the structure of **2** was identified, and named as officinoterpenoside A_2.

Officinoterpenoside B (**3**) was isolated as a white powder with negative rotation [$[\alpha]_D^{25}$ −6.9° (c = 0.38, MeOH)]. Its molecular formula was determined to be $C_{42}H_{68}O_{15}$ by negative-ion HRESI-TOF-MS (*m/z* 847.4210 $[M + Cl]^-$, calcd for $C_{42}H_{68}O_{15}Cl$ 847.4252). The IR spectrum showed absorption bands at 3367, 1733, and 1641 cm^{-1} ascribable to hydroxyl, carboxyl, and olefin functions, respectively. The ^1H NMR (C_5D_5N, Table 3) spectrum of **3** showed signals assignable to seven methyls [δ 0.96, 0.99, 1.07, 1.17, 1.38, 1.64 (3H each, all s, H_3-25, 24, 26, 23, 29, 27), 1.06 (3H, d, J = 7.0 Hz, H_3-30)], two methines bearing oxygen function [δ 3.32 (1H, d, J = 9.5 Hz, H-3), 4.03 (1H, m, H-2)], one tri-substituted olefin [δ 5.50 (1H, t, J = 3.5 Hz, H-12)], together with two anomeric proton signals [δ 5.64 (1H, d, J = 7.5 Hz, H-1″), δ 6.15 (1H, d, J = 7.5 Hz, H-1′)]. The ^{13}C NMR spectrum displayed 42 carbons including 30 carbons for the aglycon, and 12 carbons for two sugar units. ^1H and ^{13}C NMR spectra suggested that **3** was an ursolic acid type triterpene saponin derivative. In conjunction with the analysis of the HSQC spectrum, ^1H and ^{13}C NMR data for **3** were assigned as shown in Table 3. The ^1H ^1H COSY experiment on **3** indicated the presence of partial structure written in bold lines. And in HMBC experiment, long-range correlations were observed between the following proton and carbon pairs: H-18 and C-20, 28; H_3-23 and C-3–5, 24; H_3-24 and C-3–5, 23; H_3-25 and C-1, 5, 9, 10; H_3-26 and C-7–9, 14; H_3-27 and C-8, 13–15; H_3-29 and C-18–20; H_3-30 and C-19–21; H-1′ and C-28; H-1″ and C-2′ (Fig. 5). On the basis of the above mentioned evidence, the planar structure of **3** was determined, which was the same

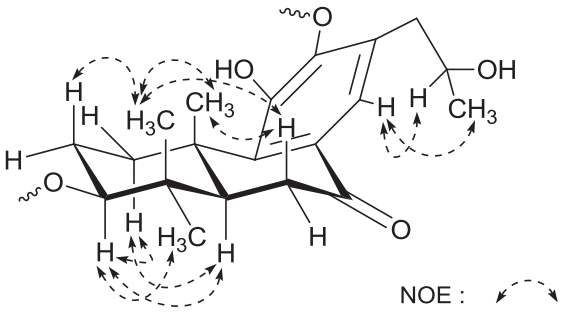

Fig. 4. The main NOE correlations for aglycon of **1** and **2**.

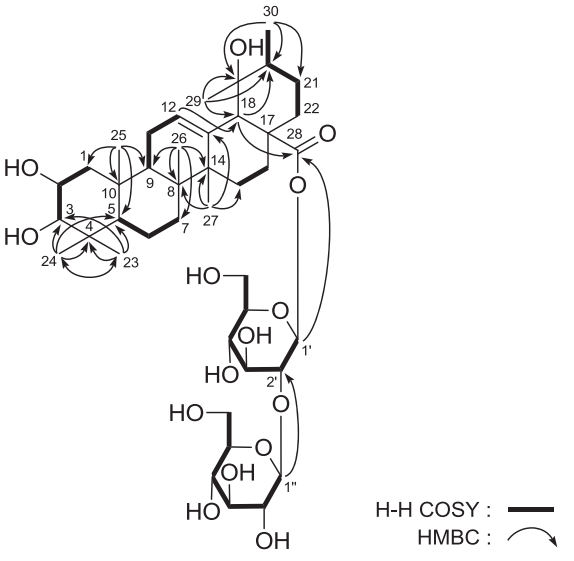

Fig. 5. The main ^1H ^1H COSY and HMBC correlations of **3**.

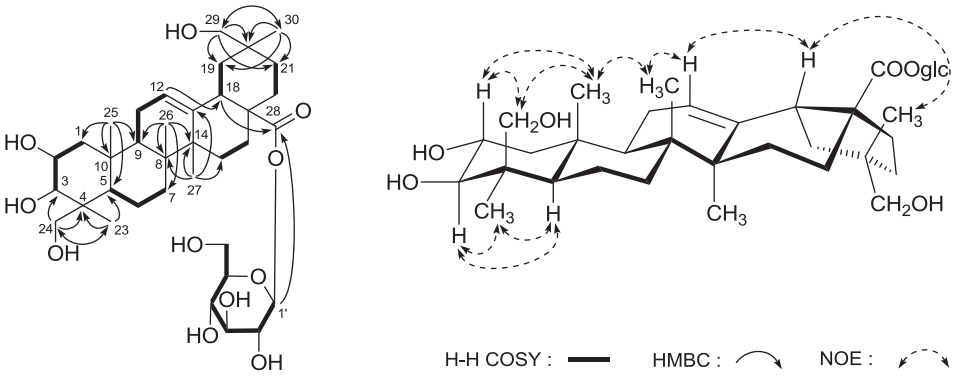

Fig. 6. The main ^{1}H ^{1}H COSY, HMBC and NOE correlations of **4**.

as pruvuloside A [22] with 2α,3α,19α-trihydroxyl groups. But the ^{13}C NMR data in 1–5 positions of **3** [δ 48.0 (C-1), 68.6 (C-2), 83.9 (C-3), 39.8 (C-4), 56.1 (C-5)] were significantly different from those of pruvuloside A [δ 43.0 (C-1), 66.2 (C-2), 79.1 (C-3), 38.7 (C-4), 48.7 (C-5)] [22], which suggested that the configuration of hydroxyl groups at 2,3-position for the two compounds was not the same as each other. Meanwhile, the coupling constant (J = 9.5 Hz) between H-2 and H-3 of **3** indicated that hydroxyl groups at 2,3-position mutually *trans* configuration. On the other hand, the proton and carbon signals in the ^{1}H and ^{13}C NMR spectra of **3** were very similar to those of glucosyl tormentate (**15**) [23], except for the signals due to another β-D-glucopyranosyl part. Finally, acid hydrolysis of it yielded D-glucose, which was identified by retention time and optical rotation using chiral detection by HPLC analysis [25]. On the basis of the above mentioned evidence, the structure of **3** was characterized to be officinoterpenoside B.

Officinoterpenoside C (**4**) was isolated as a white amorphous powder having a positive rotation [$[\alpha]_D^{25}$ +6.2° (c = 0.78, MeOH)]. The molecular formula of **4** was determined to be $C_{36}H_{58}O_{11}$ by negative-ion HRESI-TOF-MS (m/z 711.3961 $[M + COOH]^-$, calcd for $C_{37}H_{59}O_{13}$ 711.3938). The IR spectrum of **4** indicated the presence of hydroxyl, carboxyl, and olefinic groups. The ^{1}H and ^{13}C NMR (CD_3OD, Table 4) spectrum of **4** suggested that it was a triterpene monoglycoside with an oleanane skeleton. The ^{1}H ^{1}H COSY experiment on **4** indicated the presence of partial structure written in bold lines. The long-range correlations (Fig. 6) observed in HMBC experiment indicated that there were two oxymethine groups (δ_C 69.6, 85.9) and an oxymethylene group (δ_C 66.2) in ring A, and an oxymethylene group (δ_C 74.3) in ring E. Finally, the relative configuration for **4** was determined by NOESY experiment, which showed NOE correlations between the following proton pairs: H-2 and H_2-24, H_3-25; H-3 and H-5, H_3-23; H-5 and H_3-23; H-12 and H-18, H_3-26; H-18 and H_3-30; H_2-24 and H_3-25; and H_3-25 and H_3-26. Furthermore, the chemical shifts of 3, 5, 23, and 24-positions in ring A (Table 5, in C_5D_5N) were in accordance with those of known sericoside compounds with 2α,3β,24-OH [δ_C 24.4 (C-23), 56.4 (C-5), 65.4 (C-24), 85.7 (C-3)] [26], but different from those of quadranoside IV (2α,3β,23-trihydroxyurs-12-en-28-oic acid β-glucopyranosyl ester) [δ_C 14.4 (C-24), 48.2 (C-5), 66.5 (C-23), 78.2 (C-3)] [27]. In addition, comparison of the ^{13}C NMR signals for 29 and 30-positions in **4** [δ_C 19.7 (C-30), 73.7 (C-29)] with those of 3α,29-dihydroxy-23-oxo-olean-12-en-28-oic acid-28-*O*-α-L-rhamnopyranosyl(1 → 4)-β-D-glucopyranosyl(1 → 6)-β-D-glucopyranosyl ester [δ_C 19.7 (C-30), 73.6 (C-29)] and 3α,30-dihydroxy-23-oxo-olean-12-en-28-oic acid-28-*O*-α-L-rhamnopyranosyl(1 → 4)-β-D-glucopyranosyl(1 → 6)-β-D-glucopyranosyl ester [δ_C 28.2 (C-29), 65.4 (C-30)] [28], the relative configuration of hydroxymethyl group at 20-position was determined as α. The acid hydrolysis experiment gave D-glucose as the sugar moiety [25]. Consequently, the structure of officinoterpenoside C was elucidated as (**4**) 2α,3β,24,29-tetrahydroxyolean-12-en-28-oic acid-28-*O*-β-D-glucopyranosyl ester.

Officinoterpenoside D (**5**) was obtained as a white powder with a negative rotation [$[\alpha]_D^{25}$ −55.9° (c = 0.88, MeOH)]. It had the molecular formula, $C_{15}H_{24}O_7$ as deduced from the negative-ion HRESI-TOF-MS (m/z 351.1225 $[M + Cl]^-$, calcd for

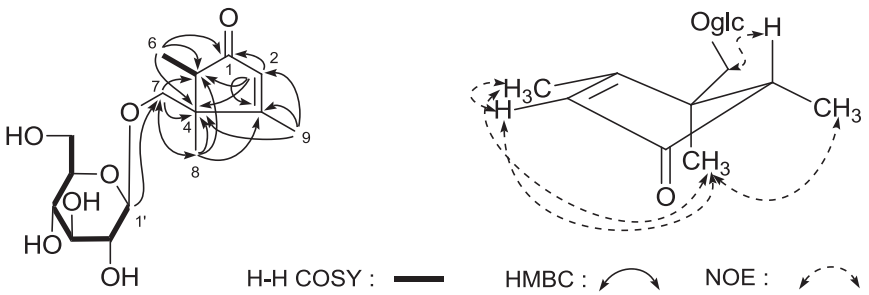

Fig. 7. The main ^{1}H ^{1}H COSY, HMBC and NOE correlations of **5**.

$C_{15}H_{24}O_7Cl$ 351.1216). The IR absorption band at 1690 cm^{-1} and the characteristic UV maxima absorption at 224 nm (4.11) revealed the presence of an α,β-unsaturated ketone group in **5**. On the acid hydrolysis, D-glucose was yielded [25]. The ^1H NMR and ^{13}C NMR (CD_3OD, Table 6) spectra of it showed signals ascribable to three methyls [δ 1.02, 2.13 (3H each, both s, H_3-8, 9), 1.05 (3H, d, $J = 7.5$ Hz, H_3-6)], one methene bearing oxygen function [δ 3.58, 3.93 (1H each, both d, $J = 9.5$ Hz, H_2-7)], one methine bearing methyl group [δ 2.67 (1H, q, $J = 7.5$ Hz, H-5)], one α,β-unsaturated ketone moiety [δ_H 5.86 (1H, d, $J = 1.0$ Hz, H-2); δ_C 130.0 (C-2), 185.4 (C-3), 213.4 (C-1)], together with a β-D-glucopyranosyl group [δ 4.26 (1H, d, $J = 8.0$ Hz, H-1′)]. In HMBC experiment, long-range correlations were observed between the following proton and carbon pairs: H-2 and C-1, 3–5; H_3-6 and C-1, 4, 5; H_2-7 and C-4, 5, 8; H_3-8 and C-3–5, 7; H_3-9 and C-2–4 (Fig. 7). On the basis of the above-mentioned evidence, the planar structure of **5** was determined as shown in Fig. 7. Furthermore, on the NOESY experiment, the NOE correlations between the following proton pairs: H-2 and H_3-8, H_3-9; H-5 and H_2-7; H_3-8 and H_3-6, H_3-9 were observed, which suggested that **5** has 2*Z*,3*S**,4*S** configuration. Consequently, the structure of officinoterpenoside D was elucidated, which is a normonoterpenoid glycoside.

Acknowledgments

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