Fitoterapia 99 (2014) 78–85

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

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

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ARTICLE INFO

Article history:
Received 26 July 2014
Accepted in revised form 23 August 2014
Available online 6 September 2014

Keywords:
Rosmarinus officinalis
Terpenoid glycosides
Normonoterpenoid
Diterpenoid
Triterpenoid

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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http://dx.doi.org/10.1016/j.fitote.2014.09.004
0367-326X/

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)			