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A-RING CONTRACTED TRITERPENOID FROM *ROSA MULTIFLORA*

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Key Word Index—*Rosa multiflora*; Rosaceae; triterpenes; rosamultic acid; 2-hydroxymethyl A(1)nor-19α,24-dihydroxyurs-2,12-dien-28-oic acid.

Abstract—A new A-ring contracted triterpene, rosamultic acid, was isolated from the roots of *Rosa multiflora*, together with five known triterpenes: sericic acid, euscaphic acid, myrianthic acid, kaji-ichigoside F1 and niga-ichigoside F2. The structure of the new compound was elucidated as 2-hydroxymethyl A(1)nor-19α,24-dihydroxyurs-2,12-dien-28-oic acid by spectroscopic methods. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Rosa multiflora Thunberg is a small tree widespread in Korea, the various parts of which have been used for several purposes in folk medicine [1]. Previous phytochemical investigations on the roots of this species led to the identification of a pentacyclic triterpene, tormentic acid and its glucoside, rosamultin [2, 3]. In this paper, we report on the isolation and structural elucidation of a new A-ring contracted triterpene, rosamultic acid (**1**), along with five known triterpenes (**2**–**6**).

RESULTS AND DISCUSSION

The methanolic extract of the roots of *R. multiflora* was diluted with water, and then extracted with chloroform and *n*-BuOH. Repeated chromatography of the two extracts on silica gel and Sephadex LH-20 afforded **1**–**4** from the chloroform extract, and **5** and **6** from the *n*-BuOH extract, respectively. Compounds **2**–**6** were identified as sericic acid (**2**), euscaphic acid (**3**), myrianthic acid (**4**), kaji-ichigoside F1 (**5**) and niga-ichigoside F2 (**6**) by comparison with previously reported physical and spectral data [4, 5]. Compound **2** has been isolated from *Terminalia sericea* [4], *T. ivorensis* (Combretaceae) [6], *Quercus ilex* (Fagaceae) [7] and *Vochysia divergens* (Vochysiaceae) [8]. However, to our knowledge, this is the first report of the ^{13}C NMR data of **2**.

Compound **1** gave a positive Liebermann-Burchard test for triterpenes and its IR spectrum showed the absorption bands for hydroxyl (3422 cm^{-1}), carboxyl (1686 cm^{-1}), and olefinic (1633 cm^{-1}) groups. The EI mass spectrum exhibited $[M]^+$ at *m/z* 486, and the HR-FAB mass spectrum $[M+Na]^-$ at *m/z* 509.3247, corresponding to the molecular formula $C_{30}H_{46}O_5$. The ^1H NMR spectrum of **1** revealed the presence of one secondary methyl (δ_H 1.07), five tertiary methyls (δ_H 1.10, 1.20, 1.38, 1.43 and 1.67), four carbinolic [δ_H 3.81, 4.00 (each 1H, *d*, J = 10.4 Hz) and δ_H 4.44, 4.59 (each 1H, *d*, J = 14.4 Hz)] and two olefinic [δ_H 5.56 (*br s*) and 6.07 (*br s*)] protons.

The ^{13}C NMR and DEPT spectra of **1** displayed signals for two trisubstituted double bonds [δ_C 128.10 (*d*), 131.22 (*d*), 140.26 (*s*) and 158.14 (*s*)], one-COOR (δ_C 180.73), two-CH_2OH (δ_C 61.01 and 66.51) and one tertiary-OH (δ_C 72.68) groups. The chemical shifts of C-12 (δ_C 128.10) and C-13 (δ_C 140.26) indicated **1** was a Δ^{12}-ursene triterpenoid [9, 10], which was further supported by the appearance of the prominent retro-Diels-Alder fragment ions (*m/z* 221 and 264) in its EI mass spectrum [11, 12].

In addition, the characteristic fragment peaks at *m/z* 264 [RDA], 246 $[264-H_2O]^+$, 201 $[264-H_2O-CO_2H]^+$, and 187, suggested that **1** was an urs-12-en-28-oic acid derivative having one free hydroxyl function in ring D or E [11, 12]. The position of the hydroxyl group was confirmed from the HMBC and NOE difference spectra of **1**. The HMBC spectrum showed cross-peaks from the one-proton singlet at δ_H 3.00 (H-18) to the quaternary carbon bearing an oxygen atom at δ_C 72.68 (C-19), and the carbonyl carbon atom at δ_C 180.73 (C-28). Moreover, irradiation of Me-29 (δ_H 1.38) showed NOEs for H-12 (δ_H 5.56), H-18β (δ_H 3.00) and Me-30 (δ_H 1.07) in its NOE difference experiments, indicating the placement of hydroxyl group at C-19α position.

The remaining trisubstituted double bond [δ_C 131.22 (*d*) and 158.14 (*s*)] and two-CH_2OH [δ_C 61.01 (*t*) and 66.51 (*t*)] groups were suggested to be present

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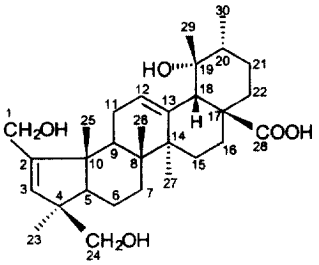

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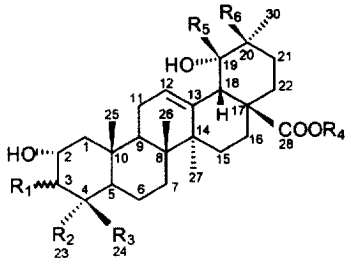

	R_1	R_2	R_3	R_4	R_5	R_6
2	β-OH	CH_3	CH_2OH	H	H	CH_3
3	α-OH	CH_3	CH_3	H	CH_3	H
4	α-OH	CH_2OH	CH_3	H	CH_3	H
5	α-OH	CH_3	CH_3	β-D-Glc	CH_3	H
6	α-OH	CH_2OH	CH_3	β-D-Glc	CH_3	H

in ring A or B based on the appearance of the retro-Diels-Alder breakdown fragment at *m/z* 221 in the EI mass spectrum. In the ^1H-^1H and ^1H-^{13}C COSY spectra of **1**, the hydroxymethylene signals at δ_H 4.44 and 4.59 [δ_C 61.01 (*t*)] showed allylic correlation peaks with the signal at δ_H 6.07 attributed to the olefinic proton on a trisubstituted double bond [δ_C 131.22 (*d*) and 158.14 (*s*)], indicating the presence of —CH=C—CH_2OH moiety in **1**. Furthermore, the signals for the A/B ring junction carbons [δ_C 63.67 (C-5) and 50.92 (C-10)] of **1** were significantly downfield shifted than those of typical ursane or oleananes [10]. These findings indicated that **1** possessed a *seco* or five-membered A-ring skeleton bearing a hydroxymethylene group attached to a trisubstituted double bond. However, the eight degrees of unsaturation from the molecular formula ($C_{30}H_{46}O_5$) suggested that **1** was a pentacyclic triterpene with two double bonds and one carboxyl group. The partial structure for the five membered A-ring was confirmed from the HMBC spectrum, which showed distinct cross-peaks of correlation through two and three bonds from δ_H 4.44 and 4.59 (H_2-1) to δ_C 158.14 (C-2), 131.22 (C-3) and 50.92 (C-10), and from δ_H 6.07 (H-3) to δ_C 63.67 (C-5), 50.92 (C-10) and 49.16 (C-4).

The second hydroxymethylene group (δ_H 3.81, 4.00 and δ_C 66.51) was located at C-4 as shown by the ^1H-^{13}C long range correlations from δ_H 3.81 and 4.00 (H_2-24) to δ_C 131.22 (C-3), 63.67 (C-5), 49.16 (C-4) and 25.11 (C-23). Moreover, NOEs were observed between Me-25 (δ_H 1.20) and H_2-24 (δ_H 3.81 and 4.00) in the NOE difference experiments, which indicated the primary hydroxyl substituent was on the axially β-oriented C-24 rather than on the equatorially α-oriented C-23. Thus, the two primary hydroxyl groups were placed at C-1 and C-24 in **1**.

From all the above data, the structure of rosamultic acid **1** was elucidated as 2-hydroxymethyl A(1)nor-19α,24-dihydroxyurs-2,12-dien-28-oic acid. In fact, this is the second example of an A-ring contracted ursene triterpene isolated from natural sources, the first having been reported from *Hyptis suaveolens* (Labiatae) [13].

EXPERIMENTAL

General

Mps: uncorr.; EIMS and HR-FABMS: VG Trio 2 and JEOL JMS AX505WA mass spectrometer, respectively; IR: JASCO 300E spectrometer; NMR: JEOL JNM-LA 300 spectrometer (300 MHz for ^1H and 75 MHz for ^{13}C) with reference to the residual solvent signals; TLC: precoated silica gel 60 F_{254} (Merck) and detection by spraying anisaldehyde-10% H_2SO_4 followed by heating.

Plant material

The roots of *Rosa multiflora* Thunberg were collected at Mt. Chunma (Korea) in July 1995, and identified by Dr Dae Suk Han, an emeritus professor of College of Pharmacy, Seoul National University. A voucher specimen (SNUPH-0026) has been deposited in the herbarium of our institute.

Extraction and isolation

The air-dried roots of *R. multiflora* (2.3 kg) were cut into pieces and extracted with 80% MeOH. The MeOH extract was evapd *in vacuo* to give a crude extract (300 g), which was successively extracted with $CHCl_3$ and *n*-BuOH. The $CHCl_3$ extract (26 g) was chromatographed over silica gel using *n*-hexane-EtOAc and $CHCl_3$-MeOH gradient to give **3** (32 mg), **4** (54 mg), and a mixture of **1** and **2** (107 mg). The mixture was subjected to the repeated CC over silica gel (*n*-hexane–Me_2CO, 4:1) and Sephadex LH-20 (MeOH) to afford **1** (49 mg) and **2** (8 mg). The *n*-BuOH extract (127 g) was chromatographed on silica

Table 1. ^{13}C NMR spectral data for compounds **1** and **2**

C	1	2
1	61.01 *t*	47.43 *t*
2	158.14 *s*	68.65 *d*
3	131.22 *d*	85.74 *d*
4	49.16 *s*	43.94 *s*
5	63.67 *d*	56.58 *d*
6	18.04 *t*	19.37 *t*
7	35.10 *t*	33.61 *t*
8	42.00 *s*	40.02 *s*
9	43.92 *d*	48.49 *d*
10	50.92 *s*	38.47 *s*
11	27.16 *t*	28.82 *t*
12	128.10 *d*	123.64 *d*
13	140.26 *s*	144.90 *s*
14	42.34 *s*	42.11 *s*
15	29.70 *t*	29.17 *t*
16	26.41 *t*	24.48 *t*
17	48.32 *s*	46.05 *s*
18	54.82 *d*	44.80 *d*
19	72.68 *s*	81.21 *d*
20	42.34 *d*	35.70 *s*
21	26.96 *t*	28.36 *t*
22	38.51 *t*	29.96 *t*
23	25.11 *q*	24.13 *q*
24	66.51 *t*	65.62 *t*
25	19.56 *q*	17.37 *q*
26	18.81 *q*	17.12 *q*
27	25.39 *q*	24.79 *q*
28	180.73 *s*	180.95 *s*
29	27.16 *q*	29.10 *q*
30	16.76 *q*	24.77 *q*

gel, eluting with $CHCl_3$–MeOH gradient to obtain **5** (379 mg) and **6** (17 mg).

Rosamultic acid (2-hydroxymethyl A(1)nor-19α,24-dihydroxyurs-2,12-dien-28-oic acid) (1). White amorphous powder, mp 239–241° (dec.). $[\alpha]_D^{25}$ +57.4° (MeOH; *c* 0.1); IR ν_{max}^{KBr} cm^{-1}: 3422 (OH), 2932, 1686 (C=O), 1633 (C=C), 1459, 1383, 1027; HR-FABMS (positive) *m/z*: 509.3247 $[M+Na]^+$, Calcd for $C_{30}H_{46}O_5Na$: 509.3243; FABMS (positive) *m/z*: 509 $[M+Na]^+$, 487 $[M+1]^+$; EIMS (70 eV) *m/z*: 486 $[M]^+$, 468, 440, 438, 437, 424, 406, 393, 376, 264, 246, 239, 231, 222, 221, 203, 201, 191, 189, 187, 185, 173, 161, 146, 119, 105, 91; ^1H NMR (pyridine-d_5): δ 1.07 (3H, *d*, *J* = 6.4 Hz, H-30), 1.10 (3H, *s*, H-26), 1.20 (3H, *s*, H-25), 1.38 (3H, *s*, H-29), 1.43 (3H, *s*, H-23), 1.67 (3H, *s*, H-27), 3.00 (1H, *s*, H-18), 3.08 (1H, *td*, *J* = 12.7, 4.4 Hz, H-16ax), 3.81 (1H, *d*, *J* = 10.4 Hz, H-24a), 4.00 (1H, *d*, *J* = 10.4 Hz, H-24b), 4.44 (1H, *d*, *J* = 14.4 Hz, H-1a), 4.59 (1H, *d*, *J* = 14.4 Hz, H-1b), 5.56 (1H, *br s*, H-12), 6.07 (1H, *br s*, H-3).

Sericic acid (2α,3β,19α,24-tetrahydroxyolean-12-en-28-oic acid) (2). White amorphous powder, mp 282–284° (dec.). $[\alpha]_D^{25}$ +37.3° (MeOH; *c* 0.15); IR ν_{max}^{KBr} cm^{-1}: 3436 (OH), 2925, 1691 (C=O), 1643 (C=C), 1463, 1380, 1051; FABMS (positive) *m/z*: 527 $[M+Na]^+$; EIMS (70 eV) *m/z*: 486 $[M-H_2O]^+$, 468, 458, 442, 424, 406, 264, 246, 239, 231, 221, 213, 203, 201, 189, 187, 173, 146, 131, 119, 105, 91; ^1H NMR (pyridine-d_5): δ 1.01 (3H, *s*, H-26), 1.03 (3H, *s*, H-25), 1.11 (3H, *s*, H-30), 1.19 (3H, *s*, H-29), 1.58 (3H, *s*, H-23), 1.62 (3H, *s*, H-27), 2.80 (1H, *m*, H-16ax), 3.55 (1H, *d*, *J* = 9.3 Hz, H-3α), 3.61 (1H, *br d*, *J* = 5.1 Hz, H-19), 3.62 (1H, *br s*, H-18), 3.73 (1H, *d*, *J* = 10.4 Hz, H-24a), 4.30 (1H, *m*, H-2β), 4.46 (1H, *d*, *J* = 10.4 Hz, H-24b), 5.54 (1H, *br s*, H-12).

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REFERENCES

1. Kim, J. G., *Illustrated Natural Drugs Encyclopedia (Color Edition)*, Vol 1. Namsandang, Seoul, 1984, p. 424.
2. Takahashi, K., Ogura, M. and Tanabe, Y., *Chemical and Pharmaceutical Bulletin*, 1969, **17**, 2223.
3. Du, H.-Q., Zhao, X., Zhao, T.-Z., Wang, M.-T., Zhang, Z.-W., Yao, M. and Yu, S.-Z., *Yaoxue Xuebao*, 1983, **18**, 314.
4. Bombardelli, E., Bonati, A., Gabetta, B. and Mustich, G., *Phytochemistry*, 1974, **13**, 2559.
5. Seto, T., Tanaka, T., Tanaka, O. and Naruhashi, N., *Phytochemistry*, 1984, **23**, 2829.
6. Iwu, M. M. and Anyanwu, B. N., *Fitoterapia*, 1982, **53**, 19.
7. Romussi, G., Parodi, B. and Falsone, G., *Pharmazie*, 1983, **38**, 787.
8. Hess, S. C., Brum, R. L., Honda, N. K., Cruz, A. B., Moretto, E., Cruz, R. B., Messana, I., Ferrari, F., Filho, V. C. and Yunes, R. A., *Journal of Ethnopharmacology*, 1995, **47**, 97.
9. Doddrell, D. M., Khong, P. W. and Lewis, K. G., *Tetrahedron Letters*, 1974, 2381.
10. Mahato, S. B. and Kundu, A. P., *Phytochemistry*, 1994, **37**, 1517.
11. Budzikiewicz, H., Wilson, J. M. and Djerassi, C., *Journal of the American Chemical Society*, 1963, **85**, 3688.
12. Ogunkoya, L., *Phytochemistry*, 1981, **20**, 121.
13. Raja Rao, K. V., Rao, L. J. M. and Prakasa Rao, N. S., *Phytochemistry*, 1990, **29**, 1326.