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Triterpenoids and other compounds from *Salvia roborowskii* Maxim

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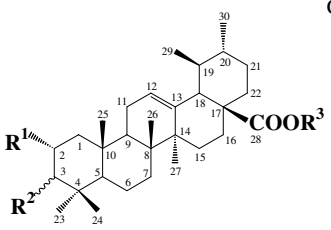
Two new triterpenoids, 2α-hydroxy-3β-methoxyurs-12-en-28-oic acid and 3α-hydroxy-2α-methoxyurs-12-en-28-oic acid as well as 25 known compounds were isolated from the whole plant of *Salvia roborowskii* Maxim. Their structures were elucidated by means of spectral data and chemical transformation. This is the first report on the chemical constituents of this plant. The presence of eugenyl β-D-glucopyranoside **22** in a plant of the genus *Salvia* is also reported for the first time.

1. Introduction

Salvia roborowskii Maxim, named "ye zhi ma" in Gansu province of China, an annual or biennial herb distributed widely in the west of China, has been used as a traditional folk medicine for the treatment of hepatitis and toothache [1]. There is no previous work on the chemical constituents of this species. We discribe here constituents isolated from the whole plant of *Salvia roborowskii* Maxim for the first time.

2. Investigations, results and discussion

From the EtOAc and n-BuOH extracts of the whole plant of *Salvia roborowskii* Maxim two new triterpenoids, 2α-hydroxy-3β-methoxyurs-12-en-28-oic acid (**1**) and 3α-hydroxy-2α-methoxyurs-12-en-28-oic acid (**2**), together with 17 known triterpenoids, 3β-hydroxy-2α-methoxyurs-12-en-28-oic acid (**3**) [2], 2α,3α-dihydroxyurs-12-en-28-oic acid (**4**) [3], 2α,3β-dihydroxyurs-12-en-28-oic acid (**5**) [3], 2α,3α,23-trihydroxyurs-12-en-28-oic acid (**6**) [3], 2α,3β,24-trihydroxyurs-12-en-28-oic acid (**7**) [4], 2α,3α,19-trihydroxyurs-12-en-28-oic acid (**8**) [5], 2α,3β,19-trihydroxyurs-12-en-28-oic acid (**9**) [6], 3-oxours-12-en-28-oic acid (**14**) [7], ursolic acid (**24**), 2α,3α-dihydroxyolean-12-en-28-oic acid (**10**) [8], 2α,3β-dihydroxyolean-12-en-28-oic acid (**11**) [8], 2α,3α,23-trihydroxyolean-12-en-28-oic acid (**12**) [9], 2α,3β,24-trihydroxyolean-12-en-28-oic acid (**13**) [10], oleanolic acid (**25**), lupenol (**15**) [11], lup-20(29)-1β,3β-diol (**16**) [12] and betulinic acid (**17**) [13], three known rosmarinic acid derivatives, rosmarinic acid (**18**), methyl rosmarinate (**19**) and ethyl rosmarinate (**20**), one phyenylpropanoid and its glycoside, methyl α,3,4-trihydroxy benzenepropanoate (**21**) [14] and eugenyl β-D-glucoside (**22**) [15], which is isolated from the genus *Salvia* for the first time, one organic acid ester, 2-hydroxyethyl linolenate (**23**), and one steroid and its glycoside, β-sitosterol (**26**) and β-sitosteryl-β-D-glucopyranoside (**27**). Among them, compounds **1**–**14**, and **17** were all isolated in methyl ester form by previous treatment with CH_2N_2/Et_2O. The structures of the known compounds were confirmed by comparing their corresponding properties (melting point, MS, IR, ^{1}H NMR and ^{13}C NMR) with the reported values in the literature or comparing with authentic samples.



Compound	R^1	R^2	R^3
1	OH	β-OMe	H
1a	OH	β-OMe	Me
2	OMe	α-OH	H
2a	OMe	α-OH	Me
2b	OMe	α-OAc	Me
4a	OH	α-OH	Me

Compound **1a** was isolated as white needle crystals (petroleum-acetone), m.p. 207–208 °C, $[\alpha]_D^{20}$ −24.0° (c 0.3, $CHCl_3$). The EI-HR MR gave a $[M]^+$ at m/z 500.3757 (calcd 500.3764), which corresponds to a molecular formula $C_{32}H_{52}O_4$. It gave a positive Liebermann-Burchard (LB) test for triterpenes. The IR spectrum showed the absorption for hydroxyl (3441 cm^{-1}), ester carbonyl (1731 cm^{-1}), ether bond (1189 cm^{-1}, 1107 cm^{-1}). Assignments of hydrogen and carbon signals were accomplished using the ^{1}H/^{1}H COSY, HMQC and HMBC spectrum. The ^{1}H NMR spectral of **1a** exhibited the following signals: the signal assignable to H-18 at δ 2.23 (d, J = 12.2 Hz), the olefinic H-12 at δ 5.25 (t, J = 3.1 Hz), the signal of carbomethoxyl δ 3.61 (s), and the signals of H_3-29 δ 0.86 (d, J = 6.8 Hz) and H_3-30 δ 0.94 (d, J = 6.1 Hz). The ^{13}C NMR spectrum showed double bond carbon signals assignable to C-12 and C-13 at δ 125.42 and 138.17, respectively, signals δ 51.44 and 178.01 due to methyl ester at C-28. These ^{1}H NMR and ^{13}C NMR spectral properties suggested the structural feature of methyl urs-12-en-28-oate. The presence of methoxyl was supported by the signal δ 3.63 (s) in the ^{1}H NMR spectrum. The ^{1}H NMR signals at δ 3.76 (ddd, J = 10.2, 9.5, 4.1 Hz) and δ 2.58 (d, J = 9.5 Hz) were attributed, respectively to the 2- and 3- protons on the carbons having hydroxyl and methoxyl functions. Methoxyl was determined to link at C-3 on the basis of the correlation beween δ 3.63 (methoxyl) and δ 95.01 (C-3) in the HMBC spectrum. The coupling contant of H-2 and H-3 (9.5 Hz) indicating both H-2 and H-3 are axial in nature thereby indicated that methoxyl and hydroxyl are α-equatorial and β-equatorial, respectively. Thus, the structure of compound **1a** was methyl 2α-hydroxy-3β-methoxyurs-12-en-28-oate, and compound **1** was 2α-hydroxy-3β-methoxyurs-12-en-28-oic acid.

Compound **2a** was isolated as colorless gum, $[\alpha]_D^{20}$ −73.7° (c 0.7, $CHCl_3$). The molecular formula, $C_{32}H_{52}O_4$, of **2a** was determined by EI-HR MS (500.3756 $[M]^+$, calcd 500.3764). It gave a positive Liebermann-Burchard (LB) test for triterpenes. Comparing the ^{13}C NMR of **2a** with that of **4a**, the most data of **2a** were in good agreement with that of **4a** except for the signals corresponding to A-ring. This was interpreted by the replacement one hydroxyl with one methoxyl, which was proved by the presence of a signal δ 3.37 (s) in the ^{1}H NMR. In order to determine the positions of methoxyl and hydroxyl, an acetylation experiment of **2a** with Ac_2O/C_5H_5N was carried out and gave a monoacetate **2b**. The ^{1}H NMR spectrum of **2b** showed that the chemical shift of H-3 shifed downfield to

Table 1: ^{13}C NMR (100.62 MHz) of compounds 1a, 2a, 2b and 4a (in $CDCl_3$, TMS as int. Standard)

C	1a	2a	2b	4a	C	1a	2a	2b	4a
1	46.62	38.38	40.15	41.85	18	52.81	52.86	52.83	52.82
2	69.04	75.19	74.59	66.43	19	39.03	39.03	39.09	39.00
3	95.01	76.09	75.79	78.83	20	38.84	38.88	38.88	38.84
4	40.38	39.80	39.67	39.64	21	30.62	30.65	30.65	30.61
5	55.42	48.08	47.41	48.04	22	36.60	36.65	36.64	36.60
6	18.11	18.04	17.94	17.97	23	28.87	28.52	28.00	28.49
7	32.86	32.71	32.69	32.71	24	17.33	22.01	21.78	21.86
8	39.84	38.01	38.16	38.26	25	16.78	16.58	16.54	16.40
9	47.61	47.28	47.41	47.23	26	16.92	16.92	16.96	16.87
10	37.95	37.66	37.89	38.19	27	23.59	23.75	23.75	23.73
11	23.36	23.27	23.36	23.25	28	178.01	178.08	178.03	178.09
12	125.42	125.36	125.43	125.34	29	17.02	17.03	17.94	16.99
13	138.17	138.29	138.18	138.21	30	21.16	21.18	21.19	21.14
14	42.10	42.14	42.08	42.07	CO_2CH_3	51.42	51.41	51.43	51.41
15	27.96	27.96	28.00	27.93	OCH_3	62.90	55.80	56.56	
16	24.19	24.22	24.23	24.18	CH_3CO			170.86	
17	48.06	48.09	48.06	48.03				21.11	

δ 5.08 while the signal of H-2 (δ 3.52) remained almost unchanged comparing with that of **2a** (H-3 δ 3.55 and H-2 δ 3.51). This evidence indicated that methoxyl and hydroxyl are linked at C-2 and C-3, respectively. The coupling of H-2 and H-3 (2.0 Hz) exclude a trans diaxial disposition but was compatible with a *cis* one, either in a 2_{ax}-3_{eq} or in a 2_{eq}-3_{ax} relative disposition. As H-2 showed a large coupling constant (10.0 Hz) with H-1_{ax}, it was axial and thus H-3 was equatorial. Thereby methoxyl and hydroxyl are α-equatorial and α-axial, respectively. Thus, the structure of compound 2a was methyl 3α-hydroxy-2α-methoxyurs-12-en-28-oate, and compound **2** was 2α-hydroxy-3β-methoxyurs-12-en-28-oic acid.

3. Experimental

3.1. Equipment

M.p.s.: Kofler apparatus, uncorr. Optical rotation: polarimeter 241 (Perkin Elmer), solvent $CHCl_3$. IR-spectra were recorded on Nicolet 170SX FT-IR instrument. ^{1}H NMR, 2D NMR (400.13 Hz) and ^{13}C NMR (100.62 Hz) were recorded on an AM-400 FT-NMR spectrometer in $CDCl_3$ with TMS as int. Standard. EI-MS spectra were determined on a MS50 (A. E. I. Brunner) mass spectrometer. FAB-MS and EI-HRMS were recorded on a VG ZAB-HS mass spectrometer. Silica gel (200–300 mesh) was used for column chromatography and silica gel GF_{254} for TLC. Spots were detected on the TLC under UV light or by heating after spraying with 5% H_2SO_4.

3.2. Plant material

The plant material was collected from Zhang county, Gansu province of P. R. China and was identified by adjunct Prof. Yong-Hong Zhang, Faculty of Pharmacy, Lanzhou Medical College of P. R. of China. A specimen has been deposited at the Lab. of Biomedicine, Faculty of Pharmacy, Lanzhou Medical college of P. R. China.

3.3. Extraction and isolation

The air-dried whole plant of *Salvia roborowskii* Maxim (5.0 kg) was powdered and extracted with EtOH at room temperature. The extract was concentrated under reduced pressure. The residue was suspended in H_2O, extracted with petroleum, EtOAc and n-BuOH, respectively. The EtOAc extract (85 g) was subjected to chromatography on a silica gel column with petroleum ether-acetone (15 : 1–1 : 1) as eluent to give eight fractions. Fr. 1 yielded **26** (21 mg) after CC on a silica gel column eluting with petroleum-acetone (15 : 1). Fr. 3 was chromatographed using $CHCl_3$–MeOH (35 : 1) as eluent to afford **15** (30 mg) and a mixture of **24** and **25**, which was further separated by recrystallization with heat MeOH several times to get **24** (2.5 g) and **25** (50 mg). Fr. 4 gave **16** (12 mg) after chromatography using petroleum-acetone (4 : 1) as eluent. Fr. 2, 5, 6 and 7 were subjected to chromatography on silica gel using $CHCl_3$–MeOH (40 : 1, 25 : 1, 20 : 1, 15 : 1) as eluents, respectively, to get four crude fractions. Each was treated with CH_2N_2/Et_2O and kept at room temperature overnight, then chromatographed on silica gel column. **14a** (20 mg) was obtained from Fr. 2. **1a** (40 mg), **2a** (15 mg), **3a** (70 mg), **4a** (25 mg), **5a** (50 mg), **10a** (5 mg) and **11a** (10 mg) were obtained from Fr. 5. **6a** (50 mg), **7a** (20 mg), **8a** (25 mg), **9a** (35 mg), **12a** (10 mg) and **13a** (5 mg) were obtained from Fr. 6, and **17a** (20 mg) from Fr. 7. Fr. 8 afforded **18** (200 mg), **19** (500 mg) and **20** (100 mg) after chromatography using $CHCl_3$–AcOH–H_2O (12 : 8 : 1) as eluent. Finally the extracts of n-BuOH (54 g) were subjected to column chromatography on silica gel and eluted with $CHCl_3$–MeOH (70 : 1), petroleum ether-acetone (5 : 2), C_6H_6–MeOH (10 : 1), successively, giving **23** (40 mg), **21** (15 mg), **22** (30 mg) and **27** (20 mg).

In order to prove that compounds **1a** and **2a** were not the products of eluent ($CHCl_3$–MeOH) reacting with compounds **4a** and **5a**, the following experiment was carried out. A part of extract of EtOAc (10 g) was subjected to chromatography on silica gel using $CHCl_3$–EtOH (15 : 1) as eluent, and then methylated with CH_2N_2/Et_2O giving Fr. 5a. Fr. 5a afforded **1a** (2 mg) and **2a** (1 mg) by chromatography using petroleum-acetone (10 : 1 and 12 : 1) as eluents. This fact confirmed that compounds **1a** and **2a** were natural products.

3.4. Methyl 2α-hydroxy-3β-methoxyurs-12-en-28-oate (**1a**)

White needle crystals (petroleum ether-acetone). m.p. 207–208 °C, $[\alpha]_D^{20}$ −24.0 (C 0.3, $CHCl_3$). IR (KBr, cm^{-1}): 3441 (OH), 2945, 1731 (O=C–O), 1458, 1380, 1189, 1107 (C–O–C). EI-HRMS: $[M]^+$ m/z 500.3757 for $C_{32}H_{52}O_4$ (calcd 500.3764). FAB-MS m/z (rel. int.): 501 (7) $[M+1]^+$, 483 (12) $[M+1-H_2O]^+$, 469 (4) $[M+1-CH_3OH]^+$, 262 (85), 238 (11), 203 (70), 189 (50), 133 (74). ^{1}H and ^{13}C NMR: see Table.

Table 2: ^{1}H NMR (400.13 MHz) of compounds 1a, 2a, 2b (in $CDCl_3$, TMS as int. standard)

H	1a	2a	2b
1α	0.94*		
1β	1.99 dd (12.9, 4.8)		
2β	3.76 ddd (10.2, 9.5, 4.1)	3.51 ddd (10.0, 2.0, 2.0)	3.52 ddd (10.0, 2.0, 2.0)
3α	2.58 d (9.5)		
3β		3.55 d (2.0)	5.08 d (2.0)
5	0.85*		
9	1.57*		
11	1.94 m		
12	5.25 t (3.1)	5.25 t (3.7)	5.25 t (3.7)
18	2.23 d (12.2)	2.22 d (12.0)	2.24 d (12.0)
23	1.01 s	1.04 s	0.99 s
24	0.79 s	0.84 s	0.88 s
25	0.97 s	1.04 s	0.93 s
26	0.73 s	0.73 s	0.74 s
27	1.08 s	1.08 s	1.11 s
29	0.86 d (6.8)	0.85 d (6.0)	0.89 d (6.1)
30	0.94 d (6.1)	0.95 d (4.0)	0.94 d (6.8)
CO_2CH_3	3.60 s	3.60 s	3.61 s
OCH_3	3.63 s	3.37 s	3.33 s
CH_3CO			2.11 s

3.5. Methyl 3α-hydroxy-2α-methoxyurs-12-en-28-oate (**2a**)

Colorless gum, $[\alpha]_D^{20}$ −73.7 (C 0.7, $CHCl_3$). EI-HRMS: $[M]^+$ m/z 500.3756 for $C_{32}H_{52}O_4$ (calcd 500.3764). IR (film, cm^{-1}): 3533 (OH), 2953, 1724 (O=C−O), 1454, 1363, 1193, 1096 (C−O−C). EI-MS (70ev) m/z (rel. int.): 500 (0.9) $[M]^+$, 482 (0.2) $[M-H_2O]$, 468 (0.4) $[M-CH_3OH]$, 262 (100), 238 (7), 203 (97), 189 (25), 133 (50). ^1H and ^{13}C NMR: see Table.

3.6. Acetylation of compound 2a

Compound **2a** was dissolved in pyridine (1 ml) and Ac_2O (0.5 ml) added. The reaction mixture was left at room temperature overnight and work-up in the usual manner affording the monoacetate **2b** as a colorless gummy residue.

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References

1 Name Reference of Chinese herbal medicine of the whole country P. 938, People's Health Press, Beijing 1996
2 Fang, Jim-Min; Wang, Kuang-Chaun; Cheng, Yu-Shia: Phytochemistry **30**, 3383 (1991)
3 Kojima, H.; Orgura, H.: Phytochemistry **28**, 1703 (1989)
4 Sashida, Y.; Ogawa, K.; Mori, N.; Yamanouchi, T.: Phytochemistry **31**, 2801 (1992)
5 Lontsi, D.; Sondengam, B.; Martin, M.; Bodo, B.: Phytochemistry **31**, 4286 (1992)
6 Stachurskl, L.; Bednarek, E.; Dobrowolski, J.; Strzelecka, H.; Mazurek, A. P.: Planta Med. **61**, 94 (1995)
7 Johns, S. R.; Lamberton, J. A.; Morton, T. C.; Suares, H.; Willing, R. I.: Aust. J. Chem. **36**, 2537 (1983)
8 Seo, S.; Tomita, Y.; Tori, K: Tetrahedron Lett. **1**, 7 (1975)
9 Kojima, H.; Orgura, H: Phytochemistry **25**, 729 (1986)
10 Hou, Zhen-Fu; Dai, Jing-Qiu; Xie, Zhi-Xiang; Tu, Yong-Qiang; Li, Yu: Indian J. Chem. Section B, submitted
11 Reynolds, W. F.; Mclean, S.; Poplawski, J.; Enriquez, R. G.; Escobar, L. I.; Leon, I.: Tetrahedron **42**, 3419 (1986)
12 Savona, G.; Bruno, M.; Rooriguez, B.; Marto, J. L.: Phytochemistry **26**, 3305 (1987)
13 Sholichin, M.; Yamasaki, K.; Kasai, R.; Tanaka, O.: Chem. Pharm. Bull. **28**, 1007 (1980)
14 Kelley, C. J.; Harruff, R. C.; Carmack, M.: J. Org. Chem. **41**, 449 (1976)
15 Orihara, Y.; Furuya, T.; Hashimoto, N.; Deguchi, Y.: Phytochemistry **31**, 827 (1992)

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