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Lupane triterpenoids from *Salvia roborowskii* Maxim

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A new lupane type triterpenoid, 3 β , 11 α -dihydroxy-30-norlupan-20-one and 6 known lupane triterpenoids were isolated from the petroleum ether (60–90 °C) extract of the whole plant of *Salvia roborowskii* Maxim. Their structures were elucidated by means of spectral methods including NMR and MS techniques.

1. Introduction

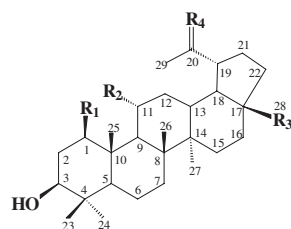
Salvia roborowskii is an important herbal plant, which has been used as a traditional folk medicine for the treatment of hepatitis and toothache in China [1]. In the investigations on chemical constituents, some natural occurring from the extract of EtOAc and n-BuOH of the plant has been found [2]. As a continuation of our phytochemical studies on *Salvia roborowskii* Maxim and cooperation with Prof. Yu Li at Lanzhou University, 7 triterpenoids with lupane carbon skeleton type have been obtained from the petroleum ether (60–90 °C) extract of this species as well. In this paper, we hope report the isolation and structural elucidation of a new triterpenoid and 6 known triterpenes.

2. Investigations, results and discussion

From the petroleum ether (60–90 °C) extracts of the whole plant of *Salvia roborowskii* Maxim, a new lupane triterpenoid, named 3 β , 11 α -dihydroxy-30-norlupan-20-one (**1**) together with 6 known lupane triterpenoids, 3 β -hydroxy-30-norlupan-20-one (**2**) [3], lupenol (**3**) [2, 4], lup-20 (29)-1 β , 3 β -diol (**4**) [2, 5], lup-20 (29)-3 β , 11 α -diol (**5**) [6, 7], lup-20 (29)-1 β , 3 β , 11 α -triol (**6**) [8], betulinic acid (**7**) [2, 9] were isolated. The structures of the known com-

pounds were confirmed by comparing their corresponding properties (melting point, MS, IR, ¹H NMR and ¹³C NMR) with the reported values in the literature.

Compound **1** was obtained as UV-inactive white needle crystals from petroleum-acetone, m. p. 149–151 °C, [α]_D²⁶ –25.0° (C, 1.0, CH₂Cl₂). Its FAB-MS gave two quasi-molecular ion peaks [M+Na]⁺ at m/z 467 and [M+Li]⁺ at m/z 451, corresponding to the formula C₂₉H₄₈O₃, indicated six degrees of unsaturation. The IR spectrum displayed strong broad absorption bands for hydroxyl group (3338 cm^{–1}) and the carbonyl group (1701 cm^{–1}). In the ¹H NMR spectrum of **1**, there were seven methyl signals at δ 0.77, 0.79, 0.98 (6H, 2 $\times$ CH₃), 1.02, 1.03 and 2.17 due to the acetylmethyl (CH₃CO–). In the ¹³C NMR spectrum of **1**, there were 29 resonant signals including 7 methyl groups, 6 quaternary carbons, 7 methines and 9 methylenes. The above properties of NMR and the positive Liebermann-Burchard (LB) test suggested the structural feature of pentacyclic triterpenoids [3–9]. Furthermore, carefully compared data of ¹H and ¹³C NMR spectra of **1** with those of **2** and **5** (Table 1) indicated that compound **1** was a pentacyclic triterpenoid with lupane type carbon skeleton. The typical carbonyl signal at δ _C 212.6 (singlet) and two carbons bearing-oxygen at δ _C 70.1 (doublet) and 78.3 (doublet) show that the molecule of **1** has one ketone function and two secondary hydroxyl functions. This conclusion should also be confirmed by clearly proton bearing-oxygen signals at δ _H 3.20 (1H) and 3.92 (1H). A remaining problem about the structure of **1** was the positions of two hydroxyl groups. In the ¹H NMR, the trip-like signal centered at 3.20 (J = 8.5 Hz, 2H) exhibited that one of the hydroxyl groups was present at the 3 β -position from the chemical shift and spin splitting pattern, as well as from the biogenetic point of view. Another signal as a six line pattern centered at δ _H 3.92 with two diaxial (J_{a,a} = 10.7 Hz) and one axial equatorial (J_{a,e} = 5.4 Hz) coupling agrees with a 11 α -OH [7, 8]. Moreover, the present of 11 α -OH was supported on the basis of the change of chemical shifts of carbons in **1**, **5**, and **2**. Besides the expected large downfield shifts of C-11 from δ _C 20.8 in **2** to δ _C 70.1 and 70.4 in **1** and **5**, respec-



Compound	R ¹	R ²	R ³	R ⁴
1	H	OH	CH ₃	O
2	H	H	CH ₃	O
3	H	H	CH ₃	CH ₂
4	OH	H	CH ₃	CH ₂
5	H	OH	CH ₃	CH ₂
6	OH	OH	CH ₃	CH ₂
7	H	H	COOH	CH ₂

Table: ^{13}C NMR data (400.13 MHz) for compounds **1**, **2** and **5**

C	1	2	5	C	1	2	5
1	39.3	38.6	41.0	16	35.1	34.9	35.4
2	27.2	27.3	27.6	17	42.9	43.0	42.9
3	78.3	78.8	78.5	18	48.6	49.6	47.7
4	39.2	38.8	39.3	19	52.1	52.6	47.7
5	55.4	55.2	55.5	20	212.6	212.6	150.2
6	18.0	18.2	18.0	21	27.5	27.6	29.8
7	34.8	34.2	35.2	22	40.9	39.6	41.0
8	42.3	40.7	42.5	23	29.2	29.1	28.2
9	55.6	50.2	55.6	24	15.5	15.3	15.5
10	39.6	37.2	38.9	25	16.3	15.8	16.3
11	70.1	20.8	70.4	26	17.0	19.0	17.2
12	38.9	27.6	37.6	27	14.4	14.3	14.4
13	35.9	37.0	37.0	28	18.0	17.9	18.0
14	42.4	42.6	42.5	29	28.3	27.9	19.3
15	27.6	27.6	29.8	30			109.8

(δ , in CDCl_3 , TMS as int. standard, types of carbons were assigned by DEPT experiments)

tively, the C-9 and C-12 signals were also significantly shifted downfield due to the 11α -configuration effect of this hydroxyl group (see Table 1). Consequently, **1** was characterised as 3β , 11α -dihydroxy-30-norlupan-20-one.

3. Experimental

3.1. Equipment

M. p. s.: Kofler apparatus, uncorr. Optical rotation: polarimeter 241 (Perkin Elmer), solvent CH_2Cl_2 . IR-spectra were recorded on a Nicolet 170SX FT-IR instrument. ^1H 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 and FAB-MS was recorded on a VG ZAB-HS mass spectrometer. Silica gel (200–300 mesh) was used for CC and silica gel GF₂₅₄ for TLC. Spots were detected on the TLC under UV light or by heating after spraying with 98% H_2SO_4 :EtOH (v : v, 95 : 5).

3.2. Plant material

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

3.3. Extraction and isolation

The plant material (5.0 kg) was extracted for three times with EtOH at room temperature. The extract was concentrated under reduced pressure. The residue (390 g) was suspended in H_2O , extracted with petroleum, EtOAc and *n*-BuOH, respectively. The petroleum ether (60–90 °C) extract (70 g) was subjected to chromatography on a silica gel column (710 g) with petroleum ether (60–90 °C) –acetone (V : V, 40 : 1–1 : 1) as eluent to give eight fractions. Fr. 3 yielded **3** (25 mg) after CC on a silica gel column eluting with petroleum ether (60–90 °C)-acetone (V:V, 12 : 1). Fr. 4 gave **2** (50 mg) after chromatography using petroleum ether (60–90 °C)-EtOAc (V : V, 8 : 1) as eluent. Fr. 5 was chromatographed using petroleum (60–90 °C)-acetone (V : V, 9 : 1) as eluent to afford a mixture of **4** and **5**, which was further separated by chromatography using petroleum ether (60–90 °C)-EtOAc as eluent to get **4** (23 mg) and **5** (18 mg). Fr. 6 was subjected to chromatography on silica gel using petroleum ether (60–90 °C)-acetone (V:V, 6 : 1) to get **1** (23 mg). Fr. 7 yielded **7** (15 mg) after chromatography using petroleum ether (60–90 °C)-acetone (V : V, 4 : 1) as eluent. Fr. 8 yielded **6** (15 mg) after chromatography using CHCl_3 -MeOH (V : V, 20 : 1) as eluent.

3.4. 3β , 11α -Dihydroxy-30-norlupan-20-one (**1**)

White needle crystals from petroleum ether (60–90 °C)-acetone. M. p. 149–151 °C, $[\alpha]_D^{26} -25.0^\circ$ (C, 1.0, CH_2Cl_2). IR (KBr, cm^{-1}): 3338, 2939, 1701, 1455, 1383, 1183, 1040. FAB-MS (70 ev) m/z : 426 (31), 411 (35), 393 (19), 365 (9), 43 (100). ^1H NMR (CDCl_3 , TMS, 400.13 MHz): δ 3.92 (dt $J = 5.4$, 10.7 Hz, H-11), 3.20 (t, $J = 8.4$ Hz, H-3 β), 2.17 (s, H-29), 1.03, 1.02, 0.98, 0.98, 0.79, 0.77 (s, H-23, 24, 25, 26, 27, 28). ^{13}C NMR: see Table 1.

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References

- Xie, Z. W.; Yu, Y. Q.: Name Reference of Chinese herbal medicine of the whole country, P. 938, People's Health Press, Beijing, 1996
- Wang, Shu-Fang; Li, Shuo; Li, Zhi-Gang; Li, Yu: *Pharmazie* **56**, 420 (2001)
- Ty, D. H. D.; Lischewski, M. et al.: *Phytochemistry* **23**, 2889 (1984)
- Enriquez, L. I.; Escobar, I. L.: *Tetrahedron* **42**, 3419 (1986)
- Savona, G.; Bruno, M.; Roriguez, B.; Marto, J. L.: *Phytochemistry* **26**, 3305 (1987)
- Ahmed, V. U.; Bano, S.; Voelter, W.; Fuchs, W.: *Tetrahedron Lett.* **22**, 1715 (1981)
- Ahmed, V. U.; Noorwala, M.; Mohammad, F. V.; Shah, M. G.; Parvez, A.: *Planta Med.* **59**, 366 (1993)
- Ahmad, V. U.; Mahammad, F. V.: *J. Nat. Prod.* **49**, 524 (1986)
- Sholichin, M.; Bruno, M.; Roriguez, B.; Marto, J. L.: *Phytochemistry* **26**, 3305 (1987)