Triterpenes and triterpene glycosides from *Salvia tricupis*

Zhen-Fu Hou, Zhi-Xiang Xie, Yong-Qiang Tu & Yu Li
National Laboratory of Applied Organic Chemistry, Lanzhou University, Lanzhou 730000, P.R.China

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Eighteen pentacyclic triterpenes and triterpene glycosides and three steroids have been isolated from *Salvia tricupis*. One of them is described for the first time as natural product, namely, 11α-hydroxyurs-12-en-3-one 1. Their structures have been determined on the basis of chemical and spectroscopic methods (MS, IR, 1H NMR, 13C NMR, and DEPT).

Some plants of the *Salvia genus* have been used as a traditional Chinese medicine for the treatment of various diseases. In order to find the active principles from the genus, studies on the chemical constituents of *Salvia tricupis* were carried out. We now report herein the isolation and structural elucidation of a new triterpene, 11α-hydroxyurs-12-en-3-one 1, in addition to seventeen known ursane and oleanane derivatives, 2α, 3β, 19-trihydroxy-24-oxo-urs-12-en-28-oic acid ester glucoside 2, 2α, 3α, 19α, 23-tetrahydroxyurs-12-en-28-oic acid ester glucoside 3, 2α, 3α, 19α-trihydroxyurs-12-en-28-oic acid ester glucoside 4, 2α, 3α, 19α-trihydroxyurs-12-en-28-oic acid 5, 2α, 3α, 23-tetrahydroxyurs-12-en-28-oic acid 6, 2α, 3β, 19-trihydroxyurs -12-en-28-oic acid 7, 2α, 3α, 19α-trihydroxyurs-12-en-28-oic acid 8, 2α, 3β-dihydroxyurs-12-en-28-oic acid 9, 2α, 3α-dihydroxyurs-12-en-28-oic acid 10, urs-12-en-3β-28-diol 11, urs-12-en-3β-ol 12, urs-12-en-3α-28-diol 13, urs-12-en-3-one 14, 2α, 3β, 24-trihydroxyolean-12-en-28-oic acid 15, 2α, 3α, 23-trihydroxyolean-12-en-28-oic acid 16, 2α, 3β-dihydroxyolean-12-en-28-oic acid 17, oleanic acid 18, and three steroids β-daucosterol 19, β-sitosterol 20, and ergost-7, 22-dien-3β-ol acetate 21.

11α-Hydroxyurs-12-en-3-one 1 was assigned molecular formula C30H4S02 by EIMS, 13C NMR and DEPT. It gave a positive Liebermann-Burchard (LB) Test for triterpenes. IR spectra showed absorption bands at 3422 (hydroxyl), 1720 (carbonyl). The 1H NMR spectrum exhibited the signals for eight skeletal groups, of which six were singlets (δ, 1.20, 1.17, 1.11, 1.11, 1.08, 0.81) and two were doublets (δ, 0.92, J=5.8 Hz, 0.86, J=5.9 Hz). The above data, coupled with the presence of 30 carbon atom signals in its 13C NMR spectrum (Table I) suggested that 1 was an ursane-type triterpenoid.

The 1H NMR spectrum of 1 further revealed a methine connected hydroxyl group (δ, 4.30, 1H, dd, J=9.3 Hz, 3.0 Hz) which must be equatorial and placed between a trisubstituted olefinic bond (δ, 5.20, 1H, d, J=3.0 Hz) and a methine group (δ, 1.61, 1H, d, J=9.3Hz). All the above data can be accommodated only on an urs-12-en triterpenoid structure for compound 1 with a hydroxyl group attached to the C-11α position.

The 13C NMR spectrum of 1 showed a carbonyl group signal (δ, 217.94). By further comparison of 1H NMR and 13C NMR spectral data of 1 with those of known compounds, urs-12-en-3α, 11α-diol 15 A and methyl urs-12-en-3-oxo-28-oate 16 B, it was found that 13C NMR spectral data at C-6 to C-22 and C-25 to C-30 of 1 were in agreement with those of compound A, and those at C-1 to C-5, C-23, C-24 of 1 corresponded with compound B. The 1H NMR data of H-9α, H-11α and H-12 of 1 were in accordance with compound A. Therefore, compound 1 was assigned the structure 11α-hydroxyurs-12-en-3-one.

**Experiment Section**

Melting points were recorded on a Kofler Melting point apparatus and are uncorrected. Mass spectra were recorded on a VG ZAB-HS mass spectrometer using 70 eV electron impact ionization. IR spectra were run on a Nicolet 170 SX FT-IR instrument; 1H NMR (400.13 Hz) and 13C NMR (100.16 MHz)
NOTES

Table 1—$^{13}$C NMR chemical shifts of compounds 1, A and B (CDCl$_3$, TMS, δ ppm)

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spectra on a Bruker AM 400 FT-NMR spectrometer in CDCl$_3$ and C$_2$D$_3$N with TMS as internal standard. Silica gel (200-300,300-400 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$_2$SO$_4$.

**Collection of plant material.** The plant material was collected in August 1996 in Zhang county, Gansu Province of China and identified by Y S Zhou of Lanzhou University. A voucher specimen has been preserved at the Herbarium of our institute.

**Extraction and isolation.** The air dried whole plants of *Salvia loricupis* (6.5 kg) were powdered and extracted two times (each 3 days) with 95% and 70% EtOH at room temperature, respectively. Filtration and evaporation of the whole solvent yielded 475 g of residue, which was dissolved in H$_2$O and partitioned with pet. ether, EtOAc and n-BuOH to give pet. ether fraction (115 g), fraction EtOAc (74 g) and n-BuOH fraction (55 g), respectively. Fraction (pet. ether) was dissolved in pet. ether and partitioned with MeOH-H$_2$O(100:1) to give MeOH-H$_2$O fraction (42g). The combined residue of fraction (EtOAc) and fraction (MeOH-H$_2$O) was subjected to column chromatography over silica gel using pet. ether-Me$_2$CO gradient to give fractions 1-10. Fraction 1 (pet ether-Me$_2$CO;30:1) was further separated by repeated column chromatography over silica gel using pet. ether-EtOAc (30:1) and pet. ether-CHCl$_3$-EtOAc (15:15:1) as eluants, and purified by recrystallization with EtOAc and pet. ether-EtOAc giving 270 mg of 14 and 50 mg of 21, respectively. Fraction 2 (pet. ether- Me$_2$CO; 20:1) on recrystallization with MeOH gave compound 20 (3.8 g) and mixture of 13 and 20, which was further separated by repeated column chromatography over silica gel using pet. ether-Et$_2$O (20:1) and pet. ether-EtOAc (30:1) as eluants giving 35 mg of 13. Fraction 3 (pet. ether-Me$_2$CO; 10:1) was further separated by column chromatography over silica gel using C$_6$H$_6$-Et$_2$O (10:1) and C$_6$H$_6$-Me$_2$CO (20:1) as eluants giving 45 mg of 1 and 30 mg of 12. Fraction 4 (pet. ether-Me$_2$CO;5:1) on repeated chromatographic purification over a silica gel column and eluting with pet. ether-CHCl$_3$-Me$_2$CO (5:5:1), and by crystallization several times with heating MeOH gave pure compounds 11 (6.8 g) and 18 (65 mg). Fraction 5,6,7 and 8 (pet. ether-Me$_2$CO; 4:1, 7:2, 3:1, 5:2) were separated by column chromatography over silica gel using C$_6$H$_6$-Me$_2$CO (8:1), CHCl$_3$-MeOH (25:1), C$_6$H$_6$-MeOH (15:1) and CHCl$_3$-MeOH (20:1) as eluants respectively, to get four crude fractions. Each was methylated with CH$_3$2N$_2$, then chromatographed on silica column and purified by recrystallization. Compounds 9a (45 mg), 17a (60 mg) and 10a (30mg) were obtained from fraction 5; compounds 7a (30 mg) and 8a (25 mg) from fraction 6, compounds 6a (35 mg) and 16a (15 mg) from fraction 7, and compounds 5a (30 mg) and 15a (15 mg) from fraction 8. Fraction 9 (pet. ether-Me$_2$CO; 2:1) was subjected to column chromatography over silica gel and eluted with CHCl$_3$-MeOH (10:1) to yield 19 (1.8g). The combined residue of fraction 10 (Me$_2$CO, MeOH) and fraction (n-BuOH) were subjected to column...
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chromatography over silica gel and eluted with CHCl₃-MeOH-H₂O (4:1:0.1), EtOAc-MeOH-H₂O (8:1:0.1) and EtOAc-n-BuOH-H₂O (15:1:0.1) successively giving compounds 2 (45 mg), 3 (50 mg) and 4 (40 mg).

The known compounds were identified either by comparing their corresponding properties (melting point, mass, IR, ¹H NMR and ¹³C NMR) with literature values or comparing with authentic samples.

11α-Hydroxyurs-12-en-3-one 1. White crystals, mp 132-34°C; IR (KBr): 3422, 2927, 2856, 1720, 1586, 1462, 1380, 1192, 1141, 1038; EI MS (70 eV): m/z 673 [M+Na]⁺, 671 [M+Li]⁺, 502 [M+Gluc]⁺; ¹H NMR (CDCl₃, TMS, δ, ppm): 10.38 (IH, s, H-24), 6.18 (1H, d, J=8.2 Hz, H-l), 5.45 (IH, br. s, H-12), 4.0-4.7 (5H, m, H-2 to H-6), 3.97 (1H, dd, J=9.3, 3.0 Hz, H-11α), 1.61 (1H, d, J=9.3 Hz, H-9 α), 1.20, 1.17, 1.11, 1.11, 1.08, 0.81 (3H each, s, Me-23 to Me-28), 0.92 (3H, d, J=5.8 Hz, Me-29 or 30), 0.86 (3H, d, J=5.9 Hz, Me-29 or 30); ¹³C NMR and DEPT: see Table I.

2α, 3β, 19α-trihydroxy-24-oxo-urs-12-en-28-oic acid ester glucoside 2. White amorphous powder, mp 200-3°C; FABMS: m/z 687 [M+Na]⁺, 671 [M+Li]⁺, 502 [M+Gluc]⁺; ¹H NMR (CD₂D₅N, TMS, δ, ppm): 10.38 (IH, s, H-24), 6.18 (1H, d, J=8.2 Hz, H-l), 5.45 (IH, br. s, H-12), 4.0-4.7 (5H, m, H-2 to H-6), 3.97 (1H, dd, J=9.3, 3.0 Hz, H-11α), 1.61 (1H, d, J=9.3 Hz, H-9 α), 1.20, 1.17, 1.11, 1.11, 1.08, 0.81 (3H each, s, Me-23 to Me-28), 0.92 (3H, d, J=5.8 Hz, Me-29 or 30), 0.86 (3H, d, J=5.9 Hz, Me-29 or 30); ¹³C NMR data were identical with those reported in the literature.

2α, 3β, 19α-trihydroxyurs-12-en-28-oic acid ester glucoside 3. White amorphous powder, mp 213-16°C; FABMS: m/z 689 [M+Na]⁺, 673 [M+Li]⁺, 504 [M+Gluc]⁺; ¹H NMR (CD₂D₅N, TMS, δ, ppm): 6.93 (IH, d, J=8.1 Hz, H-l), 5.46 (IH, br. s, H-12), 2.86 (1H, s, H-18), 1.65, 1.45, 1.28, 1.06, 0.88 (3H each, s, Me-24 to Me-27, Me-29), 1.03 (3H, d, J=6.3 Hz, Me-30); ¹³C NMR data were identical with those reported in the literature.

2α, 3β, 19α-trihydroxyurs-12-en-28-oic acid ester glucoside 4. White amorphous powder, mp 203-6°C; FABMS: m/z 673 [M+Na]⁺, 657 [M+Li]⁺, 488 [M+Gluc]⁺; ¹H NMR (CD₂D₅N, TMS, δ, ppm): 6.19 (1H, d, J=8.2 Hz, H-l), 5.52 (IH, br. s, H-12), 4.0-4.7 (6H, m, H-2 to H-6'), 3.70 (IH, br. s, H-3β), 3.00 (1H, s, H-18), 1.46, 1.29, 1.10, 0.96, 0.88, 0.80 (3H each, s, Me-23 to Me-27, Me-29), 1.01 (3H, d, J=6.5 Hz, Me-30); ¹³C NMR data were identical with those reported in the literature.

Methyl 2α, 3β, 24-trihydroxy-olean-12-en-28-oate 15a. ¹³C NMR (CDCl₃, TMS): 46.12 (C-1, CH₂), 68.91 (C-2, CH₂), 85.23 (C-3, CH), 43.10 (C-4, C), 55.78 (C-5, CH), 18.32 (C-6, CH₃), 32.72 (C-7, CH₂), 39.23 (C-8, CH), 47.55 (C-9, CH), 37.97 (C-10, C), 23.65 (C-11, CH₂), 121.95 (C-12, CH₂), 143.78 (C-13, C), 41.57 (C-14, C), 27.56 (C-15, CH₂), 24.10 (C-16, CH₂), 46.63 (C-17, C), 41.17 (C-18, CH), 45.77 (C-19, CH₂), 30.56 (C-20, C), 33.77 (C-21, CH₂), 32.29 (C-22, CH₂), 23.00 (C-23, CH₃), 65.47 (C-24, CH₂), 16.76 (C-25, CH₃), 16.72 (C-26, CH₃), 25.87 (C-27, CH₃), 178.30 (C-28, C), 33.04 (C-29, CH₃), 23.56 (C-30, CH₃). The ¹³C NMR spectral data have not been reported in the literature so far.

Reference
8 Zhen-Fu Hou, Yan-Ping Shi, Xiong-Fang Li & Yu Li, Indian J Chem, 36B, 1997, 293.