TRITERPENOIDS AND STEROIDS FROM *Ixeridium gracile*

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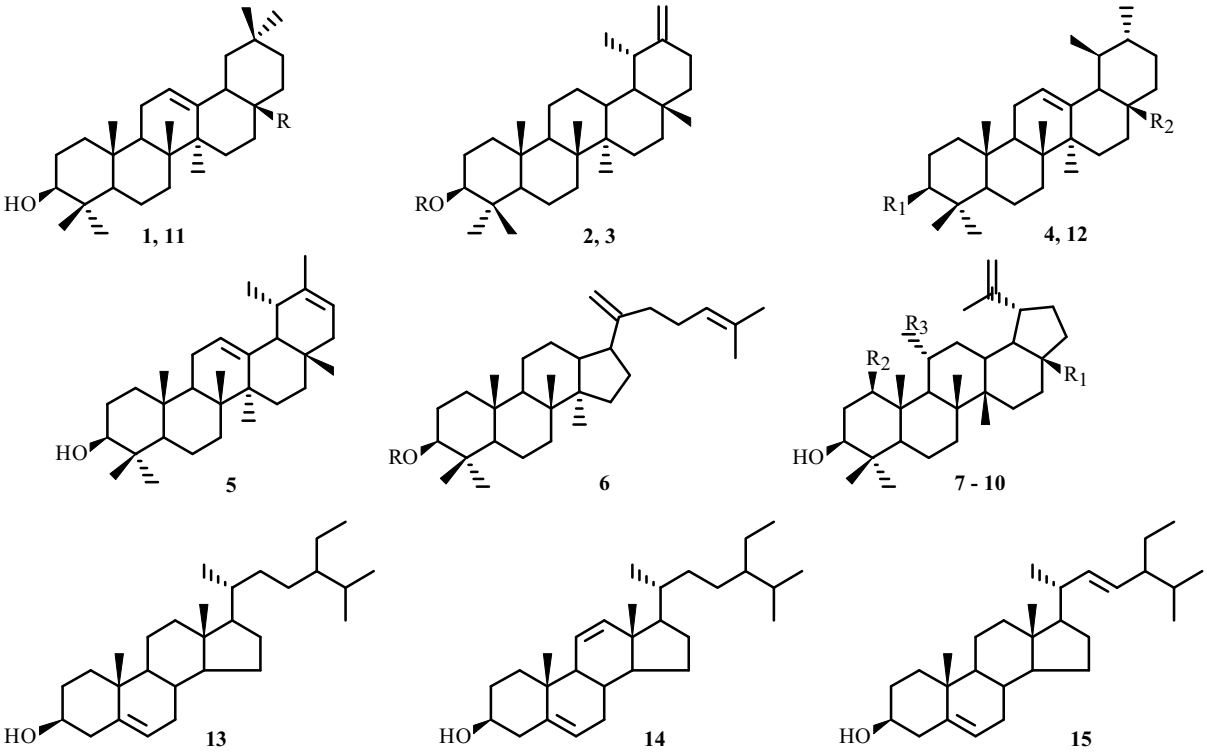
Ixeridium gracile, a herbaceous perennial plant belonging to the Compositae family, is the only species of *Ixeridium* Cass distributed in Sitsang region used as a representative Tibetan herbal medicine and is also valued as a delicious and nutritional potherb [1]. Up to now, its chemical constituents have not been investigated. In continuing our investigation on natural products for terpenoids and steroids [2-5], eleven triterpenoids compounds and three steroids were isolated from an alcoholic extract of the whole plant of *I. gracile*. We report herein the isolation and structural elucidation of all these compounds.

From an alcoholic extract of the whole plant of *I. gracile* (DC.) Shih, β-amyrin (**1**) [5], taraxasterol (**2**) [5], taraxast-20(30)-ene-3β-*O*-palmitate (**3**) [4], α-amyrin (**4**) [5], pseudotaraxasterol (**5**) [5], dammara-20,24-dien-3β-ol (**6**) [6], lupeol (**7**) [2, 7], lup-1β,3β,11α-triol (**8**) [2, 8], lup-3β-ol-28-carboxylate (**9**) [8], betulinic acid (**10**) [2, 8], oleanolic acid (**11**) [9], ursolic acid (**12**) [9], β-sitosterol (**13**), stigmast-5,11-dien-3β-ol (**14**), and stigmasterol (**15**) were isolated and purified by repeated chromatography over silica gel column. The structure of every compound was postulated on the basis of spectroscopic analysis.



1: R = CH_3; **2:** R = H; **3:** R = $OCO(CH_2)_{14}CH_3$; **4:** R_1 = OH, R_2 = CH_3; **7:** R_1 = CH_3, R_2 = R_3 = H; **8:** R_1 = CH_3, R_2 = R_3 = OH; **9:** R_1 = $COOCH_3$, R_2 = R_3 = H; **10:** R_1 = COOH, R_2 = R_3 = H; **11:** R = COOH; **12:** R_1 = OH, R_2 = COOH

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β-Amyrin (1). Colorless needles. The data of ^{1}H NMR and ^{13}C NMR spectrum were identical to that in the literature [5].

Taraxasterol (2). Colorless needle crystal, mp 130-132°C. IR (KBr, cm^{-1}): 3236 (OH), 1637 (C=C). ^{1}H NMR (400 MHz, TMS, $CDCl_3$, δ, J/Hz): 4.66 (H-30α), 4.54 (H-30β), 3.21 (H-3, dd, J = 11.2, 4.4). ^{13}C NMR (100 MHz, TMS, $CDCl_3$, δ): 38.82 (C-1), 26.90 (C-2), 79.20 (C-3), 38.88 (C-4), 55.51 (C-5), 17.98 (C-6), 33.78 (C-7), 39.92 (C-8), 50.10 (C-9), 37.18 (C-10), 21.42 (C-11), 26.78 (C-12), 38.76 (C-13), 42.22 (C-14), 26.89 (C-15), 38.70 (C-16), 33.22 (C-17), 48.64 (C-18), 39.45 (C-19), 154.55 (C-20), 35.42 (C-21), 38.76 (C-22), 27.95 (C-23), 15.59 (C-24), 16.81 (C-25), 16.08 (C-26), 14.63 (C-27), 19.26 (C-28), 25.42 (C-29), 107.12 (C-30).

Taraxast-20(30)-ene-3β-*O*-palmitate (3). Colorless oil. ^{1}H NMR (400 MHz, TMS, $CDCl_3$, δ, J/Hz): 4.64 (H-30α), 4.60 (H-30β), 4.50 (H-3, dd, J = 10.8, 5.6 Hz). ^{13}C NMR (100 MHz, TMS, $CDCl_3$, δ): 38.28 (C-1), 23.90 (C-2), 80.65 (C-3), 39.88 (C-4), 55.51 (C-5), 17.98 (C-6), 33.98 (C-7), 39.98 (C-8), 50.16 (C-9), 37.28 (C-10), 21.42 (C-11), 25.78 (C-12), 38.76 (C-13), 42.22 (C-14), 26.89 (C-15), 38.70 (C-16), 33.32 (C-17), 48.64 (C-18), 39.45 (C-19), 154.55 (C-20), 35.42 (C-21), 38.76 (C-22), 27.95 (C-23), 15.59 (C-24), 16.81 (C-25), 16.08 (C-26), 14.23 (C-27), 20.26 (C-28), 25.22 (C-29), 107.32 (C-30); 173.80 (C-1′), 34.78 (C-2′), 25.26 (C-3′), 29.34 (C-4′), 29.76 (C-13′), 31.96 (C-14′), 22.72 (C-15′), 14.10 (C-16′).

α-Amyrin (4). Colorless needles, mp 184~185°C. The data of ^{1}H NMR and ^{13}C NMR spectrum were identical to that in the literature [5].

Pseudotaraxasterol (5). Colorless power, mp 196~198°C. ^{1}H NMR (400 MHz, TMS, $CDCl_3$, δ, J/Hz): 3.20 (dd, J = 2.40, 10.20 Hz, 3-H), 1.02 (3H, s, 23-H), 0.77 (3H, s, 24-H), 0.83 (3H, s, 25-H), 1.04 (3H, s, 26-H), 0.90 (3H, s, 27-H), 0.93 (3H, s, 28-H), 1.63 (3H, s, 29-H), 4.61 (2H, m, 30-H). ^{13}C NMR (100 MHz, TMS, $CDCl_3$, δ): 38.75 (C-1), 27.40 (C-2), 79.12 (C-3), 40.14 (C-4), 55.18 (C-5), 21.42 (C-6), 34.40 (C-7), 41.06 (C-8), 50.28 (C-9), 37.91, (C-10), 21.53 (C-11), 27.89 (C-12), 39.38 (C-13), 42.14 (C-14), 27.13 (C-15), 38.30 (C-16), 36.31 (C-17), 48.64 (C-18), 37.13 (C-19), 139.78 (C-20), 117.94 (C-21), 41.05 (C-22), 34.02 (C-23), 15.38 (C-24), 18.30 (C-25), 16.14 (C-26), 14.71 (C-27), 18.29 (C-28), 26.23 (C-29), 25.60 (C-30).

Dammara-20,24-dien-3β-ol (6). Colorless needles, mp 135°C. IR (KBr, ν_{max}, cm^{-1}): 3380 (OH), 1640 (C=C). ^{1}H NMR (400 MHz, TMS, $CDCl_3$, δ, J/Hz): 0.75 (3H, s), 0.82 (3H, s), 0.84 (3H, s), 0.95 (6H, s), 1.58 (3H, br s), 1.66 (3H, br s), 3.18 (1H, dd, J = 5.16, 11.00 Hz), 4.67, 4.68 (each 1H, each, s, 21-H), 5.16 (1H, s, 24-H). ^{13}C NMR (100 MHz, TMS, $CDCl_3$, δ): 35.31 (C-1), 34.08 (C-2), 78.89 (C-3), 40.41 (C-4), 55.81 (C-5), 18.22 (C-6), 28.82 (C-7), 38.90 (C-8), 50.81 (C-9), 37.16 (C-10), 21.32 (C-11), 24.92 (C-12), 45.11 (C-13), 49.42 (C-14), 31.33 (C-15), 27.32 (C-16), 47.78 (C-17), 15.56 (C-18), 15.02 (C-19), 152.67 (C-20), 107.14 (C-21), 39.51 (C-22), 27.21 (C-23), 124.46 (C-24), 131.42 (C-25), 25.56 (C-26), 17.12 (C-27), 16.22 (C-28), 25.30 (C-29), 15.62 (C-30).

Lupeol (7). White needles, mp 208~210°C. The data of ^{1}H NMR and ^{13}C NMR spectrum were identical to that in the literature [2, 7].

Lup-1β,3β,11α-triol (8). ^{1}H NMR (400 MHz, TMS, $CDCl_3$, δ): 3.22 (1H, m, 1-H), 3.17 (1H, m, 3-H), 3.12 (1H, m, 3-H), 0.88 (3H, s, 23-H), 0.79 (3H, s, 24-H), 0.86 (3H, s, 25-H), 1.01(3H, s, 26-H), 0.94 (3H, s, 27-H), 0.78 (3H, s, 28-H), 4.66 (1H, br s, 29-H), 4.52 (1H, br s, 29-H′), 1.09 (3H, s, 30-H). ^{13}C NMR (100 MHz, TMS, $CDCl_3$, δ): 79.02 (C-1), 37.55 (C-2), 75.74 (C-3), 38.88 (C-4), 55.02 (C-5), 18.32 (C-6), 34.17 (C-7), 40.18 (C-8), 50.03 (C-9), 37.21 (C-10), 70.52 (C-11), 27.76 (C-12), 37.78 (C-13), 42.10 (C-14), 27.03 (C-15), 35.13 (C-16), 42.56 (C-17), 48.65 (C-18), 47.77 (C-19), 150.24 (C-20), 30.25 (C-21), 38.67 (C-22), 27.93 (C-23), 15.31 (C-24), 16.30 (C-25), 15.98 (C-26), 14.68 (C-27), 17.33 (C-28), 109.16 (C-29), 20.72 (C-30).

Lup-3β-ol-28-carboxylate (9). ^{1}H NMR (400 MHz, TMS, $CDCl_3$, δ): 3.15 (1H, m, 3-H), 0.85 (3H, s, 23-H), 0.79 (3H, s, 24-H), 0.83 (3H, s, 25-H), 1.01 (3H, s, 26-H), 0.92 (3H, s, 27-H), 3.65 (3H, s, OCH_3), 4.65 (1H, br s, 29-H), 4.54 (1H, br s, 29-H′), 1.10 (3H, s, 30-H). ^{13}C NMR (100 MHz, TMS, $CDCl_3$, δ): 38.62 (C-1), 27.48 (C-2), 78.88 (C-3), 38.62 (C-4), 55.18 (C-5), 18.58 (C-6), 34.20 (C-7), 40.77 (C-8), 50.20 (C-9), 37.16 (C-10), 21.19 (C-11), 25.78 (C-12), 38.46 (C-13), 42.42 (C-14), 30.51 (C-15), 32.15 (C-16), 56.37 (C-17), 48.81 (C-18), 39.25 (C-19), 150.15 (C-20), 29.57 (C-21), 37.11 (C-22), 27.88 (C-23), 15.32 (C-24), 16.12 (C-25), 16.53 (C-26), 14.77 (C-27), 176.12 (C-28), 109.28 (C-29), 19.46 (C-30).

Betulinic Acid (10). The data of ^{1}H NMR and ^{13}C NMR spectrum were identical to that in the literature [2, 8].

Oleanolic Acid (11). White powder, IR (KBr, ν_{max}, cm^{-1}): 3400 (OH), 1690 (C=C). The data of ^{1}H NMR and ^{13}C NMR spectrum were identical to that in the literature [9].

Ursolic Acid (12). White powder, IR (KBr, ν_{max}, cm^{-1}): 3400 (OH), 1690 (C=C). The data of ^{1}H NMR and ^{13}C NMR spectrum were identical to that in the literature [9].

β-Sitosterol (13). White needle crystals from Me_2CO, mp 139-140°C. TLC and IR spectrum were identical with those of an authentic sample.

Stigmast-5,11-dien-3β-ol (14). White needle crystals, mp 146-148°C. TLC and IR spectrum were identical with those of an authentic sample.

Stigmasterol (15). Colorless needles, mp 149-151°C. TLC and IR spectrum were identical with those of an authentic sample.

The plant material was bought from Tibetan Hospital of Qinghai province, China, in August 2004 and was identified by Dr. Huan-Yang Qi. A voucher specimen has been deposited at Key Laboratory for Natural Medicine of Gansu Province, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, P.R. China.

Extract, Isolation, and Purification of Compounds. Air-dried and ground whole plants of *Ixeridium gracile* (DC.) Shih (3.0 kg) were extracted seven times with 85% EtOH at room temperature, each time lasting three days. The combined extracts were evaporated to dryness under reduced pressure. The residue (120 g) was then suspended in H_2O (1.0 L) and extracted with petroleum ether (60-90°) (1.5 L × 8), ethyl acetate (1.5 L × 6), and *n*-butanol (1.5 L × 4). The petroleum ether extract (60 g) was subjected to column chromatography on silica gel (200-300 mesh, 600 g) using petroleum ether with increasing volume of acetone (v/v, from 30:1 to 1: 1) as eluent to give six fractions (Fr. 1 - Fr. 6). Fraction 1 (v/v, from 30: 1 to 25: 1) was chromatographed on silica gel column using petroleum ether-chloroform (v/v, 10: 1) as eluent to yield pure compound **1** (10 mg) , compound **2** (9 mg), and compound **13** (40 mg). Fraction 2 (v/v, from 25: 1 to 20: 1) was eluted with petroleum ether-ethyl acetate (v: v = 18: 1) to give Fr. 2a, Fr. 2b, and Fr. 2c. Fraction 2a was repeatedly purified by CC over silica gel with petroleum ether-acetone (v/v, 20: 1) as eluent to give pure compound **3** (10 mg) and compound **14** (35 mg). Fraction 2b was chromatographed on silica gel column with petroleum ether-ethyl acetate (v/v, 12 :1) as eluent to give compound **4** (17 mg). Fraction 2c was chromatographed on silica gel column with petroleum ether-ethyl acetate (v/v, 10 :1) as eluent to give compound **5** (12 mg) and compound **15** (10 mg). Fraction 3 (v/v, from 20: 1 to 15: 1) was eluted with petroleum ether-ethyl acetate (v: v = 15: 1) to give compound **6** (17 mg) and compound **7** (15 mg). Fraction 4 (v/v, from 15: 1 to 12: 1) gave compound **8** (9 mg) and compound **9** (10 mg) after CC on silica gel eluted with petroleum ether-acetone (v/v, 10: 1). Fraction 5 (v/v, from 12: 1 to 10: 1) was chromatographed using petroleum ether-acetone (v/v, 10: 1) as eluent to afford compound **10** (10 mg). Fraction 6 (v/v, from 10: 1 to 5: 1) was chromatographed using petroleum ether-acetone (v/v, 8: 1) as eluent to afford compound **11** (10 mg) and compound **12** (15 mg).

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REFERENCES

1. *Flora Xizangica*, Tibet Plateau Scientific Expedition Team of the Chinese Academy Sciences. Beijing: Science Press, **4**, 961 (1985).
2. Y. Li, Q. X. Wu, and Y. P. Shi, *Pharmazie*, **58,** 937 (2003).
3. Y. P. Jin and Y. P. Shi, *Pharmazie*, **59,** 855 (2004).
4. J. T. Feng and Y. P. Shi, *Pharmazie*, **60,** 464 (2005).
5. A. M. Yang, X. Liu, R. H. Lu, and Y. P. Shi, *Pharmazie*, **61,** 70 (2006).
6. Q. F. Zhang, S. D. Luo, and H. Y. Wang, *Acta Botanica Yunnanica*, **20** (3), 362 (1998).
7. R. H. Liu and L. G. Kong, *Nat. Prod. Res. Dev.*, **17** (4), 437 (2005).
8. S. B. Mahato and A. P. Kundu, *Phytochemistry*, **37,** 1517 (1994).
9. Y. Luo, J. Tian, and F. E. Wu, *Nat. Prod. Res. Dev.*, **15** (6), 502 (2003).