Five Triterpene Glycosides from *Oxytropis myriophylla*

Masafumi OKAWA,[a] Rie YAMAGUCHI,[a] Hurlad DELGER,[a] Ryota TSUCHIHASHI,[a] Toshihiro NOHARA,*[,a] Junei KINJO,[b] Susumu ISODA,[c] and Yoshiteru IDA[c]

[a] *Faculty of Pharmaceutical Sciences, Kumamoto University; 5–1 Oe-honmachi, Kumamoto 862–0973, Japan:* [b] *Faculty of Pharmaceutical Sciences, Fukuoka University; 8–19–1 Nanakuma, Jyonan-ku, Fukuoka 814–0133: and* [c] *Showa University, School of Pharmaceutical Sciences, 1–5–8 Hatanodai, Shinagawa-ku, Tokyo 142–0064, Japan.*
Received November 16, 2001; accepted April 11, 2002

Four new triterpene glycosides: one cycloartane-type glycoside and three azukisapogenol glycosides were isolated together with one known oleanene bisdesmoside from the Mongolian natural medicine, *Oxytropis myriophylla.*

Key words *Oxytropis myriophylla*; Leguminosae; Mongolian natural medicine; triterpene saponin; cycloartane glycoside; azukisapogenol glycoside

The whole plant of *Oxytropis myriophylla* (PALL.) DC. (Leguminosae) is an important Mongolian medicine and its antifebrile, detoxication and hemostatic properties have been used in the treatment of rubella and influenza, applied to swelling and throat pain, and various bleeding owing to poor blood circulation.

In our search for bioactive substances among leguminous plants,[1,2] we have studied the constituents of the title plant. Whole plants collected in a suburb of Hailar City were extracted with MeOH. The methanolic extract was separated and purified with the aid of various column chromatographies to provide five new triterpene glycosides, referred to as myriosides A—D (**1**—**4**, respectively), together with pericarsaponin Pk.[3,4] This paper deals with their chemical structures.

Myrioside A (**1**) was obtained as an amorphous powder showing $[\alpha]_D$ −10.1° (MeOH). The positive FAB-MS exhibited a peak at *m/z* 941 due to $[M+Na]^+$ and the high-resolution (HR) positive FAB-MS gave a quasimolecular ion at *m/z* 941.5078 (Calcd for $C_{46}H_{78}O_{18}Na$: 941.5086). The ^{1}H-NMR spectrum showed six tertiary methyl signals between δ 1.13 and 1.51, one secondary methyl signal at δ 1.00 (d, J=6.1 Hz) and characteristic signals at δ 0.46 and 0.96 (each 1H, d, J=4.3 Hz) for a cyclopropane ring. Therefore, **1** was deduced to be a cycloartane triterpene derivative. Moreover, the ^{1}H-signals displayed the presence of three anomeric protons at δ 5.02 (1H, d, J=7.3 Hz), 5.16 (1H, d, J=7.9 Hz) and 5.68 (1H, d, J=2.4 Hz). The ^{13}C-NMR showed 46 carbon signals in total, and measurement of heteronuclear multiple bond connectivity (HMBC) led to the assignment of five hydroxy groups at C-1, -3, -7, -24 and -25 as shown in Fig. 1. The aglycone moiety of **1** was deduced to be identical with that reported in a saponin obtained from *Astragalus oleifobius*[5] by comparing their ^{13}C-NMR spectra, and was substantiated by measurement of proton–proton chemical shift correlation spectroscopy (^{1}H–^{1}H COSY), heteromolecular multiple quantum coherence (HMQC) and HMBC (Fig. 1). Acid hydrolysis of **1** provided D-apiose, D-xylose and D-glucose by gas-liquid chromatography (GLC),[6] and inspection of the ^{13}C signals due to the sugar moiety resulted in assignment of the respective sugar components of a terminal D-apiofuranosyl (C-1—5, δ 108.9, 77.7, 80.3, 75.2, 65.2), a 4-substituted D-xylopyranosyl (C-1—5, δ 105.1, 74.8, 76.1, 76.0, 64.3) and a 4-substituted D-glucopyranosyl (C-1—6, δ 98.5, 75.0, 76.5, 80.7, 76.3, 61.7) moiety. Various two-dimensional (2D) NMR spectra (^{1}H–^{1}H COSY, HMQC, HMBC, Fig. 1) also enabled assignment of the ^{1}H signals and connectivities of the respective sugar components. The ^{1}H assignments for the sugar moiety [terminal apiosyl H-1—2, H_2-4, H_2-5: δ 5.68 (1H, d, J=2.4 Hz), 4.65 (1H, d, J=2.4 Hz), 4.30, 4.67 (each 1H, d, J=9.2 Hz), 4.13 (2H, s), respectively, xylosyl H-1—4, H_2-5: δ 5.02 (1H, d, J=7.3 Hz, 3.93 (1H, dd, J=7.3, 8.7 Hz), 4.05 (1H, t-like, J=8.7 Hz), 4.18 (1H, overlapped), 3.49 (1H, t-like, J=10.6 Hz), 4.13 (1H, overlapped), glucosyl H-1—5, H_2-6: δ 5.16 (1H, d, J=7.9 Hz), 4.00 (1H, t-like, J=7.9 Hz), 4.21 (1H, overlapped), 4.22 (1H, t-like, J=8.0 Hz), 4.21 (1H, overlapped), 4.42, 4.48 (each 1H, d, J= 9.2 Hz)], respectively, also supported the above sugar structure deduced from the ^{13}C-NMR spectrum. The respective sugar anomeric configurations were decided based on the J values of their anomeric protons and chemical shifts on the ^{13}C-NMR spectra.[7] Consequently, the structure of **1** was determined to be 25-*O*-β-D-apiofuranosyl(1→4)-β-D-xylopyranosyl(1→4)-β-D-glucopyranosyl 1α,3β,7β,24(*S*),25-pentahydroxycycloartane.

Myrioside B (**2**) was obtained as an amorphous powder

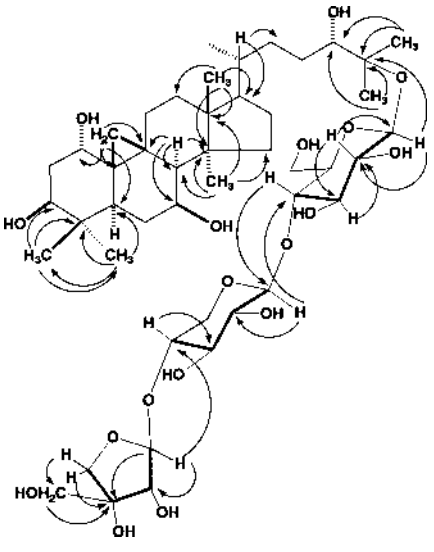

Fig. 1. Structure and Significant HMBC of **1**

* To whom correspondence should be addressed. e-mail: none@gpo.kumamoto-u.ac.jp

showing $[\alpha]_D$ +2.3° (MeOH). The positive FAB-MS exhibited peaks at *m/z* 693 and 671 due to $[M-H+2Na]^+$ and $[M+Na]^+$, respectively. The HR positive FAB-MS gave a quasimolecular ion at *m/z* 693.3639 (Calcd for $C_{36}H_{55}O_{10}Na_2$: 693.3591). The ^{1}H-NMR spectrum showed six tertiary methyl signals between δ 0.80 and 1.49 and one anomeric proton signal at δ 4.98 (1H, d, J=7.3 Hz). The ^{13}C-NMR spectrum exhibited 36 signals constituted of a triterpene moiety, including an oxygen-bearing carbon (δ 88.9), a hydroxymethyl group (δ 63.1) and a carboxylic group (δ 181.6), and a β-D-glucuronopyranosyl moiety (C-1—6: δ 105.9, 75.0, 76.9, 73.5, 77.8, 175.1). The ^{13}C-signals which arose from the sapogenol were identical with those except that of C-3 of the sapogenol, azukisapogenol, reported for the constituents in *Phaseolus azuki*.[8,9] The structure of the sapogenol was verified by 2D NMR techniques. Moreover, the H-1 (d, J=7.3 Hz at δ 4.98) of glucuronic acid correlated with the C-3 (δ 88.9) of the aglycone in the HMBC. Therefore, it was shown that the glucuronic acid linked to C-3-OH. Thus, **2** was characterized as 3-*O*-β-D-glucuronopyranosyl azukisapogenol.

Myrioside C (**3**), obtained as an amorphous powder, showed $[\alpha]_D$ −2.2° (MeOH) and a peak at *m/z* 855 and 833 due to $[M-H+2Na]^+$ and $[M+Na]^+$, respectively, in the positive FAB-MS. The HR positive FAB-MS gave a quasimolecular ion at *m/z* 855.4154 (Calcd for $C_{42}H_{65}Na_2$: 855.4119). The ^{1}H-NMR spectrum showed six tertiary methyl signals between δ 0.78 and 1.50 and two anomeric proton signals at δ 5.01 (1H, d, J=7.3 Hz) and 6.29 (1H, d, J=7.9 Hz). The ^{13}C-NMR spectrum of **3** exhibited 42 signals composed of a azukisapogenol, a terminal β-D-glucuronopyranosyl moiety (C-1—6: δ 106.4, 75.3, 77.9, 73.3, 78.0, 172.5) and a terminal β-D-glucopyranosyl moiety (C-1—6: δ 96.0, 74.2, 78.6, 70.9, 79.4, 62.1). A newly occurring glucosyl moiety was found to connect to the C-29 carboxylic group since the carboxylic carbon shifted +3.9 ppm by glycosidic esterification in comparison with that of **2**. The HMBC showed correlations between the H-1 (d, J=7.3 Hz, δ 5.01) of glucuronic acid and the C-3 (δ 89.0) of the aglycone, and between the H-1 (d, J=7.9 Hz, δ 6.29) of glucose and the C-29 COOH (δ 177.7) of the aglycone. Therefore, **3** was represented as 3-*O*-β-D-glucuronopyranosyl azukisapogenol 29-*O*-β-D-glucopyranosyl ester.

Myrioside D (**4**), obtained as an amorphous powder, showed $[\alpha]_D$ −4.0° (MeOH) and a peak at *m/z* 987 and 965 due to $[M-H+2Na]^+$ and $[M+Na]^+$, respectively, in the positive FAB-MS. The HR positive FAB-MS gave a quasimolecular ion at *m/z* 987.5257 (Calcd for $C_{47}H_{73}O_{19}Na_2$: 987.4541). The ^{1}H-NMR spectrum showed six tertiary methyl signals between δ 0.72 and 1.41 and three anomeric proton signals at δ 4.84 (1H, d, J=7.3 Hz), 5.38 (1H, d, J=7.3 Hz) and 6.31 (1H, d, J=7.9 Hz). The ^{13}C-NMR spectrum of **4** exhibited 47 signals in total composed of a azukisapogenol, a terminal β-D-xylopyranosyl(1→2)-β-D-glucuronopyranosyl moiety (xyl C-1—5: δ 104,6, 75.4, 78.0, 70.5, 66.9, glc A C-1—6: δ 105.0, 80.6, 75.0, 70.8, 76.2, 175.0) and a terminal β-D-glucopyranosyl moiety (C-1—6: δ 95.8, 73.9, 78.2, 70.8, 79.2, 61.9). The HMBC were observed between the H-1 (d, J=7.3 Hz, δ 4.83) of glucuronic acid and the C-3 (δ 90.4) of the aglycone, between the C-2 (δ 80.6) of glucuronic acid and the H-1 (d, J=7.3 Hz, δ 5.38) of xylose, and between the H-1 (d, J=7.9 Hz, δ 6.31) of glucose and the C-29 COOH (δ 178.0) of the aglycone. Consequently, the structure of **4** was elucidated as 3-*O*-β-D-xylopyranosyl(1→2)-β-D-glucuronopyranosyl azukisapogenol 29-*O*-β-D-glucopyranoside ester.

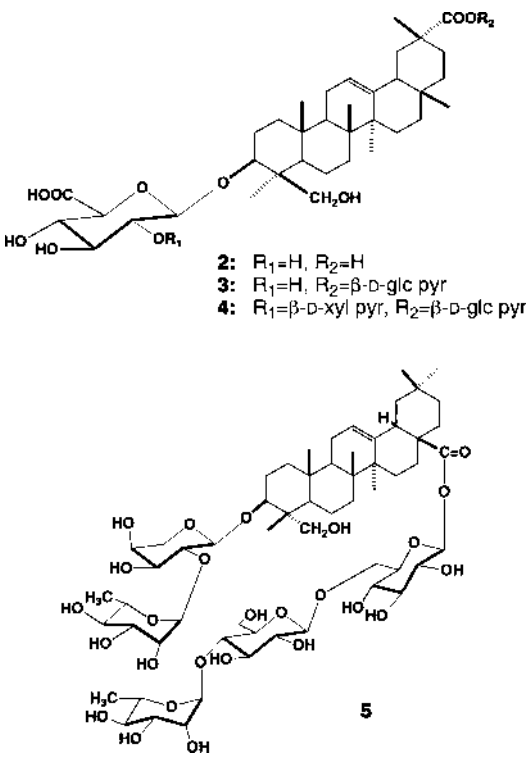

Fig. 2. Structures of **2**, **3**, **4** and **5**

Pericarsaponin Pk (**5**),[3,4] obtained as an amorphous powder, showed $[\alpha]_D$ +16.2° (MeOH) and a quasimolecular peak at *m/z* 1244 due to $[M+Na]^+$ in the positive FAB-MS, and was identified by ^{1}H-, ^{13}C-NMR and HMBC spectra.

Experimental

General Procedures Optical rotations were determined on a JASCO DIP-1000 polarimeter (l=0.5). FAB-MS were obtained in a glycerol matrix in the positive ion mode using a JEOL SX102A mass spectrometer. NMR spectra were measured in pyridine-d_5 on a JEOL α-500 spectrometer (500 MHz) and chemical shifts were referenced to tetramethylsilane (TMS). Column chromatography was carried out on silica gel 60 (230—400 mesh ASTM, Kanto Kagaku), Diaion HP-20 (Mitsubishi Kasei), Sephadex LH-20 (25—100 nm, Pharmacia Fine Chemicals), MCI gel CHP 20P (75—150 mm, Mitsubishi Kagaku), Bondapak ODS (Waters), and TLC was performed on precoated silica gel $60F_{254}$ (0.2 mm, Merck). GC was performed with a Hewlett-Packard HP-5890, detection: FID. column: silicone OV-1 (0.32 mm i.d.×30 m, Ohio Valley Specialty Chem.), column temp.: 230 °C, carrier gas: He 30 ml/min.

Plant Material Whole plants of *Oxytropis myriophylla* (PALL.) DC. (Leguminosae) were collected in a suburb of Hailar City in August 2000 and were identified by Mr. S. Isoda, Botanical Garden of the School of Pharmaceutical Sciences of Showa University. A voucher specimen has been deposited at this Botanical Garden.

Extraction and Isolation The collected plants were ground to powder (412.62 g), and hot MeOH provided an extract (35.33 g) after evaporation. The MeOH extract was partitioned between hexane and 80% MeOH, and the lower phase was taken up and evaporated to give a residue (19.07 g), which was then subjected to Diaion HP-20P to remove the sugar part eluting first with water and then with MeOH. The MeOH portion (6.68 g) was then applied to Toyo Pearl to collect a saponin fraction (3.47 g) by eluting with MeOH. The saponin fraction (3.47 g) was subjected to silica gel column chromatography with $CHCl_3$–MeOH–water=7 : 3 : 0.5 to give six fractions (Fr. 1—6). Fraction 1 (600.4 mg) was further chromatographed on silica

gel ($CHCl_3$–MeOH–water=7 : 3 : 0.5→6 : 4 : 1) and ODS (50% MeOH→MeOH) to provide compounds **1** (40 mg) and **2** (21 mg). Fraction 3 (363.3 mg) was also chromatographed on ODS (40% MeOH–MeOH) and silica gel (butanol–AcOH–water=4 : 1 : 5) to afford compounds **3** (24.2 mg) and **5** (7.2 mg). Fraction 4 (88.5 mg) was subjected to ODS (50% MeOH→MeOH) column chromatography to give compound **4** (15.8 mg).

Myrioside A (**1**): An amorphous powder, $[\alpha]_D^{16}$ −10.1° (c=0.10, MeOH). ^{1}H-NMR spectrum (in pyridine-d_5) δ: 0.45, 0.96 (each 1H, AB q, J=4.3 Hz, H_2-19), 1.00 (3H, d, J=6.1 Hz, H_3-21), 1.13 (3H, s, H_3-18), 1.16 (3H, s, H_3-28), 1.27 (3H, s, H_3-30), 1.30 (3H, s, H_3-29), 1.47, 1.52 (each 3H, s, H_3-26, -27), 3.80 (1H, br d, J=9.8 Hz, H-24), 3.86 (1H, overlapped, H-1), 3.99 (1H, overlapped, H-7), 4.39 (1H, overlapped, H-3). ^{13}C-NMR spectrum (in pyridine-d_5) δ: 72.7, 39.0, 73.0, 41.0, 39.4, 32.3, 70.2, 55.2, 21.1, 34.1, 26.6, 33.3, 46.0, 49.1, 37.8, 28.9, 52.4, 17.9, 28.2, 37.0, 19.0, 34.5, 29.2, 78.6, 81.1, 24.4, 20.9, 14.0, 26.2, 19.1 (aglycone moiety C-1—30, respectively).

D,L-Determination of Sugar Components for 1—4 A small amount of **1** (3 mg) in MeOH (0.5 ml) was heated in 2 M HCl/H_2O at 90 °C for 3 h. The hydrolysate was subjected to MCI gel CHP 20P and Amberlite IRA-400 to give a sugar fraction. This fraction was dissolved in pyridine (0.1 ml), then the solution was added to a pyridine solution (0.2 ml) of L-cystein methyl ester hydrochloride (0.1 mol/l) and heated at 80 °C for 1 h. The solvent was evaporated under a N_2 stream and dried *in vacuo* with heating. The remaining syrup was trimethylsilylated with trimethylimidazole (0.1 ml) at 60 °C for 1 h. After the addition of hexane and H_2O, the hexane layer was taken out and checked by GC. A respective solution of **2**—**4** (3 mg each) in MeOH (0.5 ml) was methylated with (trimethylsilyl)CH_2N_2 (2.0 M solution in hexane, 0.5 ml). To a solution of the methylated samples of **2**—**4** was added $NaBH_4$, and mixture was kept at room temperature for 30 min. The reaction mixture was worked up with MCI gel CHP 20P. The MeOH eluate was evaporated and heated in 2 M HCl/H_2O at 90 °C for 3 h. Each sugar fraction was trimethylsilylated in accordance with the above method and analyzed by GC. The reaction times of peaks coincided with those of D-glucose (t_R 9.59 min), D-xylose (t_R 5.33 min) and D-apiose (t_R 8.14 min).

Myrioside B (**2**): An amorphous powder, $[\alpha]_D^{15}$ +2.3° (c=0.11, MeOH). ^{13}C-NMR spectrum (in pyridine-d_5) δ: 38.6, 26.4, 88.9, 42.8, 56.0, 18.7, 33.1, 40.0, 47.6, 36.5, 24.0, 122.9, 144.6, 41.9, 26.6, 27.1, 32.7, 46.5, 41.4, 42.8, 29.4, 35.5, 23.3, 63.1, 15.4, 16.9, 26.1, 28.4, 181.6, 19.9 (aglycone moiety C-1—30, respectively).

Myrioside C (**3**): An amorphous powder, $[\alpha]_D^{14}$ −2.2° (c=0.12, MeOH). ^{13}C-NMR spectrum (in pyridine-d_5) δ: 38.7, 26.3, 89.0, 44.4, 56.1, 18.1, 33.1, 40.0, 47.6, 36.5, 24.0, 123.1, 144.6, 41.8, 26.8, 27.1, 32.6, 46.3, 40.6, 43.1, 29.5, 36.1, 23.3, 63.2, 15.4, 16.9, 26.0, 28.2, 177.7, 19.5 (aglycone moiety C-1—30, respectively).

Myrioside D (**4**): An amorphous powder, $[\alpha]_D^{15}$ −4.0° (c=0.10, MeOH). ^{13}C-NMR spectrum (in pyridine-d_5) δ: 38.8, 26.2, 90.4, 44.0, 56.3, 18.6, 33.0, 39.9, 47.5, 36.4, 24.0, 123.1, 144.2, 41.7, 26.4, 27.0, 32.6, 46.2, 40.5, 43.1, 29.4, 36.0, 22.7, 62.8, 15.5, 16.8, 25.9, 28.2, 178.0, 19.5 (aglycone moiety C-1—30, respectively).

Acknowledgements We wish to thank Mr. K. Takeda and Mr. T. Iriguchi of the Central Analytical Laboratory of this University for the measurements of NMR and MS spectra.

References and Notes

1) Kinjo J., Nohara T., "Towards Natural Medicine Research in the 21st Century," ed. by Ageta H., Aimi N., Ebizuka Y., Fujita T., Honda G., Elsevier, Tokyo, 1998, pp. 237—249.
2) Kinjo J., Nohara T., "Studies in Natural Products Chemistry," ed. by Atta-ur-Rahman, Vol. 25, Elsevier Science B. V., 2001, pp. 89—124.
3) Higuchi R., Kawasaki T., *Chem. Pharm. Bull.*, **24**, 1021—1032 (1976).
4) Compound **5**, an amorphous powder, $[\alpha]_D^{20}$ +16.2° (c=0.10, MeOH). ^{1}H-NMR spectrum (in pyridine-d_5) δ: 0.87 (3H, s, H_3-30), 0.89 (3H, s, H_3-29), 0.99 (3H, s, H_3-25), 1.09 (3H, s, H_3-24), 1.13 (3H, s, H_3-26), 1.17 (3H, s, H_3-27), 3.75 (1H, d, J=10.3 Hz, H*a*-23), 4.13 (1H, overlapped, H*b*-23), 4.26 (1H, dd, J=3.7, 11.5 Hz, H-3), 5.41 (1H, br s, H-12). ^{13}C-NMR spectrum (in pyridine-d_5) δ: 38.5, 25.7, 80.5, 43.0, 47.2, 17.7, 33.5, 39.5, 47.7, 36.4, 23.3, 122.4, 143.6, 41.7, 27.8, 22.9, 46.5, 41.2, 45.7, 30.2, 32.3, 32.1, 13.5, 63.5, 15.7, 17.1, 25.3, 176.0, 23.5, 32.6 (aglycone moiety C-1—30, respectively), 101.2, 72.3, 73.4, 74.2, 69.8, 18.1, 103.8, 76.0, 73.6, 68.8, 65.1 [(3-*O*-α-L-rhamnopyranosyl-(C-1—6)-α-L-arabinopyranosyl-(C-1—5), respectively], 102.2, 72.1, 72.3, 73.6, 70.4, 18.1, 104.4, 75.3, 76.7, 78.2, 76.7, 60.8, 95.1, 73.6, 78.2, 70.4, 77.8, 68.7 [28-*O*-α-L-rhamnopyranosyl-(C-1—6)-β-D-glucopyranosyl-(C-1—6)-β-D-glucopyranosyl-(C-1—60, respectively].
5) Bedir E., Calis I., Khan I. A., *Chem. Pharm. Bull.*, **48**, 1081—1083 (2000).
6) Hara S., Okabe H., Mihashi K., *Chem. Pharm. Bull.*, **35**, 501—507 (1987).
7) Kitagawa I., Sakagami M., Hashiuchi F., Zhou J. L., Yoshikawa M., Ren J., *Chem. Pharm. Bull.*, **37**, 551—553 (1989).
8) Kitagawa I., Wang H. K., Saito M., Yoshikawa M., *Chem. Pharm. Bull.*, **31**, 674—682 (1983).
9) Sun R., Jia Z., Chen D., *Phytochemistry*, **30**, 2707—2709 (1991).