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Saponins from Chinese Medicinal Plant, *Hemsleya graciliflora* (Cucurbitaceae)

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From rhizomes of a Chinese cucurbitaceous medicinal plant, *Hemsleya graciliflora*, eight oleanolic glucuronide saponins were isolated together with three known cucurbitane-type triterpenes, dihydrocucurbitacin F, 25-*O*-acetyldihydrocucurbitacin F and its 2-*O*-β-glucosides. Of these saponins, six were known and identified as β-D-glucosyl ester of oleanolic acid, chikusetsusaponins IVa and V and hemslosides Ma1, Ma3 and H1. Structures of two new saponins called hemslosides G1 and G2 were elucidated as β-gentiobiosyl esters of oleanolic acid 3-*O*-α-L-arabinopyranosyl-(1→3)-β-D-glucuronide and oleanolic acid 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucuronide, respectively.

Keywords Chinese medicinal plant; *Hemsleya graciliflora*; Cucurbitaceae; saponin; oleanolic acid glucuronide; hemsloside; chikusetsusaponin; cucurbitane-type triterpene

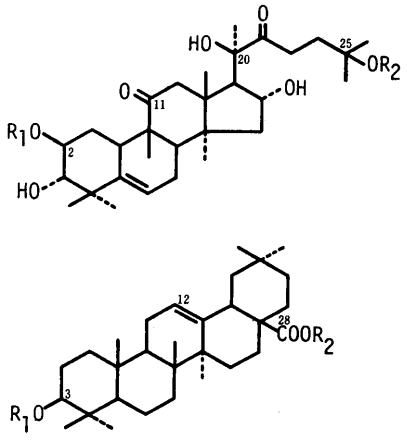
As part of our serial studies on Chinese cucurbitaceous medicinal plants,[1-8] the present paper reports isolation and structure determination of saponins from rhizomes of *Hemsleya graciliflora* (HARMS) COGN. (馬銅鈴雪胆) which grows in the southwest province of China, the northern province of India and in Vietnam.

The rhizomes collected in Sichuan, China were extracted with methanol and a suspension of the extract in water was further extracted with ethyl acetate to give an ethyl acetate-soluble fraction and a water-soluble fraction. Column chromatography of the ethyl acetate-soluble fraction afforded dihydrocurbitacin F (**1**),[1,2,5,6] 25-*O*-acetyldihydrocucurbitacin F (**2**),[1,2,5,6] 2-*O*-β-glucoside (**3**)[6] of **2** and a β-glucosyl ester of oleanolic acid (**4**)[1] which have already been isolated from other *Hemsleya* species.

The water-soluble fraction (*vide supra*) was subjected to repeated chromatography to give seven compounds **5**—**11**. Compounds **5** and **6** were respectively identified as chikusetsusaponins IVa[1,9] and V[10] which have been isolated from *Panax japonicus* C. A. MEYER (Araliaceae) and several other *Panax* species.[11] Compounds **7**, **8** and **9** were identified as hemslosides Ma1, Ma3 and H1 which have been isolated from other *Hemsleya* species.[1,2]

Compound **10**, called hemsloside G1, afforded D-glucuronic acid, D-glucose and L-arabinose on acid hydrolysis. The ^{13}C nuclear magnetic resonance (^{13}C-NMR) spectrum exhibited four anomeric carbon signals and inspection of these signals due to the aglycone moiety indicated that **10** is a 3,28-*O*-glycosylated oleanolic acid. On treatment with a anhydrous LiI–2,6-lutidine–methanol reagent,[12] **10** yielded an anomeric mixture of methyl gentiobioside and a prosapognein (**12**). By comparison of the ^{13}C-NMR spectrum and optical rotation, **12** was proved to be identical with the prosapogenin already obtained from **7** by alkaline hydrolysis.[1] Inspection of chemical shifts of the four anomeric carbon signals as well as coupling constants of four anomeric proton signals revealed the anomeric configuration of all of the sugar units. It follows that **10** can be formulated as a β-gentiobiosyl ester of oleanolic acid 3-*O*-α-L-arabinopyranosyl-(1→3)-β-D-glucuronide.

Compound **11** called hemsloside G2 yielded D-glucuronic acid and D-glucose on acid hydrolysis. In the ^{13}C-NMR spectrum, carbon signals due to the aglycone moiety indicated that **11** is a 3,28-*O*-glycosylated oleanolic acid like **5**—**10**. On treatment with the anhydrous LiI–2,6-lutidine–methanol reagent, **11** afforded an anomeric mixture of methyl gentiobioside and a prosapogenin (**13**) which was identified with the prosapogenin obtained from **6** by alka-



	R_1	R_2
1	–H	–H
2	–H	–Ac
3	–Glc	–Ac

	R_1	R_2
4	–H	–Glc
5	–GlcA	–Glc
6	–GlcA^{2}–Glc	–Glc
7	–GlcA^{3}–Ara(p)	–Glc
8	–GlcA^{2}–Glc ^{3}Ara(p)	–Glc
9	–GlcA^{2}–Glc ^{3}Ara(p)	–Glc^{6}–Glc
10	–GlcA^{3}–Ara(p)	–Glc^{6}–Glc
11	–GlcA^{2}–Glc	–Glc^{6}–Glc
12	–GlcA^{3}–Ara(p)	–H
13	–GlcA^{2}–Glc	–H

Chart 1

line hydrolysis.[10] Inspection of chemical shifts of four anomeric carbon signals as well as coupling constants of four anomeric proton signals revealed the anomeric configurations of all of the sugar units. These results led to the formulation of **11** as a β-gentiobiosyl ester of oleanolic acid 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucuronide.

Yoshikawa *et al.* reported the isolation of dammarane-saponins from *Gynostemma pentaphyllum* MAKINO (Cucurbitaceae)[13] which are structurally related to ginsenosides from *Panax ginseng* and the congeners (Araliaceae).[11] Further, it has been reported that some of Chinese cucurbitaceous plants, *Hemsleya macrosperma* C. Y. WU (羅鍋底),[1] *H. chinensis* COGN. (中華雪胆)[1,2] and *Thladiantha hookeri* C. B. CLARKE var. *pentadactyla* COGN. (五叶赤爬)[3] contain glucuronide saponins of oleanolic acid (or its 23-oxo-derivative[3]), which are closely related to the oleanane-saponins of *Panax* species such as **5** and **6**. The present result is an additional example of the similarity of cucurbitaceous plants to *Panax* species in saponin composition. On the other hand, from other Chinese cucurbitaceous plants, *Hemsleya carnosiflora* C. Y. WU *et* Z. L. CHEN. (肉花雪胆),[5] *H. panacis-scandens* C. Y. WU *et* Z. L. CHEN (藤三七雪胆),[6] *Siraitia grosvenori* SWINGLE (羅漢果),[14] and *S. siamensis* CRAIB (翅子羅漢果),[7] no oleanane- and dammarane-saponin but sweet or bitter cucurbitane glucosides such as mogroside V and carnosifloside III were isolated.

Experimental

General Procedures All melting points were uncorrected. NMR spectra were recorded on a JEOL GX 400 spectrometer in C_5D_5N (at 400 MHz for proton and at 100 MHz for carbon-13). Acid hydrolysis and subsequent identification of resulting monosaccharides including absolute configuration were carried out as described in a previous paper.[15] Each known compound was made an identification by comparison of the ^{13}C-NMR spectrum and optical rotation (and melting point in case of a crystalline form) with those of a respective authentic sample.

Extraction and Separation Dried rhizomes (2 kg) collected in Sichuan, China were extracted with hot MeOH. A suspension of the MeOH-extract in H_2O was extracted with EtOAc to give an EtOAc fraction and H_2O fraction. The EtOAc fraction was chromatographed on silica gel with C_6H_6–$(CH_3)_2CO$ (2:1, 1:1 and then 1:2) to give **1**, **2** and more polar fractions, fr. 3 and fr. 4. Fraction 3 was subjected to chromatography on silica gel with $CHCl_3$–MeOH–H_2O (60:10:1) followed by high performance liquid chromatography (HPLC) and afforded **3**; condition of HPLC: column: ODS-120T (21.5 mm × 30 cm, Tosoh Co., Ltd.), mobile phase: 60% MeOH, flow rate: 6 ml/min, detection: differential refractometer. Fraction 4 was chromatographed on LiChroprep RP-8 (Merck) with 80% MeOH to give **4**.

1 (yield: 0.4%), colorless prisms from MeOH–H_2O, mp 158—160 °C, $[\alpha]_D^{24}$ +63.2° (c=0.60, EtOH). **2** (yield: 0.9%), colorless prisms from MeOH, mp 236—239 °C, $[\alpha]_D^{24}$ +60.8° (c=1.05, EtOH). **3** (yield: 0.03%), a white powder, $[\alpha]_D^{24}$ +36.8° (c=0.73, EtOH). **4** (yield: 0.02%), colorless needles from MeOH–H_2O, mp 246 °C, $[\alpha]_D^{24}$ +49.6° (c=0.27, MeOH).

The H_2O fraction was chromatographed on silica gel with $CHCl_3$–MeOH–H_2O (10:5:1 and then 6:4:1) to be separated into five saponin-fractions-1—5 in increasing order of polarity. Saponin-fraction-1 was separated by chromatography on silica gel with $CHCl_3$–MeOH–H_2O (10:5:1 and then 20:10:1) to give **5**. Saponin-fraction-2 was chromatographed on silica gel with $CHCl_3$–MeOH–H_2O (10:5:1) to give two fractions. The fomrer was further chromatographed on LiChroprep RP-8 with 62% MeOH to afford additional **5** and the latter was chromatographed on LiChroprep RP-8 with 65% MeOH to give **7**. Saponin-fraction-3 was chromatographed repeatedly on silica gel with $CHCl_3$–MeOH–H_2O (6:4:1) to give **6** and **7**. Saponin-fraction 4 was subjected to chromatography on silica gel with $CHCl_3$–MeOH–H_2O (6:4:1) followed by HPLC to give **8**, **10** and **11**; condition of HPLC: column: ODS-120T (21.5 mm × 30 cm, Tosoh Co., Ltd.), mobile phase: 70% MeOH contained 0.05% F_3CCOOH, flow rate: 6 ml/min, detection: differential refractometer. Saponin-fraction-5 was rechromatographed on silica gel with $CHCl_3$–MeOH–H_2O (5:5:1) to give **9**.

5 (yield: 1.2%), colorless prisms from MeOH–H_2O, mp 217—219 °C, $[\alpha]_D^{24}$ +7.9° (c=0.67, MeOH). **6** (yield: 0.6%), a white powder, $[\alpha]_D^{20}$ +8.4° (c=0.94, MeOH). **7** (yield: 1.1%), a white powder, $[\alpha]_D^{24}$ +16.3° (c=0.93, MeOH). **8** (yield: 0.17%), a white powder, $[\alpha]_D^{20}$ +14.6° (c=0.82, MeOH). **9** (yield: 1.3%), a white powder, $[\alpha]_D^{20}$ +5.9° (c=0.93, MeOH).

10 (yield: 0.6%), a white powder, $[\alpha]_D^{18}$ +7.6° (c=0.9, MeOH). *Anal.* Calcd for $C_{53}H_{84}O_{23}\cdot 2H_2O$: C, 56.57; H, 7.88. Found: C, 56.79; H, 8.20. ^{1}H-NMR δ (anomeric proton): 5.00 (1H, d, J=7.6 Hz), 5.03 (1H, d, J=8.3 Hz), 5.37 (1H, d, J=7.0 Hz), 6.25 (1H, d, J=7.9 Hz). ^{13}C-NMR δ aglycone moiety: 38.9 (C1), 26.5 (C2), 89.5 (C3), 39.6 (C4), 56.0 (C5), 18.6 (C6), 33.3 (C7)*, 40.1 (C8), 48.2 (C9), 37.1 (C10), 23.6 (C11)*, 123.0 (C12), 144.2 (C13), 42.3 (C14), 28.4 (C15), 23.9 (C16)*, 47.2 (C17), 41.8 (C18), 46.5 (C19), 30.8 (C20), 34.2 (C21), 32.7 (C22), 28.3 (C23), 17.0 (C24), 15.6 (C25), 17.6 (C26), 26.1 (C27), 176.5 (C28), 33.1 (C29)*, 23.8 (C30)*. 3-*O*-arabinosyl-glucuronide moiety: GlcA: 106.6 (C1), 74.4 (C2)*, 86.1 (C3), 72.7 (C4), 77.9 (C5)*, 171.9 (C6). Ara: 105.6 (C1), 71.3 (C2), 74.6 (C3)*, 69.2 (C4), 67.1 (C5). 28-*O*-gentiobiosyl moiety: inner Glc: 95.7 (C1), 74.0 (C2), 78.7 (C3)*, 71.4 (C4), 77.3 (C5), 69.7 (C6). terminal Glc: 105.1 (C1), 75.2 (C2), 78.4 (C3)*, 71.9 (C4), 78.7 (C5)*, 62.9 (C6). (* assignment may be interchanged).

11 (yield: 0.4%), a white powder, $[\alpha]_D^{18}$ −6.1° (c=0.9, MeOH). *Anal.* Calcd for $C_{54}H_{86}O_{24}\cdot 2H_2O$: C, 56.14; H, 7.85. Found: C, 56.03; H, 7.95. ^{1}H-NMR δ (anomeric proton): 5.02 (1H, d, J=8.8 Hz), 5.04 (1H, d, J=7.8 Hz), 5.42 (1H, d, J=7.8 Hz), 6.26 (1H, d, J=8.3 Hz). ^{13}C-NMR δ aglycone moiety: 38.7 (C1), 26.6 (C2), 89.2 (C3), 39.5 (C4), 55.8 (C5), 18.5 (C6), 33.1 (C7), 39.9 (C8), 48.0 (C9), 36.9 (C10), 23.4 (C11)*, 122.9 (C12), 144.2 (C13), 42.1 (C14), 28.3 (C15), 23.7 (C16)*, 47.0 (C17), 41.7 (C18), 46.2 (C19), 30.8 (C20), 34.0 (C21), 32.5 (C22), 28.1 (C23), 16.7 (C24), 15.6 (C25), 17.5 (C26), 26.1 (C27), 176.5 (C28), 33.1 (C29), 23.7 (C30)*. 3-*O*-glucosyl-gluculonide moiety: GlcA: 105.4 (C1), 82.8 (C2), 77.7 (C3), 73.2 (C4), 77.9 (C5)*, 172.5 (C6). Glc: 106.0 (C1), 77.1 (C2), 78.3 (C3)*, 71.5 (C3), 77.9 (C5)*, 62.7 (C6). 28-*O*-gentiobiosyl moiety: inner Glc: 95.7 (C1), 73.9 (C2), 78.4 (C3)*, 70.9 (C4), 77.9 (C5), 69.4 (C6). terminal Glc: 105.3 (C1), 75.2 (C2), 78.4 (C3)*, 71.7 (C4), 78.7 (C5)*, 62.6 (C6). (* assignment may be interchanged).

Selective Cleavage of Ester-Type Glycosyl Linkage A mixture of **10** (61 mg), LiI (70 mg, dried at 150 °C under reduced pressure for 2 h) and anhydrous 2,6-lutidine (1.5 ml) in anhydrous MeOH (1.5 ml) was refluxed under Ar-gas for 42 h. To the mixture was added 50% MeOH (1 ml) and the solution was deionized with Amberlite MB-3 and then concentrated to dryness. The residue was chromatographed on silica gel with $CHCl_3$–MeOH–H_2O (10:5:1 then 6:4:1) to give an anomeric mixture of methyl gentiobioside (6 mg) and **12** [23 mg, a white powder, $[\alpha]_D^{18}$ +34.7° (c=0.90, MeOH)]. By the same procedure, **11** (60 mg) yielded an anomeric mixture of methyl gentiobioside (8 mg) and **13** [16 mg, a white powder, $[\alpha]_D^{18}$ +15.5° (c=0.84, MeOH)].

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