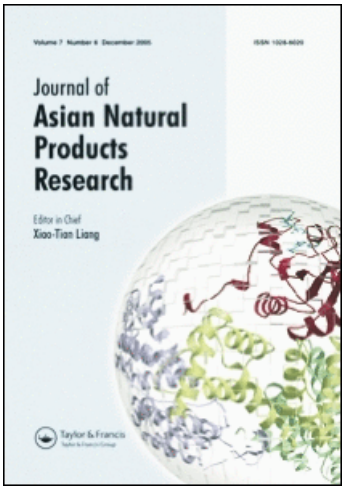

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Four new glycosides from Pleurospermum franchetianum

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FOUR NEW GLYCOSIDES FROM *PLEUROSPERMUM FRANCHETIANUM*

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Four new glycosides, pleurofranosides I–IV, together with eight known compounds were isolated from the whole plants of *Pleurospermum franchetianum* Hemsl. Based on the spectral data and chemical evidence, the structures of pleurofranosides I, II, III and IV were elucidated to be 16β, 21β, 23, 28-tetrahydroxyolean-12-ene-3β-yl-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranoside, 13β, 28-epoxy-16β, 23-dihydroxyolean-11-ene-3β-yl-*O*-β-D-glucopyranosyl-(1 → 4)-[β-D-glucopyranosyl-(1 → 2)]-β-D-fucopyranoside, 13β, 28-epoxy-16β, 23-dihydroxyolean-11-ene-3β-yl-*O*-β-D-glucopyranosyl-(1 → 4)-[β-D-fucopyranosyl-(1 → 2)]-β-D-glucopyranoside and 13β, 28-epoxy-16β, 23-dihydroxyolean-11-ene-3β-yl-*O*-β-D-glucopyranosyl-(1 → 3)-[β-D-glucopyranosyl-(1 → 2)]-β-D-glucopyranoside. The known compounds identified were octadecyl caprate, β-sitosterol, (22*E*, 20*S*, 24*R*)-5α, 8α-epidioxy-ergosta-6, 22-dien-3-β-ol, daucosterol, α-spinasterol-3-*O*-β-D-glucopyranoside, quercetin-3, 7-di-*O*-β-D-glucopyranoside, kaempferol-3, 7-di-*O*-α-L-rhamnopyranoside and kaempferol-3-*O*-β-D-glucopyranosyl-7-*O*-α-L-rhamnopyranoside, respectively.

Keywords: *Pleurospermum franchetianum* Hemsl; Umbelliferae; Pleurofranosides I–IV; Glycosides

INTRODUCTION

The plants of the genus *Pleurospermum* (Umbelliferae) are mainly distributed in North Asia and East Europe [1]. Some of the 32 species registered in China are used as folk medicine. Pharmacological study revealed that the water extract of the whole plants of *P. rivulorum* Diels is antiarrythmic [2]. *P. govanianum* (Wall) Benth ex C.B. Clark var. Bicolor Wolff. is used as antiasthmatic, expectorant and smooth muscle relaxant [3]. *P. lindleyanum* (Lipsky) B. Fedtsch is adapted to hypertension, coronary heart disease [4]. Various compounds including fatty acids and their esters [2,5,6], coumarins [4–6], flavonoids [4], monoterpenes [7] and steroids [2,5] were isolated from the plants of this genus. *P. franchetianum* Hemsl. distributed in Southwest and Northwest China [8] has not been chemical investigated. In the study on the whole plant of *P. franchetianum*, four new glycosides, pleurofranosides I–IV, were isolated. Their structures were elucidated to be 16β, 21β, 23, 28-tetrahydroxyolean-12-ene-3β-yl-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranoside (**8**), 13β, 28-epoxy-16β,

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23-dihydroxyolean-11-ene-3β-yl-*O*-β-D-glucopyranosyl-(1 → 4)-[β-D-glucopyranosyl-(1 → 2)]-β-D-fucopyranoside (**10**), 13β, 28-epoxy-16β, 23-dihydroxyolean-11-ene-3β-yl-*O*-β-D-glucopyranosyl-(1 → 4)-[β-D-fucopyranosyl-(1 → 2)]-β-D-glucopyranoside (**11**) and 13β, 28-epoxy-16β, 23-dihydroxyolean-11-ene-3β-yl-*O*-β-D-glucopyranosyl-(1 → 3)-[β-D-glucopyranosyl-(1 → 2)]-β-D-glucopyranoside (**12**). Eight known compounds were determined to be octadecyl caprate (**1**), β-sitosterol (**2**), (22*E*, 20*S*, 24*R*)-5α, 8α-epidioxyergosta-6, 22-dien-3-β-ol (**3**), daucosterol (**4**), α-spinasterol-3-*O*-β-D-glucopyranoside (**5**), quercetin-3, 7-di-*O*-β-D-glucopyranoside (**6**), kaempferol-3, 7-di-*O*-α-L-rhamnopyranoside (**7**) and kaempferol-3-*O*-β-D-glucopyranosyl-7-*O*-α-L-rhamnopyranoside (**9**).

RESULTS AND DISCUSSION

The quasi-molecular ion peak $[M - H]^-$ at *m/z* 813.4609 in the HRFABMS (negative) of **8** suggested the molecular formula $C_{42}H_{70}O_{15}$. Only D-glucose was identified by PC and TLC after hydrolyzing **8** at 60°C with 4 M HCl (aq.) for 6 h. The acid solution was neutralized with NaOH (aq.) and then extracted with $CHCl_3$ to yield **8a**. In the FABMS (positive) of **8**, the ion peaks at *m/z* 838 ($[M + Na + H]^+$), 815 ($[M + H]^+$), 653 ($[M + H - glc]^+$), 473 ($[M - (glc-glc)]^+$), 455 ($[M - (glc-glc) - H_2O]^+$) and 437 ($[M - (glc-glc) - 2 \times H_2O]^+$) suggested the presence of two glucose moieties. The ^1H NMR signals δ 5.32 (1 H, d, $J = 7.6$ Hz) and 5.04 (1 H, d, $J = 7.2$ Hz) indicated two β-D-glucopyranosyl moieties. Six methyl groups resonated at δ 1.31, 1.30, 1.19, 1.04, 1.00 and 0.97 (each 3 H, s) were recognized in **8**. The cross signal at δ 5.26 (1 H, t, $J = 3.2$ Hz, H-12) /123.7 d (C-12) in HMQC and the ^{13}C NMR signal δ 142.7 suggested compound **8** was a derivative of olean-type triterpene. The chemical shifts C-3, C-16, C-21 and other C-atoms could be assigned on the basis of HMQC, HMBC and $^1H-^1H$ COSY starting from the cross signals δ 5.26 (H-12)/47.1 d (C-9) and 43.6 d (C-14, 18) in HMBC. The key correlations were described in Fig. 1. It is reported that C-3, 16 and 21 resonated at δ 73.4, 67.7 and 72.7 in gymnestrogenin and the configurations of 3-, 21- and 16-OH were determined based on NOE experiments [10]. The ^1H and ^{13}C NMR data of **8a** were in accordance with those of gymnestrogenin [9,10]. The β-oriented 3-, 21- and 16-OH in compound **8** should also be confirmed by the difference of chemical shift of C-3 [about (β-) − (α-) = 2–5 ppm], C-21 [about (α-) − (β-) = 3–6 ppm] and C-16 [about (α-) − (β-) = 6–7 ppm] between α- and β-configurated triterpenoids [11]. A downfield glycosylation shift +8.7 and +9.5 ppm of C-3 was observed in two glycosides of **8a**, gymnemic acid VII [9] and sitakisosides IX [10], respectively. The ^{13}C NMR signal of **8** at δ 82.8 could be assigned to C-3 because of the HMBC correlation δ 5.04 (H-1′)/82.8 (C-3). Thus, in **8** C-3 should be glycosylated by comparing the ^{13}C NMR data of **8** and **8a** [9,10]. The cross signal δ 5.04 (H-1′)/4.20 in $^1H-^1H$ COSY, δ 84.2 (C-2′)/4.20 (H-2′) in HMQC and δ 5.32 (H-1″)/84.2 (C-2′) in HMBC provided 1 → 2 linkage of the two sugar moieties. Thus the structure of **8** could be elucidated as 16β, 21β, 23, 28-tetrahydroxyolean-12-ene-3β-yl-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranoside, named as pleurofranoside I (Fig. 1).

Compound **10** was isolated as yellowish powder. The ion peak at *m/z* 941.5151 ($[M - H]^-$) in its HRFABMS (negative) suggested the molecular formula $C_{48}H_{78}O_{18}$. D-glucose and D-fucose were identified by PC and TLC after hydrolyzing **10** at 90°C in 5 M HCl (aq.) for 6 h. The presence of two glucose and one fucose moieties was recognized from the ion peaks at *m/z* 981 ($[M + K]^+$), 965 ($[M + Na]^+$), 763 ($[M + H - H_2O-glc]^+$), 601 ($[M + H - 2 \times glc-H_2O]^+$), 455 ($[M - 2 \times glc-fuc]^+$) and 437 ($[M - 2 \times glc-fuc - H_2O]^+$) in FABMS (positive). The ^1H NMR signals at δ 5.57, 5.28 and 4.92 (each 1 H, d, $J = 7.6$ Hz) ascribed two β-D-glucopyranosyl and one β-D-fucopyranosyl moieties.

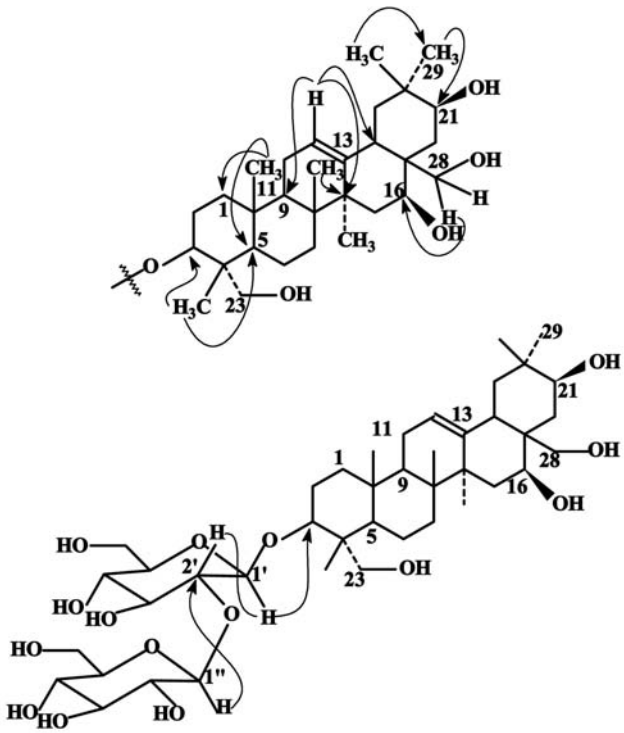
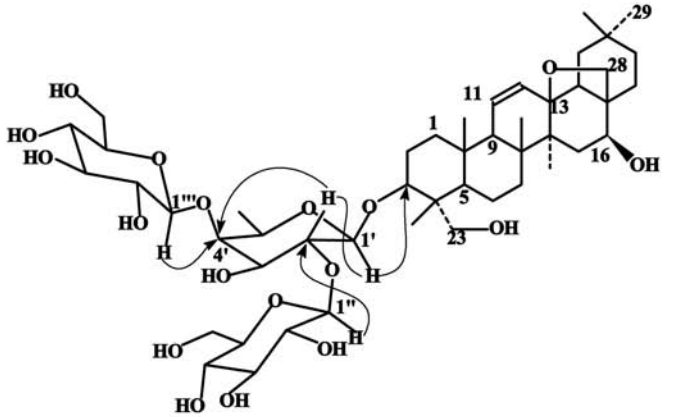


FIGURE 1 The key correlations and structure of compound **8** (→ HMBC correlations,—^{1}H–^{1}H COSY correlations).

The ^{1}H NMR signal at δ 1.38 (3 H, d, $J = 6.4$ Hz, Fuc H-6) could be assigned to the methyl group in fucose moiety. The six methyl groups resonate at δ 1.37, 1.25, 1.19, 1.10, 1.06 and 0.95 (each 3 H, s). The ^{1}H NMR signals at δ 5.63 (1 H, dd, $J = 10.0$, 2.0 Hz, H-11) and 5.96 (1 H, d, $J = 10.0$ Hz, H-12) suggested a disubstituted double bond. The aglycone was determined by comparing the NMR data of **10** with those of 13β, 28-epoxy-16β, 23-dihydroxyolean-11-ene-3β-ol [12,13]. The ^{13}C NMR signal at δ 82.6 could be assigned to glycosylated C-3 considering the HMBC correlation δ 4.92 (H-1′)/82.6 d (C-3). 3- and 16-OH should be β-oriented by comparing the ^{1}H and ^{13}C NMR data of compound **10** with those of known triterpene saponins with the same aglycone [14–18] and by considering the difference of chemical shift of C-3 [about (β-) − (α-) = 2–5 ppm] and C-16 [about (α-) − (β-) = 6–7 ppm] between α- and β-configurated compounds [11]. The ^{13}C NMR signal at δ 83.9 could be assigned to C-4′, considering the cross signal δ 4.92 (H-1′)/4.66 (H-2′) in ^{1}H–^{1}H COSY and δ 4.66/83.9 d (C-4′) in HMBC spectrum. The signal δ 4.66/77.0 d (C-2′) in HMQC along with the cross signal δ 4.66 (H-2′)/103.9 d (C-1″) and 5.57 (H-1″)/ 77.0 d (C-2′) in HMBC revealed the linkage between the first glucose and the fucose was 1 → 2. The signal δ 5.28 (H-1‴)/ 83.9 d (C-4′) in HMBC provided the linkage between the second glucose and the fucose 1 → 4. Thus compound **10** (Fig. 2) was elucidated as 13β, 28-epoxy-16β, 23-dihydroxyolean-11-ene-3β-yl-*O*-β-D-glucopyranosyl-(1 → 4)-[β-D-glucopyranosyl-(1 → 2)]-β-D-fucopyranoside, named pleurofranoside II.

The molecular formula of **11**, $C_{48}H_{78}O_{18}$, was provided by ion peak at *m/z* 941.5051 ($[M - H]^{-}$) in the HRFABMS (negative). D-glucose and D-fucose were identified by PC and TLC after hydrolyzing **11** at 95°C in 5 M HCl (aq.) for 6 h. The FABMS (positive) of **11** gave fragments ion peaks at *m/z* 982 ($[M + K + H]^{+}$), 966 ($[M + Na + H]^{+}$), 762 ($[M - glc–H_2O]^{+}$), 615 ($[M - glc–H_2O - fuc]^{+}$), 471 ($[M + H - (2 \times glc–fuc)]^{+}$), 453



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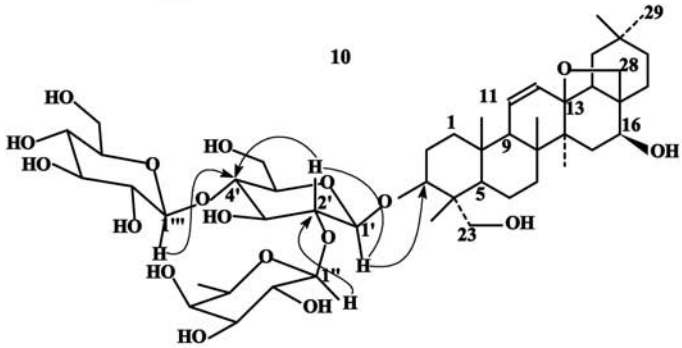

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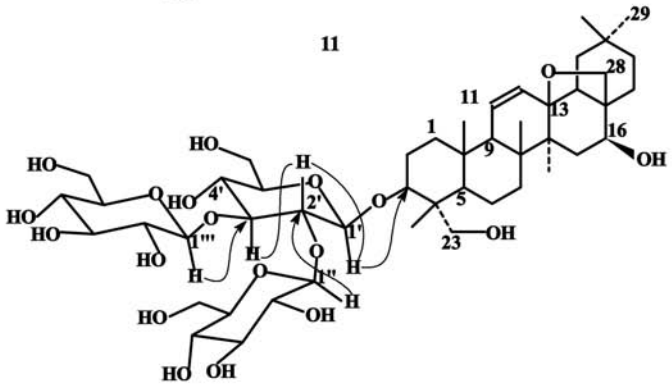

12

FIGURE 2 The Structures of **10**, **11** and **12** (→ HMBC correlations,—^{1}H–^{1}H COSY correlations).

([M + H − (2 × glc–fuc) − H_2O]$^{+}$) and 435 ([M + H − (2 × glc–fuc) − 2 × H_2O]$^{+}$). The ^{1}H NMR signals at δ 5.58, 5.30 and 4.92 (each 1 H, d, $J = 8.0$ Hz) ascribed two β-D-glucopyranosyl and one β-D-fucopyranosyl moieties. The ^{1}H NMR signal at δ 1.38 (3 H, d, $J = 6.4$ Hz, Fuc H-6) could be assigned to the methyl group in fucose moiety. In the ^{1}H NMR spectrum the six methyl groups in aglycone [at δ 1.37, 1.25, 1.19, 1.10, 1.06 and 0.95 (each 3 H, s)] and two olefinic protons [at δ 5.69 (1 H, dd, $J = 10.4$, 2.8 Hz, H-11) and 5.98 (1 H, d, $J = 10.4$ Hz, H-12)] were observed. The aglycone of **11**, 13β, 28-epoxy-16β, 23-dihydroxyolean-11-ene-3β-ol [12,13], is identical to that of **10**, in view of the ^{1}H and ^{13}C NMR data of both **10** and **11**. According to the HMBC signal δ 4.92 (H-1′)/δ 82.5 d (C-3),

C-3 was glycosylated. The ^{13}C NMR signal at δ 83.7 could be assigned to C-4′ on the basis of the $^{1}H-^{1}H$ COSY cross signal δ 4.92 (H-1′)/4.65 (H-2′) and HMBC correlation signal δ 4.65 (H-2′)/ 83.7 d. The cross signal δ 4.65 (H-2′)/77.0 d (C-2′) in HMQC along with the HMBC correlations δ 5.58 (H-1″)/77.0 d (C-2′) and 4.65 (H-2′)/103.9 d (C-1″) provided the 1 → 2 connection between fucose and the first glucose. The 1 → 4 linkage between the second glucose and the first glucose could be confirmed from the HMBC correlation δ 5.30 (H-1‴)/ 83.7 d (C-4′). Thus, the structure of **11** (Fig. 2) was elucidated as 13β, 28-epoxy-16β, 23-dihydroxyolean-11-ene-3β-yl-*O*-β-D-glucopyranosyl-(1 → 4)-[β-D-fucopyranosyl-(1 → 2)]-β-D-glucopyranoside, named as pleurofranoside III.

The molecular formula of compound **12**, $C_{48}H_{78}O_{19}$, concluded from the ion peak at *m/z* 957.5055 $[M - H]^-$ in HRFABMS (negative). D-glucose was identified by PC and TLC after hydrolyzing **12** at 98°C in 5 M HCl (aq.) for 5 h. The fragments at *m/z* 998 ($[M + K + H]^+$), 958 (M^+), 796 ($[M + H - glc]^+$), 617 ($[M + H - 2 \times glc - H_2O]^+$), 473 ($[M + H - 3 \times glc]^+$), 455 ($[M + H - 3 \times glc - H_2O]^+$), 437 ($[M - 3 \times glc - 2 \times H_2O]^+$) and 419 ($[M - 3 \times glc - 3 \times H_2O]^+$) were observed in its FABMS (negative) spectrum. From the ^{1}H NMR signals at δ 5.56, 5.28 and 4.90 (each 1 H, d, $J = 7.6$ Hz), 3 β-D-glucopyranosyl moieties could be postulated. The ^{13}C NMR data of compound **12** were in according with those of **10** and **11** except the glucose moiety, suggesting that both **12** and **11** possessed the same aglycone. The HMBC correlation δ 4.90 (H-1′)/82.5 d (C-3) showed that C-3 was glycosylated. The C-2′ resonated at δ 76.9 because of the cross signals δ 4.90 (H-1′)/4.67 (H-2′) in $^{1}H-^{1}H$ COSY and δ 4.67 (H-2′)/ 76.9 d (C-2′) in HMQC. Furthermore, $^{1}H-^{1}H$ COSY correlation δ 4.67 (H-2′)/4.09 (H-3′) and δ 4.09 (H-3′)/84.6 d (C-3′) in HMQC showed that C-3′ resonates at 84.6. The HMBC correlations δ 4.67 (H-2′)/103.9 d (C-1″) and 5.56 (H-1″)/76.9 d (C-2′) confirmed the 1 → 2 linkage between the second glucose and the first glucose. The third glucose located at C-3′ on the basis of the signals at δ 4.09 (H-3′)/105.0 d and 5.28 (H-1‴)/84.6 d (C-3′) in HMBC. Therefore, compound **12** was elucidated as 13β, 28-epoxy-16β, 23-dihydroxyolean-11-ene-3β-yl-*O*-β-D-glucopyranosyl-(1 → 3)-[β-D-glucopyranosyl-(1 → 2)]-β-D-glucopyranoside, named as pleurofranoside IV (Fig. 2).

EXPERIMENTAL SECTION

General Experiment Procedures

Mps were recorded on XRC-1 (uncorr.). UV spectra were recorded on a GBC Cintra 20 spectrometer in MeOH and IR on a Nicolet Protege 460 spectrometer with KBr discs (ν_{max} in cm^{-1}). NMR spectra were measured on Brucker AM-400 (400 MHz for ^{1}H and 100 MHz for ^{13}C, TMS as internal standard) in C_5D_5N. Column chromatography was performed on silica gel of 200–300 mesh. FABMS were carried on a VG AutoSpec-3000 (positive and negative mode, glycerol as matrix). Optical rotations were taken on a Perkin Elmer 341 Polarimeter. TLC was carried on silica gel (10–40 μ) precoated plates. Spots were detected by spraying 8% phosphomolybdic acid–ethanol solution followed by heating. Sugars were identified by PC (the upper layer of *n* − BuOH : HOAc : H_2O = 4 : 1 : 5 as eluent) and TLC [the lower layer of $CHCl_3$:MeOH:H_2O, 15:6:2-HOAc (9:1) as eluent] with the authentic monosaccharides purchased from ACROS.

Plant Material

The whole plants of *P. franchetianum* Hemsl. were collected from Miyaluo, Lixian County, Sichuan Province, China, in August 1998, and identified by Prof. F.D. Fu (Chengdu Institute

of Biology, the Chinese Academy of Sciences). A voucher specimen is deposited at the Herbarium of Chengdu Institute of Biology, the Chinese Academy of Sciences.

Extraction and Isolation

A sample of cut and dried whole plants (10 kg) was soaked with 92% EtOH (50 l × 3). After concentrated *in vacuo*, ca. 1000 g extract was obtained. The syrup was dissolved in warm H_2O 2.5 l (about 50°C) and extracted successively with petroleum ether (60–90°C) (1.0 l × 11), EtOAc (1.0 l × 10) and *n*-BuOH (1.0 l × 8).

The EtOAc extract (80 g) was divided into five fractions Fr. 1–5 by CC gradiently eluted with petroleum ether (60–90°C)–EtOAc (5:1, 3:1 and 1:1), $CHCl_3$–MeOH (10:1, 5:1 and 2:1) and MeOH, respectively. Compound **1** (28 mg), **2** (32 mg) and **3** (20 mg) were obtained from Fr. 1 by CC eluted with petroleum ether (60–90°C)–EtOAc (20:1–5:1). Fr. 3 was chromatographied with eluent $CHCl_3$–MeOH (10:1–5:1) to give compound **4** (1.5 g). Compound **5** (260 mg) was obtained from Fr. 4 by CC eluted with $CHCl_3$–MeOH (10:1). The *n*-BuOH extract (300 g) was dissolved in warm H_2O 1.0 l (about 50°C) and absorbed by macroporus resin firstly. Then, it was eluted with H_2O until no sugar was detected, then eluted by EtOH to yield 32 g fraction, which was divided into five fractions Fr. A–E by CC eluted with $CHCl_3$–MeOH (10:1, 5:1 and 2:1), MeOH and H_2O, respectively. Compound **6** (68 mg) and **7** (1.02 g) were obtained from Fr. A by CC eluted with $CHCl_3$–MeOH–H_2O (10:2:0.3, low layer). Fr. B was separated by CC with $CHCl_3$–MeOH–H_2O (10:3:0.4, low layer) to yield compound **8** (238 mg) and **9** (2.16 g), respectively. Compound **10** (985 mg) was isolated from Fr. C by CC eluted with EtOAc–MeOH (5:2). Fr. D was subjected on CC by $CHCl_3$–MeOH–H_2O (10:3:0.4, low layer), then on reverse phase C-18 with the elution of MeOH–H_2O (2:1). Thus, Compound **11** (106 mg) and **12** (256 mg) were obtained, respectively.

Compound **1** [5], **2**, **3** [19–23], **4**, **5** [24], **6** [25,26], **7** [27,28], **9** [29] were identified by co-TLC with authentic samples and/or by comparison of their spectral data with those reported.

Pleurofranoside I (**8**). White powder, mp 260–261°C, $[\alpha]_D^{20}$ + 2.0° (*c* 0.38, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3394 (–OH), 2929, 1654 (–C = C–), 1458, 1367, 1169 and 1076. HRFABMS (–) *m/z*: 813.4609 ($[M - 1]^-$, calcd. for $C_{42}H_{69}O_{15}$, 813.4636). FABMS (+) *m/z* (rel. int.): 838 ($[M + Na + H]^+$, 80), 815 ($[M + H]^+$, 95), 653 ($[M + H - glc]^+$, 1.3), 473 ($[M - (glc–glc)]^+$, 12), 455 ($[M - (glc–glc) - H_2O]^+$, 100) and 437 ($[M - (glc–glc) - 2 \times H_2O]^+$, 41). ^{1}H NMR: δ 5.32 (1 H, d, J = 7.6 Hz, H-1″), 5.26 (1 H, t, J = 3.2 Hz, H-12), 5.04 (1 H, d, J = 7.6 Hz, H-1′), 4.60 (1 H, dd, J = 11.2, 4.8 Hz, H-16), 4.36 (1 H, d, J = 9.6 Hz, H-23a), 4.33 (1 H, d, J = 10.0 Hz, H-28a), 4.25 (1 H, m, H-3), 3.69 (1 H, d, J = 10.0 Hz, H-28b), 3.71 (1 H, d, J = 9.6 Hz, H-23b), 1.31 (3 H, s, H-27), 1.30 (3 H, s, H-29), 1.19 (3 H, s, H-30), 1.04 (3 H, s, H-26), 1.00 (3 H, s, H-24), 0.97 (3 H, s, H-25). ^{13}C NMR: see Table I.

Hydrolysis of pleurofranoside I. Compound **8** (ca. 15 mg) was heated at 60°C with 4 M HCl (aq.) for 6 h. D-glucose was identified on PC and TLC with the authentic samples. Aglycone **8a** identified as gymnestrogenin was extracted with $CHCl_3$ from the acidic solution after being neutralized by aqueous sodium hydroxide.

Pleurofranoside II (**10**). Yellowish powder, mp 247–248°C, $[\alpha]_D^{20}$ +3.37° (*c* 0.624, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3422 (–OH), 2927, 1654 (–C = C–), 1458, 1383, 1256, 1164 and 1074. HRFABMS (–) *m/z*: 941.5151 ($[M - 1]^-$, calcd. for $C_{48}H_{77}O_{18}$, 941.5109). FABMS (+) *m/z* (rel. int.): 981 ($[M + K]^+$, 12), 965 ($[M + Na]^+$, 28), 763 ($[M + H - H_2O–glc]^+$, 11), 601 ($[M + H - 2 \times glc–H_2O]^+$, 10), 455 ($[M - 2 \times glc–fuc]^+$, 45) and 437 ($[M - 2 \times glc–fuc - H_2O]^+$, 35). ^{1}H NMR: δ 5.96 (1H, d, J = 10.0 Hz, H-12), 5.63 (1H,

TABLE I ^{13}C NMR data of compounds **8** and **8a** (C_5D_5N, 100 MHz)

C-atom	**8**	**8a**	C-atom	**8**	**8a**	C-atom	**8**
1	38.9	38.9	16	67.9	67.6	Glc-1	
2	27.5	27.6	17	43.9	43.9	1	104.6
3	82.8	73.3	18	43.6	43.6	2	84.2
4	42.8	42.8	19	47.6	47.6	3	78.3
5	47.6	48.5	20	36.9	36.9	4	72.4
6	18.5	18.5	21	72.4	72.6	5	77.8
7	32.6	32.7	22	33.9	34.9	6	63.7
8	40.1	40.1	23	67.9	67.9	Glc-2	
9	47.1	47.2	24	12.9	13.0	1	106.3
10	36.9	36.9	25	16.0	16.1	2	73.5
11	23.8	23.9	26	16.8	67.9	3	77.8
12	123.7	123.0	27	26.9	26.9	4	71.5
13	142.7	143.1	28	67.9	68.4	5	77.2
14	43.6	43.8	29	29.1	29.8	6	62.5
15	36.7	36.7	30	18.3	18.0		

dd, $J = 10.0, 2.0$ Hz, H-11), 5.57 (1H, d, $J = 7.6$ Hz, H-1‴), 5.28 (1H, d, $J = 7.6$ Hz, H-1″), 4.92 (1H, d, $J = 7.6$ Hz, H-1′), 4.51 (1 H, m, H-16), 4.38 (1 H, d, $J = 7.2$ Hz, H-28b), 4.37 (1 H, d, $J = 10.4$ Hz, H-23a), 4.29 (1 H, dd, $J = 12.0, 5.2$ Hz, H-3), 3.69 (1 H, d, $J = 10.4$ Hz, H-23b), 3.32 (1 H, d, $J = 7.2$ Hz, H-28b), 1.37 (3H, s, H-26), 1.25 (3H, s, H-27), 1.19 (3H, s, H-24), 1.10 (3H, s, H-25), 1.06 (3H, s, H-29) and 0.95 (3H, s, H-30). ^{13}C NMR: see Table II.

Hydrolysis of pleurofranoside II. Compound **10** (ca. 0.5 mg) was heated at 90°C with 5 M HCl (aq.) for 6 h. D-glucose and D-fucose were identified on PC and TLC with the authentic samples.

TABLE II ^{13}C NMR data of compounds **10**, **11** and **12** (C_5D_5N, 100 MHz)

C-atom	10	11	12	C-atom	10	11	12
1	38.5	38.5	38.5	25	18.5	18.5	18.5
2	26.4	26.7	26.7	26	19.9	19.9	19.9
3	82.6	82.5	82.5	27	20.7	20.6	20.6
4	43.7	43.7	43.7	28	72.9	73.1	73.1
5	47.7	47.7	47.7	29	34.6	34.8	34.8
6	17.6	17.9	17.9	30	25.9	25.9	25.9
7	31.5	31.5	31.5	1′	103.9	103.9	103.8
8	42.1	42.0	42.0	2′	77.0	77.0	76.9
9	53.0	52.9	52.9	3′	71.8	76.1	84.6
10	36.2	36.9	36.9	4′	83.9	83.7	71.6
11	132.1	132.1	132.1	5′	70.4	77.0	76.7
12	131.0	131.0	130.9	6′	17.1	62.5	63.8
13	84.8	84.8	84.8	1″	105.0	103.9	105.6
14	45.6	45.6	45.6	2″	75.2	71.5	73.0
15	36.0	36.2	36.2	3″	78.3	73.1	75.6
16	64.5	64.5	64.5	4″	71.5	72.1	71.6
17	47.7	47.7	47.7	5″	78.7	70.4	76.7
18	52.1	51.6	51.6	6″	61.6	17.1	63.0
19	37.7	37.3	37.3	1‴	103.9	105.0	103.9
20	30.9	30.4	30.4	2‴	75.2	75.3	73.7
21	34.6	34.7	34.8	3‴	78.3	77.0	76.3
22	25.9	25.9	25.9	4‴	70.4	70.4	71.5
23	64.5	65.4	65.4	5‴	78.6	77.4	76.7
24	12.6	12.6	12.6	6‴	63.0	63.0	62.7

Pleurofranoside III (**11**). White powder, mp 281.5–283°C, $[\alpha]_D^{20}$ +4.82° (*c* 0.224, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3403 (–OH), 2928, 1639 (–C = C–), 1385, 1162 and 1072. HRFABMS (–) *m/z*: 941.5051 ($[M - 1]^-$, calcd. for $C_{48}H_{77}O_{18}$, 941.5109). FABMS (+) *m/z* (rel. int.): 982 ($[M + K + H]^+$, 1.3), 966 ($[M + Na + H]^+$, 1.5), 762 ($[M - glc–H_2O]^+$, 0.6), 615 ($[M - glc–H_2O - fuc]^+$, 0.4), 471 ($[M + H - (2 \times glc–fuc)]^+$, 6), 453 ($[M + H - (2 \times glc–fuc) - H_2O]^+$, 10) and 435 ($[M + H - (2 \times glc–fuc) - 2 \times H_2O]^+$, 5). ^{1}H NMR: δ 5.98 (1H, d, J = 10.4 Hz, H-11), 5.69 (1H, dd, J = 10.4, 2.8 Hz, H-12), 5.58 (1H, d, J = 8.0 Hz, H-1″), 5.30 (1H, d, J = 8.0 Hz, H-1‴), 4.92 (1H, d, J = 8.0 Hz, H-1′), 4.49 (1 H, br, H-16), 4.38 (1 H, d, J = 10.4 Hz, H-23a), 4.37 (1 H, d, J = 6.4 Hz, H-28a), 4.29 (1 H, dd, J = 12.0, 4.8 Hz, H-3), 3.70 (1 H, d, J = 10.4 Hz, H-23b), 3.41 (1 H, d, J = 6.4 Hz, H-28b), 1.37 (3H, s, H-26), 1.25 (3H, s, H-27), 1.19 (3H, s, H-24), 1.10 (3H, s, H-25), 1.06 (3H, s, H-29) and 0.95 (3H, s, H-30). ^{13}C NMR: see Table II.

Hydrolysis of pleurofranoside III. Compound **11** (ca. 1 mg) was heated at 95°C with 5 M HCl (aq.) for 6 h. D-glucose and D-fucose were identified on PC and TLC with the authentic samples.

Pleurofranoside IV (**12**). White powder, mp 259–260°C, $[\alpha]_D^{20}$ +6.01° (*c* 0.268, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3416 (–OH), 2928, 1639 (–C = C–), 1385, 1162 and 1072. HRFABMS (–) *m/z*: 957.5055 (calcd. for $C_{48}H_{77}O_{19}$, 957.5059). FABMS (+) *m/z* (rel. int.): 998 ($[M + K + H]^+$, 0.2), 958 (M^+, 0.3), 796 ($[M + H - glc]^+$, 0.3), 617 ($[M + 2H - 2 \times glc–H_2O]^+$, 0.6), 473 ($[M + H - 3 \times glc]^+$, 6), 455 ($[M + H - 3 \times glc–H_2O]^+$, 42), 437 ($[M - 3 \times glc - 2 \times H_2O]^+$, 41) and 419 ($[M - 3 \times glc - 3 \times H_2O]^+$, 6). ^{1}H NMR: δ 5.95 (1H, d, J = 10.0 Hz, H-12), 5.62 (1H, dd, J = 10.0, 2.0 Hz, H-11), 5.56 (1H, d, J = 7.6 Hz, H-1′), 5.28 (1H, d, J = 7.6 Hz, H-1‴), 4.90 (1H, d, J = 7.6 Hz, H-1′), 4.50 (1 H, m, H-16), 4.37 (1 H, d, J = 7.2 Hz, H-28a), 4.36 (1 H, d, J = 11.2 Hz, H-23a), 4.28 (1 H, dd, J = 11.2, 4.8 Hz, H-3), 3.69 (1 H, d, J = 11.2 Hz, H-23b), 3.30 (1 H, d, J = 7.2 Hz, H-28b), 1.35 (3H, s, H-26), 1.26 (3H, s, H-27), 1.14 (3H, s, H-24), 1.09 (3H, s, H-25), 1.05 (3H, s, H-29) and 0.96 (3H, s, H-30). ^{13}C NMR: see Table II.

Hydrolysis of pleurofranoside IV. Compound **12** (ca. 1 mg) was heated at 98°C with 5 M HCl (aq.) for 5 h. Only D-glucose was identified on PC and TLC with the authentic samples.

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