# Saikosaponin Homologues from *Clinopodium* spp. The Structures of Clinoposaponins XII—XX

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**Nine new saikosaponin homologues, called clinoposaponins XII—XX, were isolated from the aerial parts of *Clinopodium vulgare*, *C. chinense* and *C. chinense* var. *parviflorum* together with nine known saikosaponin homologues. On the basis of spectral and chemical evidence, the structures of clinoposaponins XII—XX were determined to be 3-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]saikogenin F, 3-*O*-β-D-fucopyranosyl-21β-hydroxysaikogenin F, 3-*O*-[β-D-glucopyranosyl-(1→3)-β-D-fucopyranosyl]-21β-hydroxysaikogenin F, 3-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]saikogenin F, 3-*O*-{β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-fucopyranosyl}-21β-hydroxysaikogenin F, 3-*O*-{β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-fucopyranosyl}-23-oxosaikogenin E, 3-*O*-{β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-fucopyranosyl}-16-ketosaikogenin F, 3-*O*-{β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-fucopyranosyl}-30-hydroxysaikogenin F, 3-*O*-{β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-fucopyranosyl}-30-oxo-saikogenin F, respectively. The known saponins were assigned to be clinoposaponins III, V, IX, X, XI, buddlejasaponins I, IV, 3-*O*-β-D-fucopyranosylsaikogenin F and saikosaponin a.**

**Key words** *Clinopodium vulgare*; *Clinopodium chinense*; *Clinopodium chinense* var. *parviflorum*; Labiatae; saikosaponin; clinoposaponin

We previously reported[1-3] the isolation and structure elucidation of saikosaponin homologues, triterpene saponins having 13,28-epoxy-olean-11-ene skelton, isolated from *Clinopodium gracile* O. KUNTZE, *C. micranthum* (REGEL) HARA and *C. chinense* (BENTH.) O. KUNTZE var. *parviflorum* (KUDO) HARA (Labiatae). Saikosaponins are well known to have an anti-hepatotoxic activity. *Bupleurum falcatum* L. (Umbelliferae), the main source of saikosaponins, is used as an important Chinese medicine to cure hepatitis. This paper describes the isolation and structure elucidation of nine new and nine known saikosaponin homologues isolated from *C. vulgare* L., *C. chinense* (BENTH.) O. KUNTZE and *C. chinense* (BENTH.) O. KUNTZE var. *parviflorum* (KUDO) HARA. From the polar and lipophilic fraction of the aerial parts of the plants, saikosaponin homologues (**1**—**18**) were isolated by preparative HPLC using reversed phase (ODS, PhA) column. Compounds **1**, **2**, **4**, **7**, **9**, **11**, **16**—**18** were identified by comparison of the $^1$H- and $^{13}$C-NMR spectral data with reported data as clinoposaponins IX,[3] X,[3] 3-*O*-β-D-fucopyranosylsaikogenin F,[4] saikosaponin a,[5] buddlejasaponin IV,[5a] clinoposaponins XI,[3] III,[1] V[1] and buddlejasaponin I,[5a] respectively.

Clinoposaponin XII (**3**) revealed an $[M+Na]^+$ ion peak at *m/z* 819 in the FAB-MS and the high resolution mass spectral data consisted of the molecular formula $C_{42}H_{68}O_{14}$. On acid hydrolysis, only D-glucose was detected as the sugar component. The $^1$H- and $^{13}$C-NMR spectra were similar to those of clinoposaponin X (**2**), especially in the aglycone moiety. In the lower field, two anomeric proton signals at δ 5.07 (1H, d, $J=7.5$ Hz) and 5.37 (1H, d, $J=7.5$ Hz) were observed and the nuclear Overhauser effect (NOE) experiment irradiating at these signals suggested the sugar chain structure to be 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside. In the heteronuclear multiple bond connectivity (HMBC) spectrum, correlations were observed between the anomeric proton signal (δ 5.37) and the carbon signal (δ 84.2) which was assigned to the C-2 of inner glucose from the $^1$H–$^1$H correlation spectroscopy (COSY) and the heteronuclear single quantum coherence (HSQC) spectra, and between the anomeric proton signal (δ 5.07) and the carbon signal (δ 82.6) which was assigned to the C-3 of the aglycone moiety. The structure was determined to be **3**.

Clinoposaponins XIII (**5**), $C_{36}H_{58}O_9$ and XIV (**6**), $C_{42}H_{68}O_{14}$ gave D-fucose and D-fucose–D-glucose (1 : 1) on acid hydrolysis, respectively. The $^1$H-NMR spectrum of **5** showed the presence of a fucopyranosyl residue at the C-3 of the aglycone moiety compared with that of 3-*O*-β-D-fucopyranosylsaikogenin F (**4**).[4] The two methyl proton signals assigned to H-29 and H-30 were shifted downfield by 0.33 ppm and 0.25 ppm, respectively, suggesting the presence of an equatorial oxygen function at the C-19 or C-21. The carbinyl proton signal was observed at δ 4.10 as a broad double doublet ($J=11$, 3 Hz). The HMBC correlations were observed between the proton signal at δ 1.27 (H-29) and the carbinyl carbon signal at δ 73.2 which was assigned to the C-21, and between the proton signal at δ 1.16 (H-30) and the carbinyl carbon signal at δ 73.2. These data led us to conclude the structure of clinoposaponin XIII was **5**. The $^{13}$C-NMR data of **6** were superimposable on those of **5** in the aglycone moiety. The NOE experiment and the HMBC spectrum showed the sugar chain structure to be 3-*O*-β-D-glucopyranosyl-(1→3)-β-D-fucopyranoside.

Clinoposaponin XV (**8**) revealed an $[M+Na]^+$ ion peak at *m/z* 803 in the FAB-MS and elemental analysis data were consistent with the molecular formula $C_{42}H_{68}O_{13}$. Acid hydrolysis afforded D-fucose and D-glucose as the sugar moiety. The NMR data were similar to those of saikosaponin a (**7**) except for the sugar moiety. In the NOE experiment, the irradiation of the anomeric proton signal at δ 5.21 (1H, d, $J=8$ Hz) enhanced the proton signal at δ 4.46 (1H, dd, $J=9.5$, 8 Hz) due to the H-2 of

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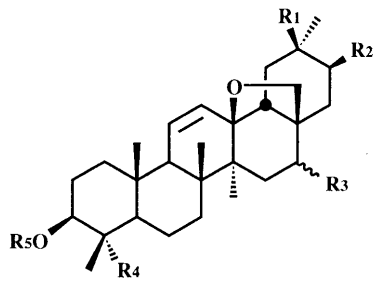


| | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ |
|---|---|---|---|---|---|
| **1** | $CH_3$ | H | β-OH | $CH_2$-OH | Glc |
| **2** | $CH_3$ | H | β-OH | $CH_2$-OH | Glc(6)—Glc |
| **3** | $CH_3$ | H | β-OH | $CH_2$-OH | Glc(2)—Glc |
| **4** | $CH_3$ | H | β-OH | $CH_2$-OH | Fuc |
| **5** | $CH_3$ | OH | β-OH | $CH_2$-OH | Fuc |
| **6** | $CH_3$ | OH | β-OH | $CH_2$-OH | Fuc(3)—Glc |
| **7** | $CH_3$ | H | β-OH | $CH_2$-OH | Fuc(3)—Glc |
| **8** | $CH_3$ | H | β-OH | $CH_2$-OH | Fuc(2)—Glc |
| **9** | $CH_3$ | H | β-OH | $CH_2$-OH | Fuc(2)—Glc<br>\|3<br>Glc |
| **10** | $CH_3$ | OH | β-OH | $CH_2$-OH | Fuc(2)—Glc<br>\|3<br>Glc |
| **11** | $CH_3$ | H | β-OH | $CH_3$ | Fuc(2)—Glc<br>\|3<br>Glc |
| **12** | $CH_3$ | H | β-OH | CHO | Fuc(2)—Glc<br>\|3<br>Glc |
| **13** | $CH_3$ | H | =O | $CH_2$-OH | Fuc(2)—Glc<br>\|3<br>Glc |
| **13a** | $CH_3$ | H | α-OH | $CH_2$-OH | Fuc(2)—Glc<br>\|3<br>Glc |
| **14** | $CH_2$-OH | H | β-OH | $CH_2$-OH | Fuc(2)—Glc<br>\|3<br>Glc |
| **15** | CHO | H | β-OH | $CH_2$-OH | Fuc(2)—Glc<br>\|3<br>Glc |
| **16** | $CH_3$ | H | β-OH | $CH_2$-OH | Fuc(2)—Glc<br>\|3<br>Glc(6)—Glc |
| **17** | $CH_3$ | H | β-OH | $CH_2$-OH | Fuc(2)—Glc<br>\|3<br>Glc(6)—Glc(4)—Glc |
| **18** | $CH_3$ | H | β-OH | $CH_2$-OH | Fuc(2)—Glc<br>\|3<br>Glc(4)—Rha |

Chart 1

fucose moiety, and the irradiation of the anomeric proton signal at $\delta$ 4.90 (1H, d, $J=8$ Hz) enhanced the proton signal at $\delta$ 4.12 which was assigned to the H-3 of the aglycone moiety by $^1H$–$^1H$ COSY and the coupling pattern (dd, $J=12$, 4.5 Hz) in the NOE difference spectrum. The structure of the sugar chain was decided as 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-fucopyranoside. The HMBC spectrum also supported this sugar sequence.

Clinoposaponin XVI (**10**) has a molecular formula $C_{48}H_{78}O_{19}$ from the high resolution mass spectral data. On acid hydrolysis D-fucose and D-glucose were detected by GC. The $^{13}C$-NMR data of the sugar moiety were superimposable on those of buddlejasaponin IV (**9**) and those of the aglycone moiety were superimposable on those of clinoposaponin XIII (**5**). The NOE experiments irradiating on the anomeric proton signal and the HMBC spectrum led to **10** as the structure of clinoposaponin XVI.

The $^{13}C$-NMR spectrum of clinoposaponin XVII (**12**) showed the presence of an aldehyde carbon at $\delta$ 209.1, which was correlated to an aldehyde proton at $\delta$ 9.81 (1H, s) in the HSQC spectrum, and the sugar chain {β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-fucopyranosyl} by comparison of the sugar carbon signals with those of buddlejasaponin IV (**9**). The aldehyde proton signal was correlated to the carbon signal at $\delta$ 83.3 and $\delta$ 55.3 due to the C-3 and C-4 of the aglycone moiety, respectively, and the upfield shifted methyl carbon signal at $\delta$ 10.1 and the downfield shifted methyl proton signal at $\delta$ 1.40 (3H, s) were correlated to the carbon signal of the C-3, C-4 and C-5 of the aglycone moiety and the aldehyde carbon signal. The methyl group was assigned to C-24 from these correlations and the chemical shift of the carbon signal. The C-23 aldehyde structure was confirmed by sodium borohydride reduction of **12** to buddlejasaponin IV (**9**).

Clinoposaponin XVIII (**13**) has the same sugar chain as that of buddlejasaponin IV (**9**) and a ketonic carbonyl carbon ($\delta$ 212.2). The carbonyl carbon was correlated to two sets of isolated methylene proton signals at $\delta$ 3.50 (1H, br d, $J=8$ Hz); 3.92 (1H, d, $J=8$ Hz) and $\delta$ 1.90 (1H, d, $J=14.5$ Hz); 2.83 (1H, d, $J=14.5$ Hz). In the HMBC spectrum, the former methylene proton signals were correlated to the C-13, C-17, C-18 and assigned to the C-28 methylene protons, and the latter methylene proton signals were correlated to the C-9, C-13, C-14, C-27 and assigned to the C-15 methylene protons. Sodium borohydride reduction of **13** afforded **13a** and the NMR data of the aglycone moiety were superimposable on those of saikosaponin d (16α-OH). These observations were compatible with those of Ogihara and Nose.[6]

The $^{13}C$-NMR spectra of clinoposaponins XIX (**14**) and XX (**15**) suggested the presence of the same sugar chain {3-*O*-β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-fucopyranosyl} and one more hydroxy methyl carbon ($\delta$ 65.3) other than the C-23 in **14** and an aldehyde carbon ($\delta$ 206.4) in **15**. The reduction of **15** with sodium borohydride afforded **14**. By comparing the C-29 ($\delta$ 29.0) and C-30 ($\delta$ 65.3) chemical shifts of **14** with those of buddlejasaponin IV (**9**), the C-29 was shifted upfield by 4.7 ppm due to the γ-effect of *O*-function, and the C-30 was shifted downfield by 41.5 ppm. These results led us to conclude the structures of clinoposaponins XIX and XX were **14** and **15**, respectively.

This is the first report of saikosaponin homologues

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Table 1. $^1$H-NMR Spectral Data of Compounds **3**, **5**, **6**, **8**, **10**, **12**, **13**, **13a**, **14**, **15** in Pyridine-$d_5$ at 35 °C

| | **3** | **5** | **6** | **8** | **10** |
|---|---|---|---|---|---|
| Aglycone moiety | | | | | |
| 3 | 4.16[a)] | 4.29 (dd, 12, 4.5) | 4.29 (dd, 12, 5) | 4.12[a)] | 4.09[a)] |
| 11 | 5.97 (br d, 10) | 6.04 (br d, 10.5) | 6.04 (br d, 10) | 5.98 (br d, 10.5) | 6.00 (br d, 10) |
| 12 | 5.65 (dd, 10, 3) | 5.72 (dd, 10.5, 3) | 5.73 (dd, 10, 2.5) | 5.63 (dd, 10.5, 2.5) | 5.69 (dd, 10, 3) |
| 16 | 4.51 (m) | 4.58 (m) | 4.59 (m) | 4.48[a)] | 4.56 (br dd, 9, 5.5) |
| 21 | | 4.10 (br dd, 11, 3) | 4.11 (br d, 11) | | 4.09[a)] |
| 23 | 3.78 (d, 10) | 3.71 (br d, 11) | 3.72 (br d, 11) | 3.75 (d, 10) | 3.71 (d, 11) |
| | 4.40 (d, 10) | 4.35 (br d, 11) | 4.37 (br d, 11) | 4.37 (d, 10) | 4.36 (d, 11) |
| 24 | 1.10 (s) | 0.94 (s) | 0.94 (s) | 1.07 (s) | 1.06 (s) |
| 25 | 0.98 (s) | 1.03 (s) | 1.02 (s) | 0.99 (s) | 0.98 (s) |
| 26 | 1.39 (s) | 1.41 (s) | 1.41 (s) | 1.38 (s) | 1.38 (s) |
| 27 | 1.10 (s) | 1.13 (s) | 1.15 (s) | 1.07 (s) | 1.12 (s) |
| 28 | 3.33 (br d, 7) | 3.43 (br d, 7) | 3.44 (br d, 7) | 3.31 (br d, 7) | 3.41 (br d, 7) |
| | 4.39 (d, 7) | 4.40 (d, 7) | 4.41 (d, 7) | 4.36 (d, 7) | 4.38 (d, 7) |
| 29 | 0.94 (s) | 1.27 (s) | 1.28 (s) | 0.91 (s) | 1.26 (s) |
| 30 | 0.90 (s) | 1.16 (s) | 1.17 (s) | 0.88 (s) | 1.15 (s) |
| Sugar moiety | | | | | |
| at C-3 | (Glc) | (Fuc) | (Fuc) | (Fuc) | (Fuc) |
| 1 | 5.07 (d, 7.5) | 4.95 (d, 8) | 4.97 (d, 8) | 4.90 (d, 8) | 4.91 (d, 7.5) |
| 2 | 4.15 (dd, 8, 7.5) | 4.34 (dd, 8, 8) | 4.53 (dd, 9.5, 8) | 4.46 (dd, 9.5, 8) | 4.65 (dd, 9.5, 7.5) |
| 3 | 4.18[a)] | 3.99[a)] | 4.05 (dd, 9.5, 3.5) | 4.03 (dd, 9.5, 3) | 4.09[a)] |
| 4 | 4.17[a)] | 4.00[a)] | 4.15 (br s) | 3.95 (br d, 3) | 4.24[a)] |
| 5 | 3.79 (m) | 3.78 (q, 6.5) | 3.69[a)] | 3.66 (q, 6.5) | 3.62 (q, 6.5) |
| 6 | 4.43[a)] | 1.54 (d, 6.5) | 1.46 (d, 6.5) | 1.48 (d, 6.5) | 1.40 (d, 6.5) |
| | 4.45[a)] | | | | |
| at C-2 of fucose or glucose | | | | | |
| | (Glc) | | | (Glc) | (Glc) |
| 1 | 5.37 (d, 7.5) | | | 5.21 (d, 8) | 5.57 (d, 8) |
| 2 | 4.11 (dd, 9, 7.5) | | | 4.08[a)] | 4.08[a)] |
| 3 | 4.21 (dd, 9, 9) | | | 4.12[a)] | 4.17[a)] |
| 4 | 4.29 (dd, 9, 9) | | | 4.24 (dd, 9, 9) | 4.17[a)] |
| 5 | | | | 3.74[a)] | 3.63 (m) |
| 6 | 4.33 (dd, 11.5, 5) | | | 4.38[a)] | 4.29[a)] |
| | 4.49[a)] | | | 4.38[a)] | 4.32[a)] |
| at C-3 of fucose | | | | | |
| | | | (Glc) | | (Glc) |
| 1 | | | 5.33 (d, 8) | | 5.28 (d, 8) |
| 2 | | | 4.04 (dd, 9, 8) | | 3.99 (dd, 8, 8) |
| 3 | | | 4.22 (dd, 9, 9) | | 4.21 (dd, 9, 8) |
| 4 | | | 4.27 (dd, 9, 9) | | 4.18 (dd, 9,9) |
| 5 | | | 4.01 (m) | | 3.93 (m) |
| 6 | | | | | 4.30[a)] |
| | | | | | 4.49 (dd, 11.5, 2.5) |

having a 16-keto or an aldehyde function being isolated from *Clinopodium* spp. The plants were found useful as a saikosaponin source.

## Experimental

**General** The instruments used in this work were: a JASCO DIP-1000 digital polarimeter for optical rotation; a JASCO J-20A spectropolarimeter for CD; a JEOL α-400 FT-NMR spectrometer for NMR spectra ($^1$H: 400 MHz, $^{13}$C: 100 MHz, in pyridine-$d_5$ at 35 °C); a JEOL JMS-SX102 spectrometer for positive mode FAB-MS; a Hitachi M-80 spectrometer for positive mode high resolution SI-MS; a Hitachi G-3000 gas chromatograph for GC; and a JASCO System 800 for HPLC.

**Extraction and Isolation** The dried aerial parts (3.1 kg) of *Clinopodium vulgare* L. (Seeds were purchased from Chiltern Seeds, England and the plants were cultivated in our botanical garden in 1995) were extracted with ether twice and the residue was extracted with methanol at room temperature three times. The methanol solution was passed through an Amberlite IRA-60E column to remove acidic compounds and the eluate was concentrated *in vacuo* at 50 °C. The residue was dissolved in water and the solution was passed through a porous polymer gel column (Mitsubishi Diaion HP-20). After washing with water, 60% methanol eluate (24 g) and methanol eluate (33 g) were obtained as a pale brown powder. A part (2.5 g) of the methanol eluate was chromatographed on a Develosil Lop-ODS column (5 cm × 50 cm × 2) using a methanol–water system (linear gradient) and Develosil PhA (2 cm × 25 cm) using an acetonitrile-water system to give compounds **7** (3 mg), **8** (3 mg), **9** (1.08 g), **10** (6 mg), **11** (104 mg), **13** (4 mg), **15** (1 mg), **16** (7 mg), **17** (15 mg) and **18** (1 mg). The aerial parts (970 g) of *C. chinense* (Benth.) O. Kuntze (collected in Jianxi, China in 1995) gave 60% methanol eluate (6 g) and methanol eluate (12.8 g). The methanol eluate afforded compounds **1** (18 mg), **2** (5 mg), **9** (1.4 g), **10** (4 mg), **11** (15 mg) and **14** (2 mg) in a similar manner as described in for *C. vulgare* L. The aerial parts (4.5 kg) of *C. chinense* (Benth.) O. Kuntze var. *parviflorum* (Kudo) Hara (cultivated in our botanical garden in 1994) were extracted with methanol at room temperature twice. The methanol extract was concentrated *in vacuo* at 50 °C and the residue was partitioned between water and ether. The water layer was passed through a polyamide column and the water eluate was passed through a porous polymer gel (Mitsubishi Diaion HP-20) column. The 60% methanol eluate (53 g) and methanol eluate (45 g) were obtained after similar treatment. The methanol eluate was chromatographed on a silica gel column using a chloroform–methanol–water system to give 12 fractions (Fr. 1—12). Fr. 2, 3, 4, 5 and 6 gave compounds **3** (19 mg), **4** (40 mg), **5** (3 mg), **6** (8 mg) and **12** (11 mg) by a similar separation method.

Clinoposaponin XII (**3**): An amorphous powder, $[\alpha]_D^{23}$ +34.3° ($c$=1.90, MeOH). High resolution positive mode SI-MS: Calcd for $C_{42}H_{68}O_{14}Na$ $(M+Na)^+$: 819.4503. Found: 819.4509. $^1$H- and $^{13}$C-NMR: Tables 1 and 2.

Clinoposaponin XIII (**5**): An amorphous powder, $[\alpha]_D^{23}$ +70.5°

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Table 1. (continued)

| | 12 | 13 | 13a | 14 | 15 |
|---|---|---|---|---|---|
| Aglycone moiety | | | | | |
| 3 | 4.05[a)] | 4.14 (dd, 12, 4.5) | | | 4.14[a)] |
| 11 | 5.93 (br d, 10) | 6.04 (br d, 10.5) | 6.02 (br d, 10.5) | 5.93 (br d, 10.5) | 5.98 (br d, 10.5) |
| 12 | 5.66 (dd, 10, 3) | 5.68 (dd, 10.5, 2.5) | 5.68 (dd, 10.5, 3) | 5.63 (dd, 10.5, 3) | 5.66 (dd, 10.5, 3) |
| 16 | 4.52 (m) | | | | 4.51 (m) |
| 21 | | | | | |
| 23 | 9.81 (s) | 3.72 (d, 11) | 3.72 (d, 11) | | 3.72 (d, 10) |
| | | 4.37 (d, 11) | 4.36 (d, 11) | | 4.37 (d, 10) |
| 24 | 1.40 (s) | 1.02 (s) | 1.01 (s) | 1.07 (s) | 1.07 (s) |
| 25 | 0.89 (s) | 0.97 (s) | 0.97 (s) | 0.96 (s) | 0.96 (s) |
| 26 | 1.31 (s) | 1.37 (s) | 1.35 (s) | 1.38 (s) | 1.35 (s) |
| 27 | 1.12 (s) | 1.07 (s) | 1.61 (s) | 1.17 (s) | 1.12 (s) |
| 28 | 3.33 (br d, 7) | 3.50 (br d, 8) | 3.32 (br d, 7) | | 3.19 (br d, 7) |
| | 4.37 (d, 7) | 3.92 (d, 8) | | | 4.33[a)] |
| 29 | 0.96 (s) | 0.87 (s) | 1.08 (s) | 1.20 (s) | 0.92 (s) |
| 30 | 0.92 (s) | 0.82 (s) | 1.03 (s) | | 9.56 (s) |
| Sugar moiety | | | | | |
| at C-3 | (Fuc) | (Fuc) | (Fuc) | (Fuc) | (Fuc) |
| 1 | 4.63 (d, 7.5) | 4.91 (d, 7.5) | 4.90 (d, 8) | 4.90 (d, 8) | 4.91 (d, 7.5) |
| 2 | 4.56 (dd, 9.5, 7.5) | 4.66 (dd, 8, 7.5) | | | 4.66 (dd, 8, 7.5) |
| 3 | 4.18[a)] | 4.10[a)] | | | 4.09[a)] |
| 4 | | 4.24 (br s) | | | 4.25[a)] |
| 5 | 3.69 (m) | | | | 3.62[a)] |
| 6 | 1.43 (d, 6.5) | 1.41 (d, 6.5) | 1.40 (d, 6.5) | 1.40 (d, 6.5) | 1.40 (d, 6.5) |
| at C-2 of fucose or glucose | | | | | |
| | (Glc) | (Glc) | (Glc) | (Glc) | (Glc) |
| 1 | 5.54 (d, 8) | 5.59 (d, 7.5) | 5.58 (d, 8) | 5.58 (d, 8) | 5.59 (d, 7.5) |
| 2 | 4.05[a)] | 4.10[a)] | | | 4.09[a)] |
| 3 | | 4.20[a)] | | | 4.16[a)] |
| 4 | 4.30[a)] | 4.20[a)] | | | 4.19[a)] |
| 5 | 3.63 (m) | 3.65 (m) | | | 3.64[a)] |
| 6 | 4.31[a)] | | | | 4.27[a)] |
| | 4.31[a)] | | | | 4.32[a)] |
| at C-3 of fucose | | | | | |
| | (Glc) | (Glc) | (Glc) | (Glc) | (Glc) |
| 1 | 5.30 (d, 8) | 5.30 (d, 7.5) | 5.29 (d, 8) | 5.29 (d, 8) | 5.30 (d, 7.5) |
| 2 | 3.98 (dd, 9, 8) | 4.00 (dd, 8, 7.5) | | | 4.00 (dd, 9, 7.5) |
| 3 | 4.20[a)] | 4.22[a)] | | | 4.22[a)] |
| 4 | 4.18[a)] | 4.22[a)] | | | 4.22[a)] |
| 5 | 3.93 (m) | 3.95 (m) | | | 3.94 (m) |
| 6 | 4.30[a)] | | | | 4.32[a)] |
| | 4.45[a)] | | | | 4.46 (dd, 12, 2.5) |

*a)* Overlapped.

($c=0.28$, MeOH). High resolution positive mode SI-MS : Calcd for $C_{36}H_{58}O_9Na$ $(M+Na)^+$: 657.3975. Found: 657.3968. $^1$H- and $^{13}$C-NMR: Tables 1 and 2.

Clinoposaponin XIV (**6**): An amorphous powder, $[\alpha]_D^{23}$ +53.3° ($c=0.83$, MeOH). High resolution positive mode SI-MS: Calcd for $C_{42}H_{68}O_{14}Na$ $(M+Na)^+$: 819.4503. Found: 819.4494. $^1$H- and $^{13}$C-NMR: Tables 1 and 2.

Clinoposaponin XV (**8**): An amorphous powder, $[\alpha]_D^{23}$ +47.7° ($c=0.32$, MeOH). *Anal.* Calcd for $C_{42}H_{68}O_{13}\cdot 4H_2O$: C, 59.13; H, 8.98. Found: C, 59.28; H, 8.76. Positive mode FAB-MS: 803 $(M+Na)^+$. $^1$H- and $^{13}$C-NMR: Tables 1 and 2.

Clinoposaponin XVI (**10**): An amorphous powder, $[\alpha]_D^{23}$ +48.9° ($c=1.35$, MeOH). *Anal.* Calcd for $C_{48}H_{78}O_{19}\cdot 3/2H_2O$: C, 58.46; H, 8.28. Found: C, 58.42; H, 8.51. Positive mode FAB-MS: 981 $(M+Na)^+$. $^1$H- and $^{13}$C-NMR: Tables 1 and 2.

Clinoposaponin XVII (**12**): An amorphous powder, $[\alpha]_D^{23}$ +70.4° ($c=1.09$, MeOH). High resolution positive mode SI-MS: Calcd for $C_{48}H_{76}O_{18}Na$: 963.4924. Found: 963.4929. $^1$H- and $^{13}$C-NMR: Tables 1 and 2.

Clinoposaponin XVIII (**13**): An amorphous powder, $[\alpha]_D^{23}$ +24.3° ($c=0.52$, MeOH). *Anal.* Calcd for $C_{48}H_{76}O_{18}\cdot 7/2H_2O$: C, 57.41; H, 8.33. Found: C, 57.39; H, 8.45. Positive mode FAB-MS: 963 $(M+Na)^+$. CD ($c=0.42$, MeOH): −118400 (298), −105000 (307), −52400 (317). $^1$H- and $^{13}$C-NMR: Tables 1 and 2.

**Clinoposaponin XIX (14)** An amorphous powder, $[\alpha]_D^{23}$ +26.5° ($c=0.13$, MeOH). High resolution positive mode SI-MS: Calcd for $C_{48}H_{78}O_{19}Na$: 981.5030. Found: 981.5063. $^1$H- and $^{13}$C-NMR: Tables 1 and 2.

Clinoposaponin XX (**15**): An amorphous powder, $[\alpha]_D^{23}$ +59.1° ($c=0.48$, MeOH). *Anal.* Calcd for $C_{48}H_{76}O_{19}\cdot 7/2H_2O$: C, 56.51; H, 8.20. Found: C, 56.35; H, 8.34. Positive mode FAB-MS: 979 $(M+Na)^+$.

**Sodium Borohydride Reduction of 12, 13 and 15** Compd. **12** (2 mg) was reduced with sodium borohydride (5 mg) in methanol (0.5 ml) for 30 min at room temperature. The reaction mixture was acidified with acetic acid and diluted with water. The solution was passed through a porous polymer gel (Mitsubishi Diaion HP-20) column. After washing the column with water, the methanol eluate was purified by HPLC (OSD column, 35% acetonitrile) to give **9** (1 mg) as an amorphous powder. The $^1$H-NMR spectrum was identical to that of buddlejasaponin IV (**9**). Compd. **13** (5 mg) and **15** (2 mg) were treated in the same manner to give **13a** (3mg) and **14** (1 mg), respectively. **13a**: Positive mode FAB-MS: 963 $(M+Na)^+$, $^1$H- and $^{13}$C-NMR: Tables 1 and 2. **14**: The $^1$H-NMR was identical to that of clinoposaponin XIX.

**Sugar Analysis of Clinoposaponins XII—XX** Each saponin (1 mg) was dissolved in 5% $H_2SO_4$ (0.05 ml) and dioxane (0.05 ml) and heated at 100 °C for 1 h. The reaction mixture was diluted with water and extracted with ethyl acetate three times. The water layer was passed through an Amberlite IRA-60E column and the eluate was concentrated. To the residue, D-cysteine (0.1 mg) and 0.02 ml of sodium acetate water solution (1 g/ml) were added and the solution was stirred for 1 h at 60 °C and

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Table 2. $^{13}$C-NMR Spectral Data of Compounds **3**, **5**, **6**, **8**, **10**, **12**, **13**, **13a**, **14**, **15** in Pyridine-$d_5$ at 35 °C

| | **3** | **5** | **6** | **8** | **10** | **12** | **13** | **13a** | **14** | **15** |
|---|---|---|---|---|---|---|---|---|---|---|
| Aglycone moiety | | | | | | | | | | |
| 1 | 38.6 | 38.7 | 38.7 | 38.7 | 38.7 | 38.0 | 38.5 | 38.7 | 38.7 | 38.7 |
| 2 | 25.9 | 26.0 | 26.1 | 26.0 | 26.0 | 24.9 | 26.0 | 26.0 | 26.0 | 26.0 |
| 3 | 82.6 | 81.7 | 81.7 | 82.3 | 82.7 | 83.3 | 82.4 | 82.8 | 82.7 | 82.6 |
| 4 | 43.7 | 43.7 | 43.8 | 43.8 | 43.9 | 55.3 | 43.8 | 43.8 | 43.9 | 43.8 |
| 5 | 48.0 | 47.5 | 47.4 | 48.1 | 47.9 | 48.0 | 47.8 | 48.0 | 47.9 | 47.9 |
| 6 | 17.7 | 17.6 | 17.6 | 17.7 | 17.7 | 20.0 | 17.5 | 17.7 | 17.7 | 17.7 |
| 7 | 31.7 | 31.7 | 31.7 | 31.7 | 31.7 | 31.3 | 31.4 | 31.6 | 31.7 | 31.6 |
| 8 | 42.3 | 42.2 | 42.2 | 42.3 | 42.2 | 42.6 | 42.1 | 41.9 | 42.3 | 42.3 |
| 9 | 53.1 | 53.1 | 53.1 | 53.1 | 53.1 | 52.8 | 52.8 | 53.0 | 53.1 | 53.1 |
| 10 | 36.3 | 36.3 | 36.4 | 36.3 | 36.3 | 35.8 | 36.2 | 36.3 | 36.6 | 36.3 |
| 11 | 132.2 | 132.2 | 132.2 | 132.2 | 132.2 | 131.5 | 133.2 | 132.0 | 132.1 | 132.5 |
| 12 | 131.2 | 131.2 | 131.2 | 131.2 | 131.1 | 131.5 | 129.4 | 132.0 | 131.2 | 130.7 |
| 13 | 84.0 | 83.8 | 83.8 | 84.0 | 83.8 | 83.9 | 84.3 | 84.9 | 84.1 | 84.0 |
| 14 | 45.7 | 45.7 | 45.7 | 45.7 | 45.7 | 45.7 | 49.8 | 43.6 | 45.8 | 45.8 |
| 15 | 36.2 | 36.3 | 36.4 | 36.3 | 36.3 | 36.1 | 44.8 | 35.5 | 36.3 | 36.2 |
| 16 | 64.1 | 65.5 | 65.5 | 64.1 | 65.5 | 64.0 | 212.2 | 77.2 | 64.3 | 64.2 |
| 17 | 47.0 | 49.3 | 49.3 | 47.0 | 49.3 | 47.0 | 56.4 | 45.4 | 47.0 | 46.4 |
| 18 | 52.2 | 51.7 | 51.7 | 52.2 | 51.7 | 52.2 | 55.1 | 51.4 | 51.9 | 53.4 |
| 19 | 37.8 | 37.4 | 37.4 | 37.8 | 37.5 | 37.8 | 39.0 | 38.4 | 31.5 | 32.1 |
| 20 | 31.6 | 37.1 | 37.1 | 31.6 | 37.1 | 31.6 | 31.6 | 31.9 | 36.3 | 47.7 |
| 21 | 34.8 | 73.2 | 73.2 | 34.7 | 73.2 | 34.7 | 35.9 | 36.9 | 30.0 | 28.8 |
| 22 | 25.8 | 34.9 | 34.9 | 25.8 | 34.9 | 25.7 | 24.5 | 31.3 | 25.6 | 26.8 |
| 23 | 64.9 | 64.5 | 64.2 | 65.0 | 64.7 | 209.1 | 64.6 | 64.9 | 64.3 | 64.7 |
| 24 | 12.9 | 13.0 | 13.0 | 12.8 | 12.7 | 10.1 | 12.7 | 12.7 | 12.7 | 12.7 |
| 25 | 18.7 | 18.8 | 18.8 | 18.7 | 18.7 | 18.2 | 18.5 | 18.8 | 18.7 | 18.6 |
| 26 | 20.1 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 19.8 | 19.5 | 20.1 | 20.0 |
| 27 | 20.9 | 20.7 | 20.7 | 20.8 | 20.7 | 20.9 | 20.2 | 18.1 | 21.0 | 20.9 |
| 28 | 73.0 | 72.7 | 72.7 | 73.0 | 72.7 | 73.1 | 75.5 | 77.8 | 73.1 | 72.8 |
| 29 | 33.7 | 30.5 | 30.5 | 33.6 | 30.5 | 33.7 | 33.3 | 33.8 | 29.0 | 24.1 |
| 30 | 23.9 | 18.0 | 18.0 | 23.8 | 18.1 | 23.9 | 23.1 | 24.4 | 65.3 | 206.4 |
| Sugar moeity | | | | | | | | | | |
| at C-3 | (Glc) | (Fuc) | (Fuc) | (Fuc) | (Fuc) | (Fuc) | (Fuc) | (Fuc) | (Fuc) | (Fuc) |
| 1 | 103.9 | 106.3 | 106.0 | 104.0 | 104.1 | 103.0 | 104.1 | 104.1 | 104.1 | 104.1 |
| 2 | 84.2 | 73.0 | 71.7 | 82.5 | 77.2 | 76.6 | 77.2 | 77.2 | 77.2 | 77.3 |
| 3 | 78.5 | 72.8 | 85.3 | 75.0 | 84.9 | 85.0 | 84.8 | 84.9 | 84.9 | 84.9 |
| 4 | 71.3 | 75.5 | 71.9 | 72.4 | 72.0 | 71.7 | 72.0 | 72.0 | 72.0 | 72.0 |
| 5 | 78.0 | 71.3 | 71.1 | 71.0 | 70.5 | 70.8 | 70.5 | 70.5 | 70.5 | 70.5 |
| 6 | 62.7 | 17.4 | 17.2 | 17.3 | 17.2 | 17.2 | 17.2 | 17.2 | 17.2 | 17.2 |
| at C-2 of fucose or glucose | | | | | | | | | | |
| | (Glc) | | | (Glc) | (Glc) | (Glc) | (Glc) | (Glc) | (Glc) | (Glc) |
| 1 | 106.0 | | | 106.2 | 104.1 | 104.0 | 104.1 | 104.0 | 104.1 | 104.1 |
| 2 | 76.9 | | | 76.8 | 76.3 | 76.1 | 76.3 | 76.2 | 76.3 | 76.3 |
| 3 | 78.1 | | | 78.2 | 78.8 | 78.8 | 78.9 | 78.8 | 78.9 | 78.9 |
| 4 | 71.5 | | | 71.4 | 72.3 | 71.9 | 72.3 | 72.2 | 72.3 | 72.3 |
| 5 | 78.3 | | | 78.1 | 77.5 | 77.6 | 77.4 | 77.4 | 77.5 | 77.4 |
| 6 | 62.8 | | | 62.5 | 63.2 | 62.9 | 63.2 | 63.1 | 63.2 | 63.2 |
| at C-3 of fucose | | | | | | | | | | |
| | | | (Glc) | | (Glc) | (Glc) | (Glc) | (Glc) | (Glc) | (Glc) |
| 1 | | | 106.7 | | 105.2 | 105.2 | 105.2 | 105.1 | 105.2 | 105.2 |
| 2 | | | 75.9 | | 75.4 | 75.4 | 75.4 | 75.4 | 75.4 | 75.4 |
| 3 | | | 78.8 | | 78.4 | 78.5 | 78.4 | 78.5 | 78.4 | 78.4 |
| 4 | | | 72.2 | | 71.7 | 71.7 | 71.7 | 71.7 | 71.7 | 71.7 |
| 5 | | | 78.5 | | 78.5 | 78.5 | 78.5 | 78.4 | 78.5 | 78.5 |
| 6 | | | 62.8 | | 62.6 | 62.6 | 62.6 | 62.6 | 62.6 | 62.6 |

Assigned by HSQC and HMBC spectra.

overnight at room temperature. After the reaction mixture was concentrated to dryness, the residual sugars were trimethylsilylated with pyridine (0.015 ml), hexamethyldisilazane (0.015 ml) and trimethylsilylchloride (0.015 ml) at 50 °C for 30 min. The supernatant of the mixture was analyzed by GC. GC condition: column, Supelco SPB™-1, 0.25 mm × 27 m, column temperature, 230 °C. D-Glucose was detected from **3**. D-Fucose was detected from **5**. D-Glucose and D-fucose were detected from **6**, **8**, **10**, **12**, **13** and **15**.

**References**


1) Yamamoto A., Miyase T., Ueno A., Maeda T., *Chem. Pharm. Bull.*, **41**, 1270—1274 (1993).
2) Yamamoto A., Suzuki H., Miyase T., Ueno A., Maeda T., *Phytochemistry*, **34**, 485—488 (1993).
3) Mori F., Miyase T., Ueno A., *Phytochemistry*, **36**, 1485—1488 (1994).
4) Ding J.-K., Fujino H., Kasai R., Fujimoto N., Tanaka O., Zhou J., Matsuura H., Fuwa T., *Chem. Pharm. Bull.*, **34**, 1158—1167 (1986).
5) *a*) Yamamoto A., Miyase T., Ueno A., Maeda T., *Chem. Pharm. Bull.*, **39**, 2764—2766 (1991); *b*) Tori K., Seo S., Yoshimura Y., Nakamura M., Tomita Y., Ishii H., *Tetrahedron Lett.*, **1976**, 4167—4170.
6) Ogihara Y., Nose M., *J. Chem. Soc., Chem. Commun.*, **1986**, 1417.


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