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Cycloartane glycosides from *Trichosanthes tricuspidata*

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Abstract

Three new cycloartane glycosides, named cyclotricuspidosides A, B and C, were isolated from the leaf and stem parts of *Trichosanthes tricuspidata*. The structure of the compounds have been determined as 28,31-di-*O*-β-D-glucopyranosides of 1α,3β,24ξ,31-tetrahydroxy-24ξ,-methyl-cycloartan-28-oic acid, 1α,3β,16β,24ξ,31-pentahydroxy-24ξ,-methylcycloartan-28-oic acid and 1α,3β,16α,24ξ,31-pentahydroxy-24ξ,-methylcycloartan-28-oic. These structural elucidations were based on analyses of chemical and spectroscopic data.

Keywords: *Trichosanthes tricuspidata*; Cucurbitaceae; Leaves and stems; Cycloartane glycosides; Cyclotricuspidosides A, B and C; Cyclotricuspidogenins A, B and C

1. Introduction

Trichosanthes species have been studied extensively and shown to contain a variety of substances, including triterpenoids and triterpenoidal glycosides (Kocor & Pyrek, 1973; Bhandari, 1983; Akihisa et al., 1988; Kitajima & Tanaka, 1989; Kitajima, Mukai, Masuda, & Tanaka, 1989), but no work has been reported on *T. tricuspidata* Lour. which is distributed in the Loochoo Islands, Japan (Hatusima & Amano, 1994).

As part of our ongoing study on the chemical constituents of cucurbitaceous plants (Kasai et al., 1987, 1988, 1989, 1990; Matsumoto, Kasai, Ohtani, & Tanaka, 1990; Fujita et al., 1995a, 1995b; Kubo et al., 1996), we have investigated this plant collected from Okinawa of the Loochoo islands. The methanol extract of the combined leaf and stem tissues afforded three new 24-hydroxymethylcycloartane glycosides, named cyclotricuspidosides A (**1**), B (**2**) and C (**3**). In this paper, we report the structural elucidation of these compounds.

2. Results and discussion

The molecular formula of glycoside **1** was determined as $C_{43}H_{72}O_{16}$ by ^{13}C NMR and HR-FAB mass spectrometry. On enzymatic hydrolysis with a mixture of crude naringinase and pectinase, glycoside **1** gave a new aglycone **1a**, $C_{31}H_{52}O_6$, named cyclotricuspidogenin A. The ^{13}C NMR spectrum of **1a** revealed 31 signals (Table 1): one carboxyl (δ 179.3), 11 methylene (one of them bearing an oxygen atom (δ 65.8)), seven methine (two of them bearing an oxygen atom (δ 72.9 and 71.2)), six quaternary (one of them bearing an oxygen atom (δ 75.8)) and six methyl carbons. Aglycone **1a** produced a monomethyl ester **1b** by methylation with CH_2N_2. The ^1H NMR spectrum of **1b** Table 2 showed two doublets at δ 0.75 and 0.50 (each $J=4$ Hz), which can be assigned to a methylene group in cyclopropane ring. These data can be accommodated on a homocycloartane triterpene having one primary, two secondary and one tertiary hydroxyl groups and one carboxyl group. The carbon signals of **1a** appeared at almost the same positions except for some resonances of the side chain as those of mollic acid (**4**), 1α,3β-dihydroxycycloart-24-en-28-oic acid, isolated from *Combretum* species (Pegel & Rogers,

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Table 1
^{13}C NMR spectral data for compounds **1**–**5**, **1a**–**3a**, **1b**–**3b** and **1c**–**3c** in pyridine-d_5

C	1	1a	4[a]	1b	1c	2	2a	5[b]	2b	2c	3	3a	3b	3c
1	72.3	72.9	72.8	72.3	72.2	72.2	72.8	32.5	72.2	72.2	72.3	72.7	72.2	72.1
2	38.3	38.0	38.5	38.6	38.6	38.2	38.2	31.3	38.6	38.6	38.2	38.4	38.6	38.6
3	71.7	71.2	70.4	70.5	71.6	71.6	71.7	78.0	70.5	71.7	71.8	71.1	70.5	71.6
4	56.3	55.4	55.7	56.0	56.0	56.3	55.5	41.1	56.0	56.0	56.4	55.5	56.1	56.0
5	37.6	37.9	37.7	37.3	37.9	37.6	37.4	47.6	38.0	37.9	37.6	37.4	38.0	37.9
6	23.1	23.2	23.4	23.3	23.3	22.9	22.8	21.4	23.3	23.2	23.1	23.3	23.4	23.3
7	25.6	26.0	25.9	25.8	25.7	25.8	25.9	26.3	25.9	25.9	25.9	26.2	26.1	26.0
8	48.2	48.4	48.2	48.1	48.1	48.2	48.5	48.2	48.3	48.2	48.3	48.3	48.3	48.2
9	20.8	20.7	21.1	20.8	20.8	20.8	20.7	20.0	20.9	20.9	20.4	20.4	20.3	20.3
10	30.0	30.3	30.5	30.0	30.1	30.1	29.9	26.8	30.0	29.9	30.2	30.2	30.2	29.9
11	26.1	26.0	26.5	26.1	26.1	25.7	26.1	26.5	25.9	25.9	26.3	26.2	26.2	26.2
12	33.2	33.3	36.9	33.2	33.1	33.1	33.2	33.3	33.2	33.1	33.2	33.1	33.1	33.1
13	45.4	45.4	45.8	45.5	45.4	45.5	45.5	45.8	45.6	45.6	47.8	47.8	47.9	47.8
14	49.0	49.1	49.3	49.1	48.1	47.0	47.1	47.1	47.2	47.0	47.0	47.0	47.0	46.9
15	35.8	35.6	33.5	35.9	35.8	48.9	49.0	48.8	49.0	48.9	48.3	48.5	48.4	48.4
16	28.4	28.3	28.4	28.4	28.4	71.5	71.6	72.0	71.8	71.5	77.0	77.1	77.0	77.0
17	52.7	52.7	52.8	52.8	52.7	57.2	57.3	57.5	57.3	57.2	61.7	60.7	60.8	61.7
18	18.7	18.5	18.3	18.4	18.3	18.4	18.6	18.3	18.7	18.4	19.2	19.1	19.1	19.1
19	30.0	29.8	29.9	29.7	29.8	30.5	30.4	30.3	30.2	30.5	30.5	30.1	30.2	30.2
20	37.3	37.2	36.3	37.9	37.3	33.3	33.3	28.7	33.5	33.3	35.9	35.0	35.2	35.9
21	19.4	19.4	19.6	19.5	19.4	19.4	19.4	19.4	19.5	19.5	19.4	19.4	19.4	19.4
22	29.9	30.2	36.3	30.1	29.7	30.1	30.4	33.1	31.5	30.1	29.5	29.0	29.4	29.4
23	31.9	31.7	25.5	31.8	31.9	32.0	32.0	27.9	32.0	32.1	32.2	30.4	30.5	32.2
24	75.5	75.8	125.9	75.8	75.3	75.7	76.3	77.2	76.3	75.7	75.6	75.9	75.9	75.3
25	33.2	33.3	130.8	33.5	33.2	31.7	31.8	72.5	32.1	31.7	33.4	34.0	34.0	33.2
26	17.3	17.4	25.9	17.5	17.3	17.3	17.6	26.2	17.7	17.4	17.4	17.5	17.6	17.3
27	17.5	17.6	17.8	17.6	17.4	17.2	17.2	25.6	17.4	17.2	17.4	17.7	17.7	17.4
28	176.7	179.3	179.9	178.2	178.1	176.6	179.5	26.5	178.1	178.1	176.6	179.3	178.1	178.1
29	9.7	10.1	9.6	9.5	9.1	9.6	10.1	14.8	9.5	9.4	9.6	10.0	9.5	9.5
30	18.4	18.3	18.7	18.7	18.6	20.2	20.3	20.3	20.3	20.2	20.4	20.4	20.4	20.3
31	75.1	65.8		65.9	75.1	75.0	66.2		66.3	75.1	75.1	65.8	65.9	75.1
OMe				51.4	51.4				51.4	51.4			51.4	51.4
G-1	106.0				106.0	105.5			105.6	105.6				105.7
G-2	75.4				75.5	75.2			75.3	75.8				75.7
G-3	78.6				78.5	78.5			78.6	78.5				78.5
G-4	70.7				70.5	70.6			70.5	70.7				70.5
G-5	78.6				78.5	78.5			78.6	78.4				78.5
G-6	62.7				62.7	62.5			62.6	62.8				62.7
G-1′	96.4					96.4				96.4				
G-2′	74.7					74.6				74.6				
G-3′	79.6					79.5				79.4				
G-4′	70.7					70.9				71.1				
G-5′	78.4					78.4				78.4				
G-6′	62.0					62.0				62.2				

[a] Data from Rogers (1989).
[b] Data from Ganenko et al. (1986).

1976; Rogers, 1989). This suggested that the only structural difference between **1a** and **4** is in the side chain, which is composed of one hydroxylated quaternary, one hydroxymethyl, two methine, two methylene and three secondary methyl carbons for **1a**. The connectivities between these carbons were corroborated by the H–H COSY and HMBC (Fig. 1) measurements of **1b**. The data revealed the structure of the side chain as shown and simultaneously supported the structure of the A–D-ring system of **1a**. Thus, the structure of aglycone **1a** was determined as 1α,3β,24ξ,31-tetrahydroxy-24ξ,-methylcycloartan-28-oic acid. For cycloartane triterpenoids, several numbering systems are in use for the carbon skeleton. This paper followed the numbering system by Connolly and Hill (1991). At the present time, the configuration at C-24 is uncertain.

Glucose was identified in the acid hydrolysate of glycoside **1**. The ^{1}H and ^{13}C NMR spectra of **1** demonstrated the presence of two β-glucopyranosyl units, one of which was identified as an ester-linked glucose from its characteristic chemical shifts of anomeric proton (δ 6.49) and carbon (δ 96.4) signals. Alkaline hy-

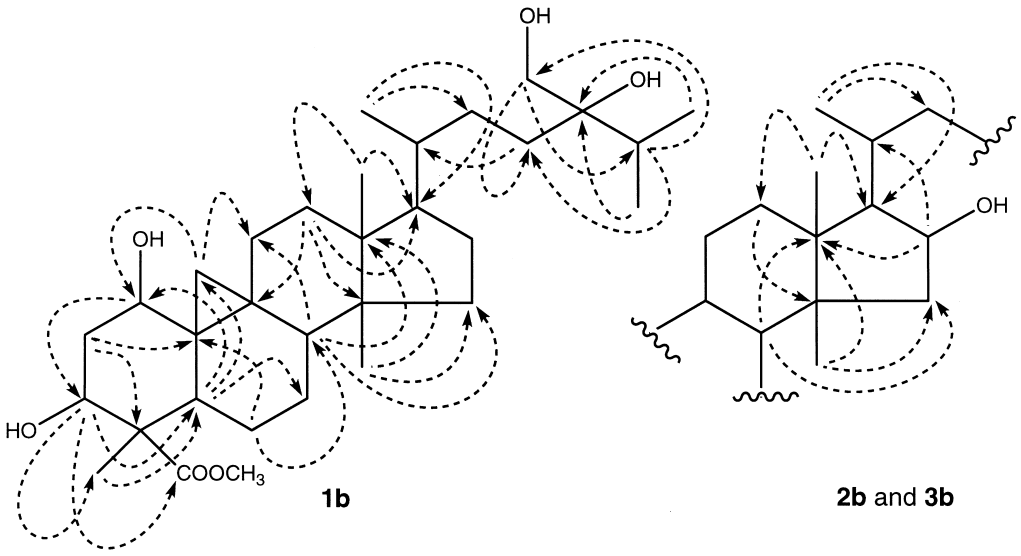
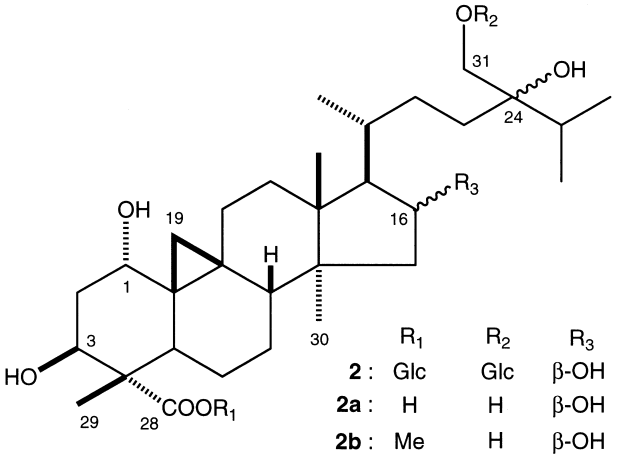


Fig. 1. HMBC correlations for **1b**, **2b** and **3b**. 3-bond couplings from ^{1}H to ^{13}C indicated by an arrow.

drolysis of glycoside **1** in methanol yielded a progenin methyl ester **1c** having one β-glucopyranosyl unit. In going from aglycone methyl ester **1b** to **1c**, the carbon signal due to the hydroxymethyl group (C-31) was significantly shifted downfield by 9.2 ppm Table 1. This suggested that another glucose is allocated to C-31 of **1**. It is obvious that these two glucose units have D-configurations by the fact that glycoside **1** was enzymatically hydrolysed (vide supra). On the basis of these data, the structure of glycoside **1** was established as 28,31-di-*O*-β-D-glucopyranoside of **1a**.

On enzymatic hydrolysis with a mixture of crude naringinase and pectinase, glycosides **2** and **3** afforded new aglycones **2a** and **3a**, named cyclotricuspidogenins B and C, respectively. The molecular formula of **2a** and **3a** was determined as $C_{31}H_{52}O_7$. The ^{1}H and ^{13}C NMR data (Table 1Table 2) of their methyl esters **2b** and **3b** resembled those of **1b**, but the signals at δ_C 71.8 and δ_H 4.67 of **2b** and δ_C 77.0 and δ_H 4.28 of **3b** indicated the presence of an additional secondary hydroxyl group at C-15 or C-16 in both compounds. The HMBC data of **2b** and **3b** (Fig. 1) confirmed the position of the hydroxyl group on C-16. The carbon signals due to the D-ring of **2a** were essentially superimposable on those of cyclofoetigenin A (**5**), cycloartane-3β,16β,24(*S*),25-tetrol, isolated from *Thalictrum foetidum* (Ganenko et al., 1986). On the basis of the above spectral analysis, the structure of **2a** and **3a** were characterized as 1α,3β,16β,24ξ,31-pentahydroxy-24ξ,-methylcycloartan-28-oic acid and its 16α-hydroxy epimer, respectively.

The ^{1}H and ^{13}C NMR spectra of **2** and **3** showed the presence of two β-glucopyranosyl units. The linkages of these two sugars in both glycosides were also determined to be C-28 and C-31 by similar means to those described above in the case of **1**. Thus, the structure of glycosides **2** and **3** was characterized as shown.



	R_1	R_2	R_3
1 :	Glc	Glc	H
1a :	H	H	H
1b :	Me	H	H
1c :	Me	Glc	H

	R_1	R_2	R_3
2 :	Glc	Glc	β-OH
2a :	H	H	β-OH
2b :	Me	H	β-OH
2c :	Me	Glc	β-OH
3 :	Glc	Glc	α-OH
3a :	H	H	α-OH
3b :	Me	H	α-OH
3c :	Me	Glc	α-OH

3. Experimental

3.1. General

NMR: 400 MHz for ^{1}H, 100 MHz for ^{13}C, in pyridine-d_5, TMS as int. standard. CC: silica gel (Kieselgel 60, 70–230 mesh, Merck) and styrene-divinylbenzene copolymer resin (Diaion HP-20, Mitsubishi Kasei Ind., Japan) were used. All solvent systems for chromatog-

Table 2
^{1}H NMR spectral data for compounds **1b**, **2b** and **3b** in pyridine-d_5

H	1b	2b	3b
1	3.86 dd (3, 3)	3.85 brs	3.85 dd (3, 3)
2a	2.42 ddd (3, 4, 13)	2.41 ddd (4, 4, 12)	2.42 ddd (3, 5, 13)
2b	2.20 ddd (3, 12, 13)	2.22[a]	2.20 ddd (3, 12, 13)
3	5.37 dd (4, 12)	5.35 dd (4, 12)	5.36 dd (5, 12)
5	3.23 dd (4, 13)	3.22 dd (4, 13)	3.24 dd (5, 13)
6a	1.40[a]	1.37[a]	1.39[a]
6b	1.13[a]	1.13[a]	1.11 dd (3, 13)
7a	1.27[a]	1.28[a]	1.31[a]
7b	1.27[a]	1.28[a]	1.27[a]
8	1.56 dd (5, 12)	1.58[a]	1.53 dd (5, 12)
11a	2.70 ddd (7, 10, 16)	2.72 ddd (7, 10, 16)	2.82 ddd (6, 11, 15)
11b	1.44[a]	1.44[a]	1.41 ddd (5, 5, 14)
12a	1.70[a]	1.72[a]	1.85[a]
12b	1.62[a]	1.72[a]	1.68[a]
15a	1.22[a]	2.06 dd (8, 12)	1.92[a]
15b	1.22[a]	1.70[a]	1.68[a]
16a	1.98[a]	4.67 ddd (5, 8, 8)	4.28 dd (6, 8)
16b	1.29[a]		
17	1.67 dd (8, 11)	1.81 dd (8, 11)	2.05 dd (6, 10)
18	0.96 s	0.99 s	1.07 s
19a	0.75 d (4)	0.76 d (4)	0.74 d (5)
19b	0.50 d (4)	90.50 d (4)	0.51 d (5)
20	[b]	2.20[a]	1.78[a]
21	0.98 d (6)	1.09 d (6)	1.04 d (6)
22a	1.94[a]	[b]	1.98[a]
22b	1.32[a]	1.30[a]	1.32[a]
23a	2.00[a]	2.07[a]	2.16[a]
23b	1.82[a]	1.95 ddd (5, 5, 13)	1.87[a]
25	2.26 qq (7)	2.26 qq (7)	2.26 qq (7)
26	1.20 d (7)	1.16 d (7)	1.18 d (7)
27	1.20 d (7)	1.16 d (7)	1.20 d (7)
29	160 s	1.58 s	1.59 s
30	1.01 s	1.41 s	1.39 s
31a	4.02 d (11)	3.99 d (11)	4.03 d (11)
31b	3.95 d (11)	3.95 d (11)	3.96 d (11)
OMe	3.63 s	3.63 s	3.65 s

[a] Overlapping signals, determined approximately by ^{1}H–^{1}H and ^{13}C–^{1}H COSY spectral data.
[b] Obscured.

raphy were homogeneous. MPLC: ODS-A-40B (26 mm × 30 cm, Yamazen, Japan).

3.2. Plant material

Aerial parts of *T. tricuspidata* were collected at Okinawa island, Japan, in January 1996 and identified by Dr. M. Yokota, Department of Biology, University of the Ryukyus. A voucher specimen is kept in the Pharmaceutical Sciences, Hiroshima University School of Medicine.

3.3. Extraction and separation

Dried and powdered aerial parts of *T. tricuspidata* (530 g) were extracted with hot MeOH. After removal of the solvent by evapn, the MeOH extract (86 g) was chromatographed on a column of silica gel with EtOAc–MeOH–H_2O (8:2:1) to give 10 frs. Fr. 7 (7.7 g) was further chromatographed on a column of silica gel with EtOAc–MeOH–H_2O (10:2:1) to give three frs 7-1, -2 and -3. Fr. 7-3 (6.9 g) was subjected to CC on styrene–divinylbenzene copolymer resin with H_2O, MeOH and Me_2CO, successively. The MeOH eluate (3.5 g) was purified by chromatography on silica gel with EtOAc–MeOH–H_2O (8:2:1) and then MPLC with 50–60% aq. MeOH to afford **1** (678 mg), **2** (310 mg) and **3** (520 mg).

3.4. Cyclotricuspidoside A (*1*)

$[\alpha]_D^{23}$ +24.5° (MeOH; *c* 1.0). HRFABMS (positive) *m/z*: 867.4719 $[M+Na]^+$ ($C_{43}H_{72}O_{16}Na$, requires 867.4717). ^{1}H NMR: δ 6.49 (1H, d, $J=8$ Hz, 28-Glc-1), 5.56 (1H, dd, $J=5$, 12 Hz, H-3), 4.96 (1H, d, $J=8$ Hz, 31-Glc-1), 3.35 (1H, dd, $J=5$, 12 Hz, H-5), 1.66 (3H, s, H-29), 1.10 (3H, d, $J=7$ Hz, H-27), 1.08 (3H, d, $J=7$ Hz, H-26), 0.95 (3H, s, H-30), 0.94 (3H, d, $J=6$ Hz, H-21), 0.84 (3H, s, H-18), 0.71 (1H, d, $J=5$ Hz, H-19a), 0.50 (1H, d, $J=5$ Hz, H-19b).

3.5. Cyclotricuspidogenin A (*1a*)

Glycoside **1** (100 mg), crude naringinase (100 mg) and crude pectinase (100 mg) were dissolved in sterilized H_2O (10 ml) in a flask equipped with a stopper. After the addition of a few drops of toluene, the soln was incubated at 37°C for 6 days. The reaction mixt. was evapd and the residue was purified by CC on silica gel using CH_2Cl_2–MeOH (10:1) to give **1a** (39 mg): $[\alpha]_D^{18}$ +35.1° (pyridine; *c* 2.1). HRFABMS (negative) *m/z*: 519.3687 $[M-H]^-$ ($C_{31}H_{51}O_6$, requires 519.3686).

3.6. Methyl ester of *1a* (*1b*)

Aglycone **1a** (30 mg) was methylated with ethereal CH_2N_2 in MeOH and then purified on a silica gel column with CH_2Cl_2–MeOH (20:1) to give **1b** (20 mg).

3.7. Alkaline hydrolysis of *1*

A soln of **1** (100 mg) in 1% NaOH in MeOH (5 ml) was kept at room temp. overnight. The reaction mixt. was acidified with 10% HCl, and then extracted with EtOAc. After washing with water, the EtOAc extract was purified by CC on silica gel with EtOAc–MeOH (8:1) to give **1c** (20 mg): $[\alpha]_D^{23}$ +35.4° (MeOH; *c* 1.0). HRFABMS (negative) *m/z*: 695.4392 $[M-H]^-$ ($C_{38}H_{63}O_{11}$, requires 695.4370). ^{1}H NMR: δ 5.36 (1H, dd, $J=4$, 12 Hz, H-3), 4.97 (1H, *d*, $J=8$ Hz, Glc-1), 3.63 (3H, s, COOMe), 3.23 (1H, dd, $J=4$, 12 Hz, H-5), 1.60 (3H, s, H-29), 1.12 (3H, d, $J=7$ Hz, H-27), 1.09 (3H, d, $J=7$ Hz, H-26), 0.98 (3H, s, H-30), 0.95

(3H, s, H-18), 0.94 (3H, d, $J=4$ Hz, H-21), 0.73 (1H, d, $J=4$ Hz, H-19a), 0.49 (1H, d, $J=4$ Hz, H-19b).

3.8. Cyclotricuspidoside B (2)

$[\alpha]_D^{23}$ +35.9° (MeOH; c 1.0). HRFABMS (positive) *m/z*: 861.4848 $[M+H]^+$ ($C_{43}H_{73}O_{17}$, requires 861.4848). ^{1}H NMR: δ 6.48 (1H, d, $J=8$ Hz, 28-Glc-1), 5.56 (1H, dd, $J=5$, 12 Hz, H-3), 4.92 (1H, d, $J=8$ Hz, 31-Glc-1), 4.65 (1H, m, H-16), 3.86 (1H, m, H-1), 3.35 (1H, dd, $J=4$, 12 Hz, H-5), 2.72 (1H, m, H-11a), 2.42 (1H, m, H-2a), 1.66 (3H, s, H-29), 1.36 (3H, s, H-18), 1.11 (3H, d, $J=6$ Hz, H-27), 1.09 (3H, d, $J=6$ Hz, H-26), 1.05 (3H, d, $J=6$ Hz, H-21), 0.89 (3H, s, H-30), 0.75 (1H, d, $J=4$ Hz, H-19a), 0.51 (1H, d, $J=4$ Hz, H-19b).

3.9. Cyclotricuspidogenin B (2a)

Glycoside **2** (100 mg) was hydrolysed with a mixture of crude naringinase (100 mg) and crude pectinase (100 mg) and then purified by the same procedure as in the case of **1** to afford **2a** (38 mg): $[\alpha]_D^{23}$ +17.0° (MeOH; *c* 0.8). HRFABMS (negative) *m/z*: 535.3638 $[M-H]^-$ ($C_{31}H_{51}O_7$, requires 535.3635).

3.10. Alkaline hydrolysis of 2

Glycoside **2** (198 mg) was hydrolysed with alkali by the same method as that of **1** to give **2c** (63 mg): $[\alpha]_D^{23}$ +16.6° (MeOH, *c* 1.0). HRFABMS (negative) *m/z*: 711.4333 $[M-H]^-$ ($C_{38}H_{63}O_{12}$), requires 711.4319). ^{1}H NMR: δ 5.34 (1H, dd, $J=4$, 12 Hz, H-3), 4.90 (1H, d, $J=8$ Hz, 31-Glc-1), 4.67 (1H, m, H-16), 3.80 (1H, m, H-1), 3.62 (3H, s, COOMe), 3.21 (1H, dd, $J=4.4$, 12.5 Hz, H-5), 2.70 (1H, m, H-2a), 1.57 (3H, s, H-29), 1.37 (3H, s, H-18), 1.09 (3H, d, $J=6$ Hz, H-27), 1.07 (3H, d, $J=6$ Hz, H-26), 1.04 (3H, d, $J=7$ Hz, H-21), 0.97 (3H, s, H-30), 0.74 (1H, d, $J=4$ Hz, H-19a), 0.48 (1H, d, $J=4$ Hz, H-19b).

3.11. Methyl ester of 2a (2b)

Progenin **2c** (63 mg) was hydrolysed with crude naringinase (100 mg) in H_2O (10 ml) at 37°C for 4 days. The reaction mixt. was subjected to a column of styrene–divinylbenzene copolymer resin with H_2O and then MeOH to give **2b** (20 mg).

3.12. Cyclotricuspidoside C (3)

$[\alpha]_D^{23}$ +12.1° (MeOH; *c* 1.0). HRFABMS (positive) *m/z*: 861.4847 $[M+H]^+$ ($C_{43}H_{73}O_{17}$, requires 861.4848). ^{1}H NMR: δ 6.42 (1H, d, $J=8$ Hz, 28-Glc-1), 5.51 (1H, dd, $J=5$, 12 Hz, H-3), 4.86 (1H, d, $J=8$ Hz, 31-Glc-1), 3.30 (1H, dd, $J=5$, 13 Hz, H-5), 1.63 (3H, s, H-29), 1.28 (3H, s, H-18), 1.10 (3H, d, $J=7$ Hz, H-27), 1.06 (3H, d, $J=7$ Hz, H-26), 1.02 (3H, s, H-30), 1.01 (3H, d, $J=6$ Hz, H-21), 0.72 (1H, d, $J=4$ Hz, H-19a), 0.50 (1H, d, $J=4$ Hz, H-19b).

3.13. Cyclotricuspidogenin C (3a)

Glycoside **3** (100 mg) was hydrolysed with a mixture of crude hesperidinase and crude pectinase to afford **3a** (52 mg): $[\alpha]_D^{23}$ +28.2° (MeOH; *c* 1.0). HRFABMS (negative) *m/z*: 535.3637 $[M-H]^-$ ($C_{31}H_{51}O_7$, requires 535.3635).

3.14. Methyl ester of 3a (3b)

Aglycone **3a** (52 mg) was methylated with CH_2N_2 to give **3b** (30 mg).

3.15. Alkaline hydrolysis of 3

Glycoside **3** (100 mg) was hydrolysed with alkali to give **3c** (37 mg): $[\alpha]_D^{23}$ +17.9° (MeOH, *c* 1.1). HRFABMS (negative) *m/z*: 711.4353 $[M-H]^-$ ($C_{38}H_{63}O_{12}$, requires 711.4319). ^{1}H NMR: δ 5.37 (1H, dd, $J=4$, 12 Hz, H-3), 4.91 (1H, d, $J=8$ Hz, Glc-1), 3.63 (3H, s, COOMe), 3.24 (1H, dd, $J=4$, 12 Hz, H-5), 1.60 (3H, s, H-29), 1.40 (3H, s, H-18), 1.11 (3H, d, $J=7$ Hz, H-27), 1.10 (3H, d, $J=7$ Hz, H-26), 1.07 (3H, s, H-30), 1.06 (3H, d, $J=6$ Hz, H-21), 0.50 (1H, d, $J=4$ Hz, H-19a), 0.73 (1H, d, $J=4$ Hz, H-19b).

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