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CARDENOLIDE GLYCOSIDES FROM SEEDS OF *CORCHORUS OLITORIUS*

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Key Word Index—*Corchorus olitorius*; Tiliaceae; tossa jute; cardenolide glycoside; boivinose; digitoxose; cannogenol; periplogenin; digitoxigenin; NMR.

Abstract—Three new cardenolide glycosides were isolated from the seeds of *Corchorus olitorius* L. On the basis of chemical and spectroscopic evidence, their structures were established as cannogenol 3-*O*-*β*-D-glucopyranosyl-(1 → 4)-*O*-*β*-D-boivinopyranoside, periplogenin 3-*O*-*β*-D-glucopyranosyl-(1 → 4)-*O*-*β*-D-digitoxopyranoside and digitoxigenin 3-*O*-*β*-D-glucopyranosyl-(1 → 6)-*O*-*β*-D-glucopyranosyl-(1 → 4)-*O*-*β*-D-digitoxopyranoside. © 1998 Elsevier Science Ltd. All rights reserved

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INTRODUCTION

Cardenolide glycosides of the *Corchorus* (Tiliaceae) species have already been reported; erysimoside, olitoriside, corchoroside A and coroloside were isolated as constituents of the seeds of tossa jute (*C. olitorius*) [1–3]. Helveticoside was also isolated from autofermented seeds [4, 5] and deglucocoroloside was detected as an artifact by hydrolysis of the seed extract [2]. In this paper, we describe the isolation and structural determination of eleven cardenolide glycosides, which involve eight known compounds including the above six and three new ones from ripe seeds.

RESULTS AND DISCUSSION

The ripe seeds of *C. olitorius* were extracted with MeOH after being delipidated and the extract was fractionated by partitioning and subsequent column chromatography to give a cardenolide fraction. This fraction was subjected to MPLC (medium pressure liquid chromatography) and subsequent preparative HPLC. Consequently, we obtained eight known compounds (**1**–**4**, **8**–**11**) and three new ones (**5**–**7**).

Compounds **1**–**4** and **8**–**11** were identified by chemical evidence and spectral analyses as erysimoside (strophanthidin 3-*O*-*β*-D-glucopyranosyl-(1 → 4)-*O*-*β*-D-digitoxopyranoside) (**1**), olitoriside (strophanthidin 3-*O*-*β*-D-glucopyranosyl-(1 → 4)-*O*-*β*-D-boivinopyranoside) (**2**), corchoroside A (strophanthidin 3-*O*-*β*-D-boivinopyranoside) (**3**), helveticoside (strophanthidin 3-*O*-*β*-D-digitoxopyranoside) (**4**), coroloside (digitoxigenin 3-*O*-*β*-D-glucopyranosyl-(1 → 4)-*O*-*β*-D-boivinopyranoside) (**8**), glucoevatromonoside (digitoxigenin 3-*O*-*β*-D-glucopyranosyl-(1 → 4)-*O*-*β*-D-digitoxopyranoside) (**9**), deglucocoroloside (digitoxigenin 3-*O*-*β*-D-boivinopyranoside) (**10**) and evatromonoside (digitoxigenin 3-*O*-*β*-D-digitoxopyranoside) (**11**). This is the first reported isolation of **4**, **9**, **10** and **11** as natural constituents from this plant.

In the high resolution negative ion FAB mass spectrum, **5** showed a $[M\text{–}H]^-$ ion peak at *m*/*z* 681.3436 ($C_{35}H_{53}O_{13}$). The fragment ion peaks of low resolution FABMS, *m*/*z* 519 for $[M\text{–}H\text{–}162]^-$ and 389 for $[\text{aglycone–H}]^-$, were observed. Compared with the spectra of **8** and **9**, the aglycone of **5** had 16 more mass units from digitoxigenin. This suggested that **5** had one extra hydroxyl group in the aglycone. In the ^1H and ^{13}C NMR spectra, **5** had spectral data for the sugar moiety very similar to those of **2** and **8**. It appeared that **5** contained the *β*-D-glucopyranosyl-(1 → 4)-*O*-*β*-D-boivinopyranoside moiety. The C-19 signal of **5** was observed at δ 66.0 shifted by +41.7 ppm from that of digitoxigenin, the aglycone of **8**. Also the signals of C-1 (δ 24.8, –6.6 ppm), C-5 (δ 30.2, –7.7 ppm) and C-10 (δ 40.4, –4.1 ppm) were significantly shifted (see Table 1). On the basis of the ^1H NMR, H–H COSY and HMQC spectra, two protons at the 19-position were assigned at δ 3.41 and 3.81 (each

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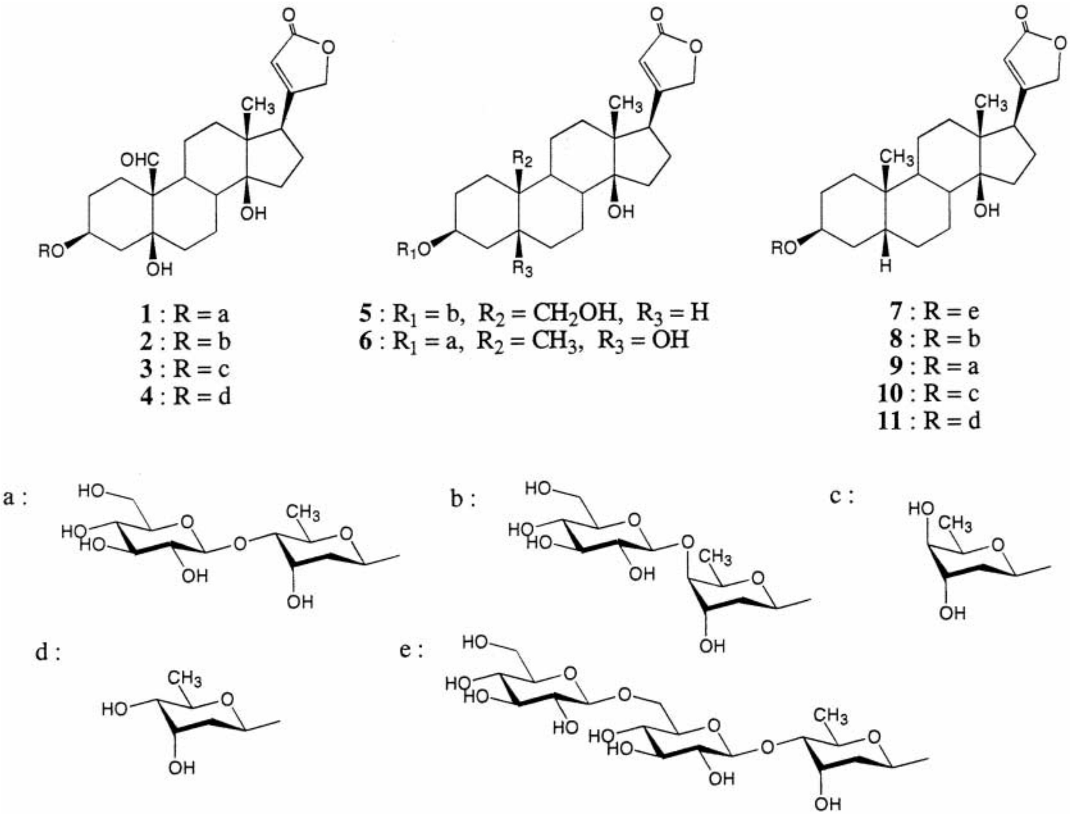

1 : R = a
2 : R = b
3 : R = c
4 : R = d

5 : R_1 = b, R_2 = CH_2OH, R_3 = H
6 : R_1 = a, R_2 = CH_3, R_3 = OH

7 : R = e
8 : R = b
9 : R = a
10 : R = c
11 : R = d

1H, *d*, J = 11 Hz). These data indicated that the aglycone was cannogenol which had one hydroxyl group at C-19 and the remainder of the signals were at the position given in the literature [6]. As the result, it was decided that the structure of **5** was cannogenol 3-*O*-*β*-D-glucopyranosyl-(1 → 4)-*O*-*β*-D-boivinopyranoside.

Compound **6** had the same molecular weight as **5** and was also 16 mass units more than **8** and **9** at the aglycone part in the FABMS data. In comparison with the ^{13}C NMR spectrum of **9**, the chemical shifts of the C-4 (δ 35.6), C-5 (δ 75.7) and C-6 (δ 35.9) positions were +4.2, +37.8 and +8.1 ppm, respectively (Table 1). This suggested that the hydroxyl group was attached to C-5 on digitoxigenin. Namely, the aglycone was identified to be periplogenin [7]. On the other hand, spectral data of **6** for the sugar moiety was very similar to those of **1** and **9** for the ^{1}H and ^{13}C NMR data. Therefore, it was revealed that **6** had *β*-D-glucopyranosyl-(1 → 4)-*O*-*β*-D-digitoxopyranoside for the sugar moiety. Consequently, the structure of **6** was established as periplogenin 3-*O*-*β*-D-glucopyranosyl-(1 → 4)-*O*-*β*-D-digitoxopyranoside.

The molecular formula of **7** was $C_{41}H_{64}O_{17}$ as determined by FABMS. The fragment ion peaks of **7** were observed at *m*/*z* 665 for $[M–H–162]^-$, 503 for $[M–H–162 \times 2]^-$ and 373 for $[\text{aglycone–H}]^-$. The ^{1}H NMR spectrum of compound **7** displayed the presence of three anomeric protons at δ 4.35 (1H, *d*, J = 8.0 Hz), δ 4.39 (1H, *d*, J = 8.0 Hz) and δ 5.00 (1H, *d*, J = 2.0, 9.5 Hz). It was presumed that **7** had one more sugar from **9**. Comparing the ^{1}H and ^{13}C NMR data of the sugar moiety with those of strophanthidin 3-*O*-*β*-D-glucopyranosyl-(1 → 6)-*O*-*β*-D-glucopyranosyl-(1 → 4)-*O*-*β*-D-digitoxopyranoside in the literature [1], they were equal. Hence, it was considered that **7** and the reference compound had the same sugar moiety. The aglycone of **7** was identified to be digitoxigenin by comparing the NMR data with those of **8**–**11**. Other 2D NMR spectra also supported the following structure. Consequently, the structure of **7** was determined to be digitoxigenin 3-*O*-*β*-D-glucopyranosyl-(1 → 6)-*O*-*β*-D-glucopyranosyl-(1 → 4)-*O*-*β*-D-digitoxopyranoside.

EXPERIMENTAL

General

Optical rotation: JASCO JASCO DIP-370 digital polarimeter. CD: J-720 spectropolarimeter. ^{1}H and ^{13}C NMR: JEOL A-600 and A-500, methanol-d_4, TMS as an internal standard. FAB-MS: JEOL JMS SX-102, glycerol as the matrix, negative ion mode.

Extraction and separation

The ripe seeds (381.9 g) of *C. olitorius* were powdered, delipidated with petroleum ether and

Table 1. ^{13}C-NMR chemical shifts for cardenolide glycosides in methanol-d_4

	$1^{\dagger}$	$2^{\dagger}$	3*	4*	$5^{\dagger}$	$6^{\dagger}$	$7^{\dagger}$	$8^{\dagger}$	$9^{\dagger}$	10*	$11^{\dagger}$
Aglycone											
1	25.2	25.2	25.1	25.1	24.8	26.6	31.5	31.4	31.4	31.4	31.4
2	25.9	26.0	25.9	25.9	27.2	26.8	27.6	27.5	27.5	27.6	27.5
3	76.3	76.4	76.0	76.2	73.7	77.3	74.6	74.3	74.4	74.2	74.49^{7}
4	36.8	36.9	36.8	36.7	30.8	35.6	31.1	31.0	31.0	31.1	31.0
5	75.3^{1}	75.4^{2}	75.30^{3}	75.30^{5}	30.2	75.7	38.0	37.9	37.9	38.0	38.0
6	37.2	37.2	37.1	37.1	27.4	35.9	27.9	27.9	27.8	27.9	27.9
7	23.3	23.3	23.2	23.2	22.4	24.8	22.6	22.6	22.5	22.6	22.6
8	42.6	42.7	42.5	42.6	42.5	41.7	42.8	42.7	42.7	42.7	42.7
9	40.4	40.5	40.4	40.4	36.5	40.2	36.9	36.9	36.9	36.9	36.9
10	56.1	56.2	56.1	56.1	40.4	41.9	36.4	36.3	36.3	36.3	36.3
11	19.0	19.0	18.9	18.9	22.1	22.7	22.4	22.3	22.3	22.4	22.4
12	40.5	40.6	40.5	40.5	41.3	41.0	41.0	41.0	41.0	41.0	41.0
13	50.7	50.8	50.7	50.7	51.0	50.9	51.1	51.0	51.0	51.1	51.1
14	85.9	86.0	85.9	85.9	86.4	86.3	86.5	86.4	86.4	86.5	86.5
15	32.5	32.5	32.4	32.4	33.1	33.4	33.5	33.4	33.4	33.4	33.4
16	27.9	28.0	27.9	27.9	28.0	28.0	28.1	28.1	28.0	28.0	28.0
17	51.8	51.8	51.7	51.7	52.1	52.0	52.2	52.1	52.1	52.2	52.1
18	16.2	16.2	16.1	16.1	16.4	16.3	16.4	16.4	16.4	16.4	16.4
19	210.2	210.0	210.1	210.0	66.0	17.3	24.3	24.3	24.3	24.3	24.3
20	178.1	178.2	178.1	178.1	178.3	178.4	178.5	178.4	178.4	178.5	178.5
21	75.2^{1}	75.3^{2}	75.27^{3}	75.26^{5}	75.3	75.4	75.4	75.3	75.3	75.4	75.4
22	117.9	118.0	117.8	117.9	117.7	117.9	117.8	117.7	117.7	117.7	117.8
23	177.2	177.2	177.2	177.2	177.2	177.3	177.3	177.3	177.2	177.3	177.3
Sugar I											
1	98.2	99.2	99.0	98.3	97.4	98.1	97.0	97.6	96.8	97.6	97.0
2	38.9	35.1	34.7	39.6	35.2	39.0	38.9	35.2	39.0	35.1	39.9
3	68.4	66.7	70.29^{4}	69.0	66.5	68.4	68.2	66.6	68.5	70.5	69.3
4	84.0	76.0	70.9	74.2	76.0	84.0	84.2	75.9	84.2	71.2	74.46^{7}
5	69.8	70.3	70.35^{4}	70.9	70.0	69.8	69.7	70.0	69.6	70.2	70.8
6	18.6	17.3	16.9	18.5	17.2	18.6	18.6	17.2	18.6	16.9	18.6
Sugar II											
1	105.8	102.5			102.2	105.8	105.2	102.1	105.7		
2	75.1	75.0			74.9	75.1	75.1	74.9	75.0		
3	77.9	78.0			77.9	78.0	78.0^{6}	77.8	77.8		
4	71.2	72.0			71.9	71.2	71.68	71.8	71.1		
5	77.7	78.2			78.1	77.8	76.8	78.0	77.7		
6	62.3	63.1			63.0	62.4	70.2	63.0	62.3		
Sugar III											
1							104.9				
2							75.2				
3							78.1^{6}				
4							71.72				
5							78.1^{6}				
6							62.9				

*Measured at 150 MHz (JEOL A-600).

†Measured at 125 MHz (JEOL A-500).

Assignments were confirmed by HMQC and HMBC analyses.

$^{1-7}$Assignments can be inverted.

extracted with MeOH. The extract (41.3 g) was partitioned between *n*-hexane and aqueous MeOH. The dried residue of the aqueous MeOH layer (26.4 g) was concentrated and partitioned again between *n*-BuOH and water. The dried residue of the BuOH layer (7.5 g) was subjected to CC over HP-20, eluting with 50 and 100% MeOH, respectively. The dried residue of the MeOH elutant (1.55 g) was subjected to CC over LH-20, eluted with 50% MeOH to provide 3 frs (fr. 1–3). Fr. 2 was chromatographed on MPLC using an ODS column (C.I.G. pre-packed column C_{18}-20, 22 × 100 mm, Kusano) to give 11 frs. and subsequent preparative HPLC (STR PREP-ODS H, 20 × 250 mm, Shimadzu) eluted 50% MeOH to give **1** (26.1 mg), **2** (17.6 mg), **3** (22.7 mg), **4** (58.2 mg), **5** (6.5 mg), **6** (3.4 mg), **7** (6.7 mg), **8** (54.7 mg), **9** (50.4 mg), **10** (1.2 mg) and **11** (2.4 mg).

Erysimoside (**1**) *and olitoriside* (**2**)

White amorphous powders. ^{13}C NMR: see Table 1. Spectroscopic data of **1** and **2** have been reported [1, 8].

Corchoroside A (**3**)

White amorphous powder. FABMS *m*/*z*: 533 $[M-H]^-$, 403 $[aglycone-H]^-$. CD (MeOH, nm, rel.int.): 245.5 (+13.0), 306 (−1.4). ^{1}H NMR: δ 0.84 (3H, *s*, H_3-18), 1.22 (3H, *d*, $J = 6.5$ Hz, boi H_3-6), 2.82 (1H, *m*, H-17), 3.21 (1H, *m*, boi H-4), 3.95 (1H, *m*, boi H-5), 3.96 (1H, *m*, boi H-3), 4.17 (1H, *m*, H-3), 4.87 (1H, *dd*, $J = 2.0$, 10.0 Hz, boi

H-1), 4.90, 5.02 (each 1H, *dd*, J = 2.0, 18.5 Hz, H_2-21), 5.89 (1H, *s*, H-22), 10.04 (1H, *s*, H_3-19). ^{13}C NMR: see Table 1.

Helveticoside (**4**)

White amorphous powder. FABMS *m*/*z*: 533 [M–H]$^-$, 403 [aglycone–H]$^-$. CD (MEOH, nm, rel.int.): 249.5 (+6.2), 306 (–1.8). ^{1}H NMR: δ 0.84 (3H, *s*, H_3-18), 1.23 (3H, *d*, J = 6.5 Hz, dig H_3-6),2.82 (1H, *m*, H-17), 3.15 (1H, *dd*, J = 3.0, 9.5 Hz, dig H-4), 3.75 (1H, *dq*, J = 6.5, 9.5 Hz, dig H-5), 4.01 (1H, *m*, dig H-3), 4.15 (1H, *m*, H-3), 4.90, 5.01 (each 1H, *dd*, J = 2.0, 18.5 Hz, H_2-21), 4.93 (1H, *dd*, J = 2.0, 10.0 Hz, dig H-1), 5.89 (1H, *s*, H-22), 10.04 (1H, *s*, H_3-19). ^{13}C NMR: see Table 1.

Cannogenol 3-O-β-D-glucopyranosyl-(1 → 4)-O-β-D-boivinopyranoside (**5**)

White amorphous powder. $[\alpha]_D^{22}$ –18.3° (MeOH; *c* 0.30). HR-FABMS *m*/*z*: 681.3436, $C_{35}H_{53}O_{13}$ requires 681.3486. FABMS *m*/*z*: 681 [M–H]$^-$, 519 [M–H–162]$^-$, 389 [aglycone–H]$^-$. CD (MeOH, nm): 243. ^{1}H NMR: δ 0.88 (3H, *s*, H_3-18), 1.25 (3H, *d*, J = 6.5 Hz, boi H_3-6), 2.82 (1H, *m*, H-17), 3.41, 3.81 (each 1H, *d*, J = 11 Hz, H_2-19), 3.45 (1H, *m*, boi H-4), 3.65 (1H, *dd*, J = 5.5, 12.0 Hz, glc H-6a), 3.86 (1H, *dd*, J = 2.0, 12.0 Hz, glc H-6b), 4.04 (1H, *m*, H-3), 4.15 (1H, *br.q*, boi H-3), 4.31 (1H, *d*, J = 8.0 Hz, glc H-1), 4.88 (1H, *dd*, J = 2.0, 10.0 Hz, boi H-1), 4.91, 5.03 (each 1H, *dd*, J = 1.5, 18.5 Hz, H_2-21), 5.89 (1H, *s*, H-22). ^{13}C NMR: see Table 1.

Periplogenin 3-O-β-D-glucopyranosyl-(1 → 4)-O-β-D-digitoxopyranoside (**6**)

White amorphous powder. $[\alpha]_D^{22}$ +16.9° (MeOH; *c* 0.20). HR-FABMS *m*/*z*: 681.3419, $C_{35}H_{53}O_{13}$ requires 681.3486. FABMS *m*/*z*: 681 [M–H]$^-$, 519 [M–H–162]$^-$, 389 [aglycone–H]$^-$. CD (MeOH, nm): 242. ^{1}H NMR: δ 0.88 (3H, *s*, H_3-18), 0.93 (3H, *s*, H_3-19), 1.30 (3H, *d*, J = 6.0 Hz, dig H_3-6), 2.84 (1H, *m*, H-17), 3.69 (1H, *dd*, J = 5.5, 12.0 Hz, glc H-6a), 3.82 (1H, *dd*, J = 2.0, 12.0 Hz, glc H-6b), 3.88 (1H, *dq*, J = 6.0, 9.5 Hz, dig H-5), 4.16 (1H, *m*, H-3), 4.29 (1H, *br.q*, dig H-3), 4.37 (1H, *d*, J = 8.0 Hz, glc H-1), 4.91, 5.03 (each 1H, *dd*, J = 1.5, 18.5 Hz, H_2-21), 4.94 (1H, *dd*, J = 2.0, 11.0 Hz, dig H-1), 5.90 (1H, *s*, H-22). ^{13}C NMR: see Table 1.

Digitoxigenin 3-O-β-D-glucopyranosyl-(1 → 6)-O-β-D-glucopyranosyl-(1 → 4)-O-β-D-digitoxopyranoside (**7**)

White amorphous powder. $[\alpha]_D^{22}$ –1.3° (MeOH; *c* 0.40). HR-FABMS *m*/*z*: 827.4045, $C_{41}H_{63}O_{17}$ requires 827.4065. FABMS *m*/*z*: 827 [M–H]$^-$, 665 [M–H–162]$^-$, 503 [M–H–162 × 2]$^-$, 373 [aglycone–H]$^-$. CD (MeOH, nm): 242.5. ^{1}H NMR: δ 0.88 (3H, *s*, H_3-18), 0.94 (3H, *s*, H_3-19), 1.29 (3H, *d*, J = 6.0 Hz, dig H_3-6), 2.83 (1H, *m*, H-17), 3.66 (1H, *m*, glc′ H-6a), 3.74 (1H, *dd*, J = 6.5, 11.5 Hz, glc H-6a), 3.85 (1H, *dq*, J = 6.5, 9.5 Hz, dig H-1), 3.86 (1H, *dd*, J = 1.5, 12.0 Hz, glc′ H-6b), 4.01 (1H, *m*, H-3), 4.13 (1H, *dd*, J = 2.0, 12.0 Hz, glc H-6b), 4.33 (1H, *br.q*, J = 3.0, Hz, dig H-3), 4.35 (1H, *d*, J = 8.0 Hz, glc′ H-1), 4.39 (1H, *d*, J = 8.0 Hz, glc H-1), 4.91, 5.04 (each 1H, *dd*, J = 2.0, 18.5 Hz, H_2-21), 5.00 (1H, *d*, J = 2.0, 9.5 Hz, dig H-1), 5.89 (1H, *s*, H-22). ^{13}C NMR: see Table 1.

Coroloside (**8**)

White amorphous powder. FABMS *m*/*z*: 665 [M–H]$^-$, 503 [M–H–162]$^-$, 373 [aglycone–H]$^-$. CD (MeOH, nm): 243. ^{1}H NMR: δ 0.88 (3H, *s*, H_3-18), 0.94 (3H, *s*, H_3-19), 1.25 (3H, *d*, J = 6.5 Hz, boi H_3-6), 2.83 (1H, *m*, H-17), 3.66 (1H, *dd*, J = 5.5, 12.0 Hz, glc H-6a), 3.87 (1H, *dd*, J = 2.0, 12.0 Hz, glc H-6b), 4.04 (1H, *m*, H-3), 4.32 (1H, *d*, J = 7.5 Hz, glc H-1), 4.87 (1H, *dd*, J = 2.0, 10.0 Hz, boi H-1), 4.92, 5.04 (each 1H, *dd*, J = 2.0, 18.5 Hz, H_2-21), 5.90 (1H, *s*, H-22). ^{13}C NMR: see Table 1.

Glucoevatromonoside (**9**)

White amorphous powder. FABMS *m*/*z*: 665 [M–H]$^-$, 503 [M–H–162]$^-$, 373 [aglycone–H]$^-$. CD (MeOH, nm): 242.5. ^{1}H NMR: δ 0.88 (3H, *s*, H_3-18), 0.94 (3H, *s*, H_3-19), 1.30 (3H, *d*, J = 6.5 Hz, dig H_3-6), 2.83 (1H, *m*, H-17), 3.70 (1H, *dd*, J = 5.5, 12.0 Hz, glc H-6a), 3.83 (1H, *dd*, J = 2.0, 12.0 Hz, glc H-6b), 4.02 (1H, *m*, H-3), 4.38 (1H, *d*, J = 7.5 Hz, glc H-1), 4.91 (1H, *dd*, J = 2.0, 10.0 Hz, dig H-1), 4.92, 5.04 (each 1H, *dd*, J = 2.0, 18.5 Hz, H_2-21), 5.90 (1H, *s*, H-22). ^{13}C NMR: see Table 1.

Deglucocoroloside (**10**)

White amorphous powder. FABMS *m*/*z*: 503 [M–H]$^-$, 373 [aglycone–H]$^-$. ^{1}H NMR: δ 0.87 (3H, *s*, H_3-18), 0.93 (1H, *s*, H_3-19), 1.20 (3H, *d*, J = 6.5 Hz, boi H_3-6), 2.82 (1H, *m*, H- 17), 4.17 (1H, *m*, H-3), 4.84 (1H, *dd*, J = 2.0, 10.0 Hz, boi H-1), 4.90, 5.02 (each 1H, *dd*, J = 2.0, 18.5 Hz, H_2-21), 5.89 (1H, *s*, H-22). ^{13}C NMR: see Table 1.

Evatromonoside (**11**)

White amorphous powder. FABMS *m*/*z*: 503 [M–H]$^-$. ^{1}H NMR: δ 0.87 (3H, *s*, H_3-18), 0.94 (1H, *s*, H_3-19), 1.22 (3H, *d*, J = 6.0 Hz, dig H_3-6), 2.82 (1H, *m*, H-17), 4.01 (1H, *m*, H-3), 4.90, 5.02 (each 1H, *dd*, J = 2.0, 18.0 Hz, H_2-21), 4.91 (1H, *dd*, J = 2.0, 9.5 Hz, dig H-1), 5.89 (1H, *s*, H-22). ^{13}C NNM: see Table 1.

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