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CYCLOARTANE TRITERPENE GLUCOSIDES FROM *CORCHORUS DEPRESSUS*

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Key Word Index—*Corchorus depressus*; Tiliaceae; Cycloartane triterpene glucoside; depressoside A and B.

Abstract—Depressoside A and B, two new cycloartane triterpene glucosides were isolated from *Corchorus depressus*. The structures of depressoside A and B were elucidated as 9,19-cyclolanosta-22(*R*),25-epoxy-3β,16β,24(*S*)-triol-3-O-β-D-glucopyranoside and 9,19-cyclolanosta-22(*R*),25-epoxy-24(*S*)-acetoxy-3β,16β-diol-3-O-β-D-glucopyranoside respectively on the basis of 1D and 2D spectroscopic studies and chemical analysis.

INTRODUCTION

The genus *Corchorus* comprises certain herbs and under shrubs. It contains about 100 species which are distributed in the tropics and subtropics, chiefly South-East Asia and South America [1]. Only six species are found in Pakistan, among which two species *Corchorus capsularis* L. and *Corchorus olitorus* L. are cultivated and are used for extracting fiber and the other four are wild species [2]. *Corchorus depressus* L. is one of the four species growing wild in sandy, clayey and saline areas of Pakistan. It is commonly known as "Bhauphali" and has been used in folk medicine. The herbalist doctors sell it under the name "Munderi". Its leaves are used as an emollient and cooling agent. The mucilage is used for the treatment of gonorrohoea and applied as a poultice for healing wounds [3]. The decoction of seeds and leaves is used as a tonic and the infusion is used as a fever drink [4]. Previous chemical investigation of this plant has resulted in the isolation of flavonoids, quercetin and kaempferol from the leaves and flowers [5]. From the whole plant of *C. depressus* α-amyrin derivatives have also been reported [6]. The medicinal importance of this plant prompted us to investigate the chemical constituents of *C. depressus*. We now report the isolation and structure elucidation of two new cycloartane triterpene glucosides depressoside A and B from the whole plant of *C. depressus*. Their structures have been established on the basis of extensive spectroscopic studies and chemical analysis.

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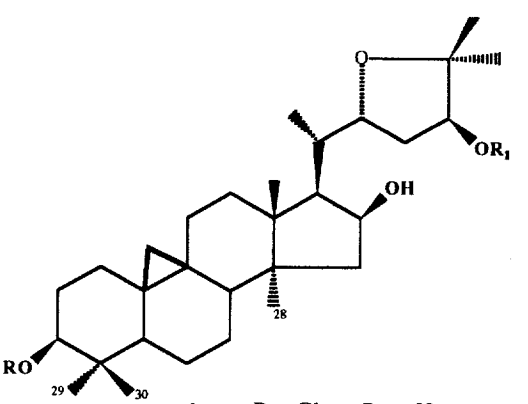
RESULTS AND DISCUSSION

The ethanol extract of the whole plants of *Corchorus depressus* was partitioned into hexane, chloroform, ethylacetate, *n*-butanol and water soluble fractions. The *n*-butanol extract on repeated CC and preparative TLC afforded two new cycloartane type triterpene glucosides depressoside A and B (**1**,**2**).



1 R = Glc; R_1 = H
1a R = H; R_1 = H
2 R = Glc; R_1 = Ac

Depressoside A (**1**) was obtained as very thin hair-like crystals mp 208–210°C. $[\alpha]^{25}_D$ −1.50°. The positive ion FAB mass spectrum of **1** exhibited a quasi molecular ion peak at *m/z* 659 (calcd. for $C_{36}H_{60}O_9Na$) $[M+Na]^+$. The fragment ions at *m/z* 497 $[M+Na-162]^+$, 479 $[M+Na-162-H_2O]^+$ indicated the loss of one hexose unit. The negative ion FAB mass spectrum showed a peak at *m/z* 599 $[M-H-2H_2O]^-$. The EI mass

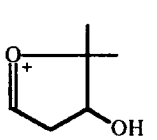
spectrum of the intact saponin showed significant fragments at m/z 456 [(M-162-H_2O)$^+$, 7.17], 438 [(M-162-2H_2O)$^+$, 12.83], 143 (10.17) and 115 (100).

^{1}HNMR spectrum of **1** (400 MHz, CD_3OD) showed two one-proton doublets at δ 0.37 (J=4.0 Hz, H-19A), 0.57 (J=4.0 Hz, H-19B) characteristic of geminal cyclopropane protons, suggesting the cycloartane skeleton in the molecule. Six tertiary methyl signals at δ 0.90 (s, 3 × H-30), 0.96 (s, 3 × H-28), 1.08 (s, 3 × H-29), 1.18 (s, 3 × H-18), 1.19 (s, 3 × H-27), 1.20 (s, 3 × H-26) and a methyl group as a doublet at δ 0.93 (d, J=7.0 Hz, 3 × H-21) were observed. One anomeric proton signal at δ 4.45 (d, J=7.65 Hz, H-1′) indicated the presence of a β-linked sugar. The ^{13}CNMR spectrum of **1** (100 MHz, CD_3OD) showed 36 carbon resonances. DEPT experiment suggested the presence of 13 methine, 10 methylene and 7 methyl groups and by difference from the broad band 6 quaternary carbon atoms were identified. The anomeric signal was located at δ 104.16. The signals of the upfield quaternary carbon atoms resonating at δ 21.08 and 27.44 and a methylene carbon at δ 31.01 confirmed the presence of cycloartane type skeleton in the aglycone.

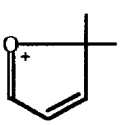
^{13}C, DEPT, ^{1}H-^{1}H-COSY and HMQC experiments were employed in assigning all the ^{1}H and ^{13}C values as shown in Tables 1 and 2. By comparison of respective ^{13}CNMR spectral data **1** differed from other known cycloartanes only in the side chain.

Acid hydrolysis of **1** afforded D-glucose and the aglycone which was a new cycloartane type triterpene and we named it as depressogenin (**1a**), mp 195–197°C. $[\alpha]^{25}_D$ +53.8. The EI mass spectrum of **1a** showed a molecular ion peak at m/z 474 (calcd. for $C_{30}H_{50}O_4$). The most prominent ions were due to the loss of water (m/z 456 and 438), and (i) cleavage of the side chain (m/z 313), (ii) cleavage of the ring D (m/z 245), (iii) The side chain (m/z 142), (iv) and the base peak due to the cleavage of five membered furan type ring (m/z 115) as shown in Fig. 1. This fragmentation pattern is in good agreement with that already reported for 22, 25-epoxy cycloartanes [7].

The structure of **1a** was established by the combination of 1D and 2D NMR techniques. The ^{1}HNMR spectrum of **1a** (500 MHz, $CDCl_3$) contained six methyl singlets, one methyl doublet, signals of two high field cyclopropane protons and four protons on oxygenated carbons. These data, together with ^{1}H decoupled ^{13}CNMR and DEPT spectra, accounted for a 9,19-cyclolanostane skeleton with three hydroxyl groups, two of which can be easily located at C-3 and C-16, after comparison with known compounds [8, 9]. The chemical shift value at δ 71.85 is attributed to C-16. In the ^{1}HNMR spectrum the signal for H-16 appeared at δ 4.45 as ddd (J=5.8, 8.0, 8.5 Hz, H-16α), the chemical shift value and the coupling constants are in good agreement with the published data for a 16β-hydroxyl group [10]. In ^{1}H-^{1}H COSY-45° spectrum H-16α was coupled with H-15α (δ 1.38), H-15β (δ 1.80) and it was also coupled with H-17 (δ 1.93). The latter further showed coupling with the proton at δ 2.44 (H-20) which in turn showed coupling with the proton of H_3-21 (δ 0.89) and H-22 (δ 3.92). The proton at δ 3.92 (H-22) was further coupled with two geminal protons at δ 2.25 and δ 1.79 (H_2-23). Both these geminal protons further showed coupling with the proton at δ 4.03 (H-24).



m/z 115 (100%) in (1) & (1a)



m/z 97 (100%) in (2)

Fig. 1. Fragment ions produced in the mass spectra of compounds **1**, **1a** and **2**.

The site of attachment of the glucose moiety in **1** was determined by means of the usual glycosidation shift, which was observed at C-3. In the ^{13}CNMR spectrum of **1** C-3 resonated at δ 91.02 and at δ 78.85 in **1a**. No glycosidation shift was observed at C-16 and C-24. The anomeric signal which appeared at δ 4.50 as doublet (J=7.65 Hz, H-1′) showed long range correlation with the carbon at δ 91.02 in HMBC experiment confirming the position of glucose at C-3. The remaining two downfield methine signals in the aglycone resonating at δ 80.90 and 78.30 were assigned to C-22 and C-24, respectively, on the basis of HMQC and ^{1}H-^{1}H COSY experiments. The presence of an oxygen atom at C-22 was confirmed from the downfield shift for C-20 which appeared at δ 33.37 as compared to δ 28.0 and a subsequent upfield shift for C-17 and C-21 resonating at δ 52.94 and δ 15.9 compared to δ 58.0 and δ 18.0 respectively as reported earlier for 16β, 22-hydroxy cycloartanes [11, 12].

The presence of a very downfield quaternary carbon at δ 84.0 compared to δ 71-73 observed in 24,25-hydroxy or 20-24-epoxycylocartanes [13, 14] suggested that **1** contains neither acyclic nor 20-24-epoxy side chain. On the basis of above accumulated evidence we propose a 22-25-epoxy side chain in **1**.

All the assignments were further confirmed through a HMBC experiment (Fig. 2). C-21 methyl protons at δ 0.93 (d, J=7.0 Hz, 3 × H-21) showed correlation both with C-17 (δ 52.94) and C-22 (80.90). The C-23 methylene protons at δ 1.79 and 2.23 were correlated with C-24 (δ 78.30) and C-25 (δ 84.0). Both the methyl protons of H_3-26 and H_3-27 at δ 1.20 and 1.19 showed correlations with C-24 (δ 78.30) and C-25 (δ 84.0). The stereo-chemistry of C-22 and C-24 chiral centres was fixed by comparing the ^{13}C values with the known compounds having acylic side chain. C-24 having the S-configuration resonates at δ 77.1. In contrast, the atom having the R-configuration resonates at δ 80.5 [15]. The signal of the C-24 atom in the ^{13}CNMR spectrum of **1** and **1a** was located at δ 78.30 and 77.68, respectively, suggesting the 24S configuration. The configuration at C-22 and C-24 in **1a** was confirmed from NOE difference spectra as shown in Fig. 3. The

Table 1. ^{1}HNMR spectral data of depressoside A (**1**), B (**2**) (400 MHz, CD_3OD) and depressogenin **1a** (500 MHz, $CDCl_3$) from 1D and 2D experiments

	1	**1a**	**2**
1	1.26	1.19	1.28
	1.54	1.46	1.56
2	1.70	1.57	1.68
	2.05	1.74	2.05
3	3.25 *dd* (J=4.3, 11.0 Hz)	3.28 *dd* (J=4.5, 11.5 Hz)	3.24 *dd* (J=4.5, 12.0 Hz)
5	1.34	1.28	1.35
6	0.82	0.80	0.85
	1.62	1.58	1.63
7	0.93	1.08	1.08
	1.30	1.34	1.30
8	1.64	1.61	1.68
11	1.15	1.10	1.20
	2.05	1.95	2.05
12	1.33	1.25	1.36
	1.66	1.58	1.65
15	1.38	1.38	1.40
	1.94	1.80	1.97
16	4.50 *ddd* (J=5.5, 8.0, 8.0 Hz)	4.45 *ddd* (J=5.8, 8.0, 8.5 Hz)	4.47 *ddd* (J=5.5, 8.0, 8.5 Hz)
17	1.95 *dd* (J=6.8, 11.4 Hz)	1.93 *dd* (J=7.0, 12.0 Hz)	1.94 *dd* (J=6.8, 11.5 Hz)
18	1.18 *s*	1.18 *s*	1.18 *s*
19	0.37 *d* (J=4.0 Hz)	0.32 *d* (J=4.0 Hz)	0.35 *d* (J=4.0 Hz)
	0.57 *d* (J=4.0 Hz)	0.57 *d* (J=4.0 Hz)	0.58 *d* (J=4.0 Hz)
20	2.26 *m*	2.44 *m*	2.28 *m*
21	0.93 *d* (J=7.0 Hz)	0.89 *d* (J=7.0 Hz)	0.92 *d* (J=7.0 Hz)
22	4.00 *ddd* (J=3.2, 7.0, 8.9 Hz)	3.92 *ddd* (J=3.2, 7.0, 8.9 Hz)	4.20 *ddd* (J=3.2, 7.0, 9.0 Hz)
23	1.79 *m*	1.79 *m*	1.85 *m*
	2.23 *m*	2.25 *m*	2.45 *m*
24	4.03 *dd* (J=4.2, 6.8 Hz)	4.03 *dd* (J=4.2, 6.8 Hz)	5.02 *dd* (J=2.8, 7.05 Hz)
26	1.20 *s*	1.16 *s*	1.20 *s*
27	1.19 *s*	1.26 *s*	1.22 *s*
28	0.96 *s*	0.89 *s*	0.94 *s*
29	1.08 *s*	0.96 *s*	1.07 *s*
30	0.90 *s*	0.80 *s*	0.89 *s*
Glc 1′	4.50 *d* (J=7.65 Hz)	–	4.45 *d* (J=7.5 Hz)
2′	4.05 *dd* (J=7.6, 9.0 Hz)	–	4.05 *dd* (J=8.0, 9.2 Hz)
3′	3.68 *t* (J=9.0 Hz)	–	3.65 *t* (J=9.0 Hz)
4′	3.42 *t* (J=9.66 Hz)	–	3.40 *t* (J=9.5 Hz)
5′	3.32 *ddd* (J=2.5, 5.5, 8.5 Hz)	–	3.28 *ddd* (J=2.5, 5.4, 8.6 Hz)
6′	3.68 *dd* (J=5.0, 11.9 Hz)	–	3.66 *dd* (J=4.5, 12.0 Hz)
	3.84 *dd* (J=2.5, 11.9 Hz)	–	3.82 *dd* (J=2.0, 11.5 Hz)
$OCOCH_3$	–	–	1.90 *s*

NOE clearly indicated the opposite orientation of H-22 (δ 3.92) and H-24 (δ 4.03) in space. Thus, depressoside A **1** was identified as 9,19-cyclolanosta-22(*R*), 25-epoxy-3β,16β,24(*S*)-triol, 3-O-β-D-glucopyranoside.

Depressoside B (**2**) was isolated as crystalline solid mp 218–220°C. $[\alpha]_D^{25}$ −1.72°. A sharp absorption at 1735 cm^{-1} in IR indicated an ester carbonyl functionality. The positive ion FAB mass spectrum exhibited a quasi molecular ion peak at m/z 701 $(M+Na)^+$, molecular ion peak at m/z 679 $[(M+H)^+$, calcd. for $C_{38}H_{62}O_{10}$] and the fragment ions at m/z 540 $(M+H+Na\text{-}162)^+$ and 517 $(M+H\text{-}162)^+$, and 479 $(M+Na\text{-}162\text{-}CH_3COOH)^+$. The negative ion FAB mass spectrum showed peaks at m/z 599 $(M\text{-}H\text{-}H_2O\text{-}CH_3COOH)^-$, 515 $(M\text{-}H\text{-}162)^-$, 497 $(M\text{-}H\text{-}H_2O\text{-}162)^-$, indicating the presence of one hexose and an ester moiety in the molecule. The ^{1}HNMR and ^{13}CNMR spectra of **2** were identical with those of **1** except in the side chain region. The ^{1}HNMR spectrum of depressoside B (**2**) showed signals due to cyclopropane methylene at δ 0.35 *d* (J=4.0 Hz, H-19A) and 0.58 *d* (J=4.0 Hz, H-19B), seven tertiary methyl singlets at δ 0.89 (*s*, 3 × H-30), 0.94 (*s*, 3 × H-28), 1.07 (*s*, 3 × H-29), 1.18 (*s*, 3 × H-18), 1.20 (*s*, 3 × H-26), 1.22 (*s*, 3 × H-27), 1.90 (*s*, $OCOCH_3$) and a secondary methyl signal at δ 0.92 (*d*, J=7.0 Hz, 3 × H-21). The additional methyl singlet at δ 1.90 compared to **1** was due to the ester moiety. The other ^{1}HNMR signals were identical to those of **1** except in the side

Table 2. ^{13}CNMR spectral data of depressoside A **1**, B **2** (100 MHz, CD_3OD) and depressogenin **1a** (125 MHz, $CDCl_3$)

C.No.	DEPT	1	1a	2
1	CH_2	33.10	32.00	33.08
2	CH_2	30.50	30.38	30.29
3	CH	91.02	78.85	90.90
4	C	42.05	40.48	42.02
5	CH	48.88	47.18	49.50
6	CH_2	22.07	21.07	22.01
7	CH_2	27.20	26.07	27.60
8	CH	49.64	47.83	50.05
9	C	21.08	20.68	21.01
10	C	27.44	26.21	27.45
11	CH_2	27.31	26.14	27.79
12	CH_2	34.37	33.28	34.49
13	C	49.90	46.93	46.95
14	C	47.91	47.31	47.97
15	CH_2	47.65	46.24	47.83
16	CH	73.04	71.85	73.12
17	CH	52.94	51.28	52.71
18	CH_3	19.42	18.90	19.36
19	CH_2	31.01	30.38	30.69
20	CH	33.37	31.68	33.55
21	CH_3	15.9	16.56	15.44
22	CH	80.90	80.55	80.27
23	CH_2	36.27	35.18	34.30
24	CH	78.30	77.68	81.87
25	C	84.0	83.00	83.85
26	CH_3	26.0	24.98	22.62
27	CH_3	23.08	22.28	24.76
28	CH_3	20.42	20.04	20.54
29	CH_3	26.14	25.42	26.03
30	CH_3	15.48	13.97	15.13
1′	CH	104.16	–	104.17
2′	CH	81.77	–	81.01
3′	CH	77.73	–	77.86
4′	CH	71.64	–	71.64
5′	CH	77.11	–	77.29
6′	CH_2	62.63	–	62.76
$OCOCH_3$	–	–	–	182.19
$OCOCH_3$	–	–	–	23.88

chain region. H-24 which appeared at δ 4.03 in **1** was shifted downfield due to the α-effect of acetyl group, and appeared at δ 5.02 in **2** [16]. In the ^{13}CNMR spectrum the signal due to C-24 was also shifted downfield and appeared at δ 81.87, compared to 78.30 in **1**. Carbon signals for C-23, C-25, C-26 and C-27 were shifted slightly upfield and resonated at δ 34.30, 83.85, 22.62 and 24.76, respectively. These changes suggested that the 24-hydroxyl group in **1** has been acetylated in **2**. This fact was further supported from the base peak observed in the EI mass spectrum of **2**. In **1** the base peak was observed at *m/z* 115 but in **2** base peak was observed at *m/z* 97 after the removal of the $OCOCH_3$ group as CH_3COOH from the fragment at *m/z* 157 as shown in Fig. 1.

Tables 1 and 2 show the ^{1}H and ^{13}CNMR assignments which were successfully made with the help of DEPT., HMQC, ^{1}H-^{1}H COSY-45° and finally these connectivities were confirmed by a HMBC experiment, thus depressoside B(**2**) was identified as 9,19-cyclolanosta-22(*R*)-25-epoxy-24(*S*)-acetoxy-3β,16β-diol-3-O-β-D-glucopyranoside.

Recently cyclokirilodiol and isocyclokirilodiol two novel cycloartane triterpene alcohols possessing the same monohydroxy-tetrahydrofuran ring in the side chain as in depressoside A and B have been reported from the seeds of *Trichosanthes kirilowii* [17]. Isocyclokirilodiol has the same 22(*R*),24(*S*)-configuration as we have confirmed from NOE difference specta for depressogenin **1a** (Fig. 3).

EXPERIMENTAL

General

Mps (Uncorr.) were determined on a Buchi 535 melting point apparatus.$[\alpha]_D$ values were measured on a JASCO DIP-360 instrument. ^{1}HNMR (400/500 MHz), ^{13}CNMR (100/125 MHz), Bruker AM-400 and AM-500 spectrometers. Chemical shifts were reported in δ ppm, with TMS as internal standard. EIMS: Finnigan MAT-312 double focusing mass spectrometer. FABMS: Jeol JMS HX-110 spectrometer. UV Hitachi U-3200. IR JASCO A-302. Kieselgel 60 (35-70) Mesh was used for CC. Precoated Kieselgel 60, F_{254} aluminium sheets (E. Merck, Art. No. 1.05554) was used for TLC. Purity of the compounds was checked on HPTLC (E. Merck, Art. No. 5556).

Plant material

The whole plant of *Corchorus depressus* L. was collected in June, 1995 from Karachi. A voucher specimen was deposited in the Herbarium of Department of Botany, University of Karachi.

Extraction and isolation

Air dried plant of *Corchorus depressus* (20 kg) was extracted 3 × with EtOH. The ethanolic extract was evaporated *in vacuo* to afford a gummy residue. This residue was partitioned between H_2O and hexane (550 g). The aq. layer was then extracted 3 × each with $CHCl_3$, EtOAc and then with *n*-BuOH. The *n*-BuOH extract (182 g) was subjected to CC on silica gel using a gradient of MeOH in $CHCl_3$. A mixture of saponins was obtained with $CHCl_3$–MeOH (4:1) which was further subjected to CC on Sephadex LH-20 using MeOH–H_2O (1:1) system. Saponin **1** was finally purified by repeated CC on silica gel $CHCl_3$–MeOH–H_2O. Saponin **2** which was a minor constituent and was purified by prep. TLC on silica gel with solvent system $CHCl_3$–MeOH–H_2O (79.5:20:0.5). The purity of the compounds was checked on HPTLC plates using *n*-BuOH–AcOH–H_2O (12:3:5) and $CHCl_3$–MeOH–H_2O (20:79.5:0.5). The spots were visualized

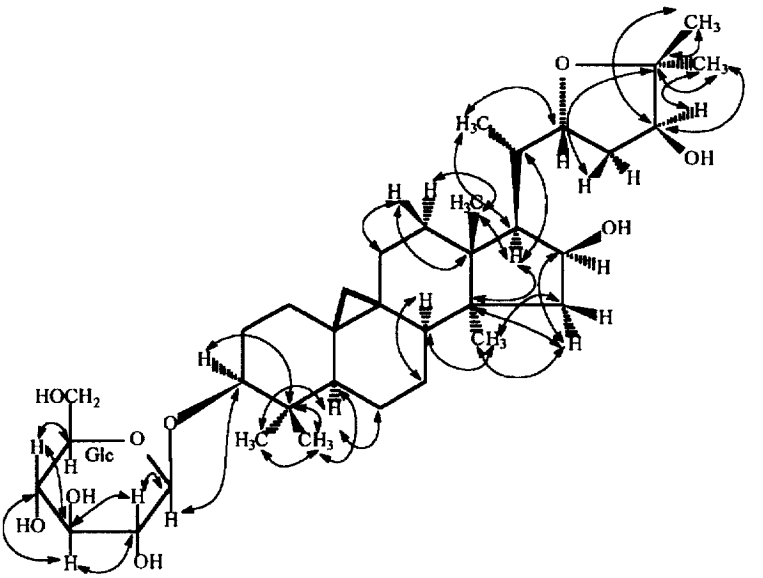


Fig. 2. Long-range HMBC correlations in depressoside A (1).

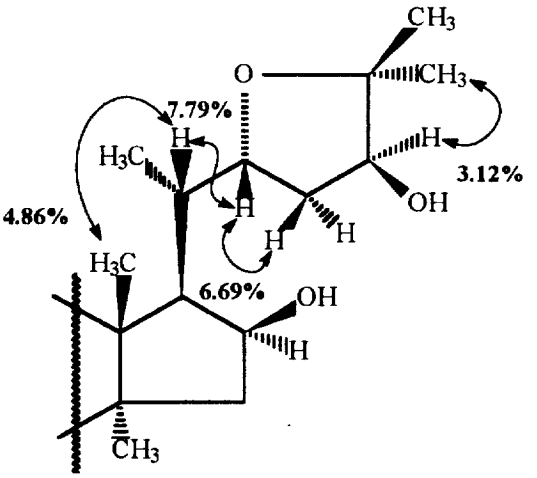


Fig. 3. NOE difference spectra of **1a**.

by spraying with ceric ammonium sulphate reagent followed by heating.

Depressoside A (**1**)

Mp 208–210°C; $[\alpha]_D^{28}-1.50°$ ($C=0.17$, MeOH). FAB-MS (Pos. ion mode): *m/z* 659 $(M+Na)^+$, 497 $(M+Na\text{-}162)^+$, 479 $(M+Na\text{-}162\text{-}H_2O)^+$. FAB-MS (Neg. ion mode): *m/z* 599 $(M\text{-}H\text{-}2H_2O)^-$. EIMS: *m/z* 456 (7.17), 438 (12.83), 395 (5.60), 313 (3.29), 295 (7.90), 273 (4.74), 255 (3.82), 245 (5.25), 203 (6.98), 143 (10.17), 142 (27.30), 115 (100). ^{1}HNMR: Table 1. ^{13}CNMR: Table 2. IRν_{max}^{KBr}cm^{-1}: 3380–3450 (OH), 3840 (CH_2 of cyclopropane ring).

Depressoside B (**2**)

Mp 218–220°C; $[\alpha]_D^{28}-1.70°$ ($C=0.07$, MeOH). FAB-MS (Pos. ion mode): *m/z* 701 $(M+Na)^+$, 679 $(M+H)^+$, 540 $(M+H+Na\text{-}162)^+$, 517 $(M+H\text{-}162)^+$, 499 $(M+H\text{-}H_2O\text{-}162)^+$, 479 $(M+Na\text{-}162\text{-}CH_3COOH)^+$. FAB-MS (Neg. ion mode): *m/z* 599 $(M\text{-}H\text{-}H_2O\text{-}CH_3COOH)^-$, 515 $(M\text{-}H\text{-}162)^-$, 497 $(M\text{-}H\text{-}162\text{-}H_2O)^-$. EIMS: *m/z* 498 (2.57), 454 (1.76), 341 (1.30), 313 (2.96), 295 (1.59), 247 (3.42), 203 (1.26), 157 (10.22), 97 (100). ^{1}HNMR: Table 1. ^{13}CNMR: Table 2. IRν_{max}^{KBr}cm^{-1}: 3350–3450 (OH), 1735 (C=O).

Acid hydrolysis of depressoside A (**1**)

Compound **1** (60 mg) was dissolved in MeOH and 5 ml of 20% HCl was added. The reaction mixture was refluxed for 6 hr. After cooling MeOH was evaporated *in vacuo*. Distilled H_2O (5 ml) was added and mixture was extracted 3 × with EtcOAc. The aq. layer was neutralized with Ag_2CO_3, filtered and concentrated under red. pres. The residue obtained contained only the D-glucose.

Depressogenin (**1a**)

The EtOAc layer was concentrated. The aglycone was purified on prep. TLC by using solvent system $CHCl_3$-EtOAc (6:4). The compound was recrystallized from $CHCl_3$ and its purity was checked on HPTLC with the same solvent system. Mp 195–197°C.$[\alpha]_D+53.8°$($C=0.13$, $CHCl_3$). EIMS (70 eV): *m/z* 474 $[M]^+$ (2.53), 456 (6.11), 438 (1.10), 385 (2.28), 334 (2.23), 313 (4.02), 295 (1.68), 245 (3.01), 143 (7.22), 142 (26.32), 115 (100). ^{1}HNMR: Table 1. ^{13}CNMR: Table 2.

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