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Polyhydroxypregnane glycosides from *Oxystelma esculentum* var. *alpini*

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Abstract

Three new polyhydroxypregnane glycosides named alpinoside A [kidjolanin 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-thevetopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside], alpinoside B [kidjolanin 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-thevetopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside], and alpinoside C [kidjolanin 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside] were isolated from the leaves of *Oxystelma esculentum* var. *alpini*. The structure elucidation was accomplished by extensive spectroscopic analysis and acid-catalyzed hydrolysis.

Keywords: *Oxystelma esculentum* var. *alpini*; Asclepiadaceae; Polyhydroxypregnane glycosides; NMR

1. Introduction

Oxystelma esculentum var. *alpini* (Asclepiadaceae) is an Egyptian twining glabrous perennial herb growing in waste moist ground (Boulos, 2000), with milky juice, whose local Arabic name is '*Libben*' (El-Hadidi and Fayed, 1994). This plant is also found in India, China and South Indonesia. A decoction is used as a gargle, the latex as a dressing for ulcers, and the roots in the treatment of jaundice. The sap mixed with *Styrax benzoin*, *Citrus aurantifolia* and *Santalum album* juice, is drunk to treat cholera; the bark, with vinegar and spices, is smeared on the body (Perry and Metzger, 1980).

In earlier chemical investigations on the roots of *Oxystelma esculentum* the presence of pregnane oligoglycosides (Trivedi et. al., 1988, 1989, 1990) and two cardenolide glycosides (Srivastava et. al., 1991, 1993) were reported. Since to date no work has been done on the leaves of this plant growing in Egypt and elsewhere, we have now examined the chemical constituents of the aerial parts.

2. Results and discussion

The $CHCl_3$-soluble portion of the methanol extract of the aerial parts of *Oxystelma esculentum* var. *alpini* was chromatographed on Si gel to obtain three new glycosides namely alpinoside A (**1**), alpinoside B (**2**), alpinoside C (**3**). The glycosides showed positive Liebermann–Burchard and Keller–Kiliani reactions which indicated the presence of steroidal glycosides with 2-deoxy sugars. Compound **1** had a molecular formula $C_{63}H_{94}O_{27}$, as determined from ^{13}C and ^{13}C DEPT NMR and ESIMS in negative-ion mode. The ESIMS of **1** showed an $[M-H]^-$ ion at m/z 1281 and prominent fragments at m/z 1133 $[(M-H)-148]^-$ attributable to the loss of a cinnamic acid residue, m/z 1119 $[(M-H)-162]^-$ and m/z 957 $[(M-H)-(162\times2)]^-$ due to the cleavage of one and two hexose units, respectively. Also evident were peaks at m/z 797 and 653 arising from the sequential losses of a methyldeoxyhexopyranose (160 mass units) and a methyldideoxyhexopyranose (144 mass units) from the ion at m/z 957.

The ^{13}C and DEPT ^{13}C NMR spectra showed 63 carbon signals, of which 21 carbon signals were assigned to a pregnane aglycone, 9 to a cinnamoyl residue and 33 to a sugar portion.

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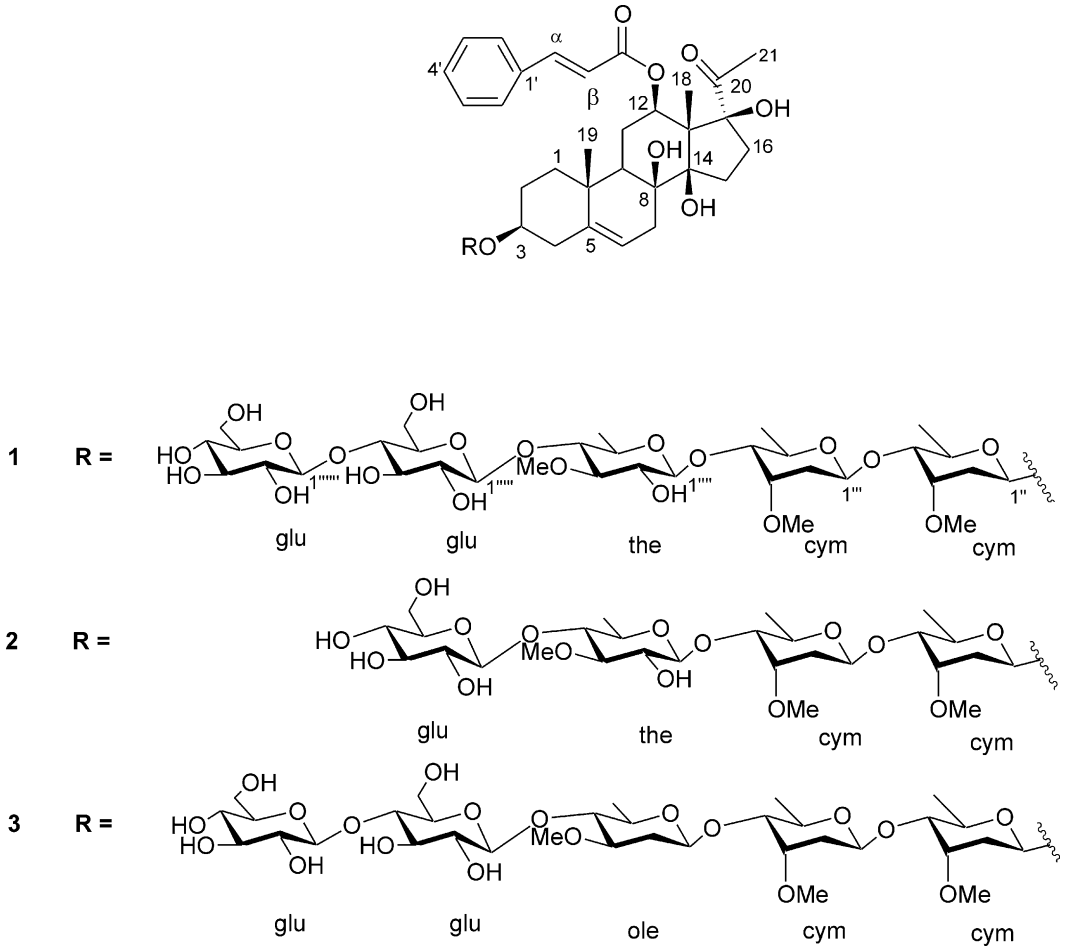

The ^{1}H NMR spectrum of **1** showed for the aglycone moiety three tertiary methyl proton signals at δ1.19, 1.63 and 2.26 (each *s*), two methine proton signals indicative of secondary alcoholic functions at δ 3.56 (1H, *br m*) and 4.68 (1H, *dd*, J = 4.0 and 10.0 Hz) and an olefinic proton signal at δ 5.38 (H-6, *m*). Also evident were the signals of a *trans* cinnamoyl residue [δ 6.43 (1H, *d*, J = 16.0 Hz, H-2′), 7.44 (3H overlapped, H-6′, H-7′, H-8′), 7.65 (3H overlapped, H-3′, H-5′, H-9′)]. The ^{13}C NMR spectrum of **1** also suggested for the aglycon moiety a pregnane skeleton with two secondary alcoholic functions (δ 79.3 and 74.9) and three tertiary alcoholic functions (δ 74.3, 89.9 and 93.0). On the basis of HSQC and HMBC data the aglycon of **1** was identified as the 12-*O*-cinnamoyl ester of 3β,8β,12β,14β,17β-tetrahydroxypregnan-20-one, named kidjolanin (Sasaki et al., 1972). The ^{13}C NMR spectrum of **1** showed, in addition to the aglycone signals, 33 signals ascribable to a sugar portion made up of two 3-*O*-methyl-2,6-dideoxyhexopyranose units, one 3-*O*-methyl-6-deoxyhexopyranose and two hexopyranose units. Five signals were assigned to anomeric carbons (δ 97.2, 101.1, 104.2, 104.7 and 106.0), three to methoxyl groups (δ 58.4, 58.6 and 61.0) and four to methyl groups (δ 18.5, 18.6, 18.7). In the ^{1}H NMR spectra three methoxyl- (δ 3.44 × 2 and 3.65), three secondary methyl- (δ 1.21, 1.33 and 1.41) and five anomeric proton (δ 4.38, 4.44, 4.50, 4.83 and 4.91) signals also supported the above results. The structure elucidation of the sugar portion was achieved by 1D-TOCSY, DQF–COSY, HSQC and HMBC experiments (Table 1). The isolated anomeric proton signals resonating at the uncrowded region of the spectrum (between δ 4.38 and 4.91) were the starting point for the 1D-TOCSY experiments. The 1D-TOCSY subspectra obtained by irradiating the anomeric proton signal at δ 4.44 and 4.50 allowed us to establish these protons as belonging to two glucose units. In the case of the 2,6-dideoxyhexoses and the 6-deoxyhexose, an easier identification of all the proton signals was obtained by recording the 1D-TOCSY experiments also irradiating the methyl doublets.

The identity of each proton signal in 1D-TOCSY spectra was deduced by a DQF–COSY experiment which allowed the sequential assignments of all proton resonances within each sugar residue, starting from the well isolated anomeric proton signals (Table 1). The β-configurations of the five sugar units were shown by the large (J = 7.5 for the two glucose units and the thevetose

unit) and J between 9.0 and 9.5 for the 2,6-dideoxyhexoses) coupling constants of the anomeric proton signals (Agrawal, 1992). A HSQC experiment which correlated each hydrogen signal to the corresponding carbon signal allowed the assignment of all the carbon resonances and therefore the identification of the sugars as a terminal β-glucopyranosyl unit (H-1 = δ 4.44), and a second β-glucopyranosyl unit (H-1 = δ 4.50), two β-cymaropyranosyl units (H-1 = δ 4.83; H-1 = δ 4.91) and one β-thevetopyranosyl unit (H-1 = δ 4.38). All the substituted sugars were glycosidated at C-4. The sugar sequence was deduced from the HMBC spectrum which showed long-range correlations between C-3_{agl} (δ 79.3) and H-1_{cymI} (δ 4.91), C-4_{cymI} (δ 83.8) and H-1_{cymII} (δ 4.83), C-4_{cymII} (δ 84.1) and H-1_{the} (δ 4.38), C-4_{the} (δ 83.0) and H-1_{gluI} (δ 4.50), C-4_{gluI} (δ 81.1) and H-1_{gluII} (δ 4.44).

The absolute configurations of the sugar units were assigned after acid hydrolysis and fractionation of the hydrolysate by silica gel column chromatography which

Table 1
^{1}H NMR and ^{13}C NMR data of sugar moieties of compounds **1**–**3**[a]

	1 (Alpinoside A)		**2** (Alpinoside B)		**3** (Alpinoside C)	
	^{1}H NMR	^{13} C NMR	^{1}H NMR	^{13} C NMR	^{1}H NMR	^{13} C NMR
	β-D-cymarose		β-D-cymarose		β-D-cymarose	
1″	4.91 *dd*, *J* = 9.5, 2.0	97.2	4.91 *dd*, *J* = 9.6. 1.8	97.2	4.91 *dd*, *J* = 9.5, 2.0	97.3
2″	2.10 *m*, 1.58 *m*	36.6	2.10 *m*, 1.58 *m*	36.6	2.10 *m*, 1.58 *m*	36.6
3″	3.90 *q*, *J* = 3.0 Hz	78.1	3.86 *q*, *J* = 3.0	78.7	3.82 *q*, *J* = 3.0	78.0
4″	3.29 *dd*, *J* = 9.5, 3.0	83.8	3.24 *dd*, *J* = 9.5, 3.0	83.8	3.25 *dd*, *J* = 9.5, 3.0	83.9
5″	3.87 *dq*, *J* = 9.5, 6.5	69.9	3.83 *dq*, *J* = 9.5, 6.5	69.9	3.84 *dq*, *J* = 9.5, 6.5	69.8
6″	1.21 *d*, *J* = 6.5	18.5	1.21 *d*, *J* = 6.5	18.5	1.21, *d*, *J* = 6.5	18.5
OMe	3.44 *s*	58.4	3.44 *s*	58.5	3.44 *s*	58.4
	β-D-cymarose		β-D-cymarose		β-D-cymarose	
1‴	4.83 *dd*, *J* = 9.6, 1.8	101.1	4.83 *dd*, *J* = 9.6, 1.8	101.2	4.84 *dd*, *J* = 9.6, 1.8	101.2
2‴	2.18 *m*, 1.62 *m*	36.4	2.18 *m*, 1.62 *m*	36.4	2.18 *m*, 1.61 *m*	36.4
3‴	3.89 *q*, *J* = 3.0	78.1	3.85 *q*, *J* = 3.0	78.0	3.88 *q*, *J* = 3.0	78.4
4‴	3.32 *dd*, *J* = 9.5, 3.0	84.1	3.32 *dd*, *J* = 9.5, 3.0	83.8	3.29 *dd*, *J* = 9.5, 3.0	83.9
5‴	3.92 *dq*, *J* = 9.5, 6.5	70.0	3.92 *dq*, *J* = 9.5, 6.5	69.9	3.88 *dq*, *J* = 9.5, 6.5	69.8
6‴	1.33 *d*, *J* = 6.5	18.7	1.33 *d*, *J* = 6.5	18.5	1.32 *d*, *J* = 6.5	18.5
OMe	3.44 *s*	58.6	3.44 *s*	58.5	3.44 *s*	58.5
	β-D-thevetose		β-D-thevetose		β-D-oleandrose	
1⁗	4.38 *d*, *J* = 7.0	106.0	4.35 *d*, *J* = 7.0	106.1	4.63 *dd*, *J* = 9.0, 1.5	102.7
2⁗	3.34 *dd*, *J* = 7.0, 9.5	71.4	3.30 *dd*, *J* = 7.0, 9.5	71.4	2.36 *ddd*, *J* = 1.5, 4.0, 13.0 1.47 *ddd J* = 9.0,9.5, 13.0	37.8
3⁗	3.22 *dd*, *J* = 9.5, 9.5	86.0	3.22 *dd*, *J* = 9.5, 9.5	86.1	3.43 *ddd*, *J* = 4.0, 9.5, 9.5	80.3
4⁗	3.41 *dd*, *J* = 9.5, 9.5	83.0	3.38 *dd*, *J* = 9.5, 9.5	82.7	3.30 *dd*, *J* = 9.5, 9.5	83.7
5⁗	3.50 *m*	72.4	3.48 *m*	72.6	3.43 *dq*, *J* = 9.5, 6.2	72.4
6⁗	1.41 *d*, *J* = 6.5	18.6	1.39 *d*, *J* = 6.5	18.67	1.42 *d*, *J* = 6.2	18.5
OMe	3.65 *s*	61.0	3.65 *s*	61.0	3.48 *s*	58.0
	β-D-glucose		β-D-glucose		β-D-glucose	
1⁗′	4.50 *d*, *J* = 7.0	104.2	4.46 *d*, *J* = 7.0	104.3	4.52 *d*, *J* = 7.0	103.8
2⁗′	3.29 *dd*, *J* = 7.0,9.5	74.9	3.21 *dd*, *J* = 7.0,9.5	75.5	3.27 *dd*, *J* = 7.0,9.5	75.0
3⁗′	3.55 *dd*, *J* = 9.5, 9.5	76.4	3.37 *dd*, *J* = 9.5, 9.5	78.0	3.55 *dd*, *J* = 9.5, 9.5	76.4
4⁗′	3.57 *dd*, *J* = 9.5, 9.5	81.1	3.24 *dd*, *J* = 9.5, 9.5	71.6	3.54 *dd*, *J* = 9.5, 9.5	80.8
5⁗′	3.42 *ddd*, *J* = 3.0, 5.0, 9.5	76.8	3.28 *ddd*, *J* = 3.0, 5.0, 9.5	78.1	3.42 *ddd*, *J* = 3.0, 5.0, 9.5	76.7
6⁗′	3.83 *dd*, *J* = 12.0, 5.0 3.98 *dd*, *J* = 12.0, 3.0	62.3	3.67 *dd*, *J* = 12.0, 5.0 3.89 *dd*, *J* = 12.0, 3.0	62.6	3.84 *dd*, *J* = 12.0, 5.0 3.96 *dd*, *J* = 12.0, 3.0	62.0
	β-D-glucose				β-D-glucose	
1⁗″	4.44 *d*, *J* = 7.0	104.7			4.44 *d*, *J* = 7.0 Hz	104.6
2⁗″	3.25 *dd*, *J* = 7.0,9.5	74.9			3.25 *dd*, *J* = 7.0,9.5	75.3
3⁗″	3.33 *dd*, *J* = 9.5, 9.5	77.8			3.33 *dd*, *J* = 9.5, 9.5	78.0
4⁗″	3.35 *dd*, *J* = 9.5, 9.5	71.4			3.35 *dd*, *J* = 9.5, 9.5	71.4
5⁗″	3.38 *ddd*, *J* = 3.0, 5.0, 9.5	77.9			3.38 *ddd*, *J* = 3.0, 5.0, 9.5	78.0
6⁗″	3.67 *dd*, *J* = 12.5, 5.0 3.92 *dd*, *J* = 12.5, 3.5	62.5			3.67 *dd*, *J* = 12.5, 5.0 3.92 *dd*, *J* = 12.5, 3.5	62.3

[a] Assignments were confirmed by 1D-TOCSY, DQF–COSY, HSQC and HMBC experiments. *J* values are reported in Hz.

afforded cymarose and thevetose (Abe et al., 1994). This sugars were believed to be in the D-form on the basis of their optical rotation values. In the case of glucose the absolute configuration was assigned after hydrolysis of **1** with 1 N HCl, trimethylsilation of the hydrolisate, and GC analysis on a Chirasil-Val column. The absolute configuration of glucose was established as D by comparison with retention times of authentic samples of D and L glucose (Lee et al., 2000). Thus compound **1** was defined as the new kidjolanin 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-thevetopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside, named alpinoside A.

Compound **2** ($C_{57}H_{84}O_{22}$) showed an $[M-H]^-$ ion at *m/z* 1119, 162 mass units lower than that observed for **1**, and fragment at *m/z* 971 $[(M-H)-148]^-$ attributable to the loss of a cinnamic acid residue, and *m/z* 957 $[(M-H)-162]^-$ due to the cleavage of one hexose unit. Also evident were peaks at *m/z* 797 and 653 arising from the sequential losses of a methyldeoxyhexopyranose (160 mass units) and a methyldideoxyhexopyranose (144 mass units) from the ion at *m/z* 957.

The analysis of the NMR data of **2** revealed the same aglycone as in **1** and a sugar chain made up of 4 monosaccharides (Table 1), differing from that of **1** for the absence of the terminal glucopyranosyl unit. The sugar sequence was confirmed from the HMBC spectrum which showed long-range correlations between C-3_{agl} (δ 79.3) and H-1_{cymI} (δ 4.91), C-4_{cymI} (δ 83.8) and H-1_{cymII} (δ 4.83), C-4_{cymII} (δ 83.8) and H-1_{the} (δ 4.35), C-4_{the} (δ 82.7) and H-1_{glu} (δ 4.46).

Thus compound **2** was defined as the new kidjolanin 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-thevetopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside, named alpinoside B.

Compound **3** had the molecular formula $C_{63}H_{94}O_{26}$, as determined from ^{13}C and ^{13}C DEPT NMR and ESIMS in negative-ion mode. The ESIMS of **1** showed an $[M-H]^-$ ion at *m/z* 1265 and prominent fragments at *m/z* 1117 $[(M-H)-148]^-$ attributable to the loss of a cinnamic acid residue, *m/z* 1103 $[(M-H)-162]^-$ and *m/z* 941 $[(M-H)-(162\times2)]^-$ due to the cleavage of one and two hexose units, respectively. Also evident were peaks at *m/z* 797 and 653 arising from the sequential losses of two methyldideoxyhexopyranose (144 mass units) from the ion at *m/z* 941. Comparison of ^1H and ^{13}C NMR data of **3** with those of **2** clearly showed that the difference between the two compounds should be confined to a sugar residue. In the ^1H NMR of **3** the anomeric proton signal at δ 4.38 ascribable to thevetose in **1** was absent while an anomeric proton signal at δ 4.63 was evident. The 1D-TOCSY spectrum obtained by irradiating this signal clearly showed the spin system of an oleandropyranose unit. The absolute configurations of the sugar units were assigned after acid hydrolysis and fractionation of the hydrolysate by silica gel column chromatography which afforded cymarose and oleandrose. This sugars were believed to be in the D-form on the basis of their optical rotation values. The absolute configuration of glucose was established as D after hydrolysis and GC analysis. Analysis of HMBC data which showed the long range correlations between C-3_{agl} (δ 79.3) and H-1_{cymI} (δ 4.91), C-4_{cymI} (δ 83.9) and H-1_{cymII} (δ 4.84), C-4_{cymII} (δ 83.9) and H-1_{ole} (δ 4.63), C-4_{ole} (δ 83.7) and H-1_{gluI} (δ 4.52), C-4_{gluI} (δ 80.8) and H-1_{gluII} (δ 4.44) allowed the identification of **3** as kidjolanin 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside, named alpinoside C.

Compounds **1**–**3** are closely related to pregnane glycosides previously reported in the roots of *O. esculentum* some of which possess kidjolanin as aglycon but different sugar chains (Trivedi et al., 1988).

3. Experimental

3.1. General

NMR spectra in CD_3OD were obtained using a Brüker DRX-600 spectrometer, operating at 599.19 MHz for ^1H and 150.86 MHz for ^{13}C. 2D experiments: ^1H–^1H DQF–COSY (double filtered direct chemical shift correlation spectroscopy), inverse detected ^1H–^{13}C HSQC (heteronuclear single quantum coherence), HMBC (heteronuclear multiple bond connectivity), were obtained using UXNMR software. Selective excitation spectra, 1D-TOCSY were acquired using waveform generator-based GAUSS shaped pulses, mixing time ranging from 100 to 120 ms and a MLEV-17 spinlock field of 10 kHz preceded by a 2.5 ms trim pulse. ESIMS were performed on a Finnigan LC-Q Deca Ion Trap mass spectrometer scanned from 150 to 1200 Da. The mass spectral data were acquired and processed using Xcalibur software. Samples were dissolved in MeOH and infused in the ESI source by using a syringe pump at a flow rate of 3 μl/min. The capillary voltage was 5 V, the spray voltage 5 kV and the tube lens offset 50 V. The capillary temperature was 220 °C. Exact masses were measured by a Q-Star Pulsar (Applied Biosystems, Foster City, CA) triple-quadrupole orthogonal time-of-flight (TOF) instrument. Electrospray ionization was used in TOF mode at 10.000 resolving power. Samples were dissolved in TFA 0.1% acetonitrile/water (1:1), mixed with the internal calibrant, and introduced directly into the ion source by direct infusion. Optical rotations were measured on a Perkin-Elmer 192 polarimeter equipped with a sodium lamp (589 nm). GC analysis was performed on a Termo Finnigan Trace GC apparatus using an 1-Chirasil-Val column (0.32 mm × 25 m).

3.2. Plant material

The plant material was collected at Gazal Island (Aswan, Egypt) in December 2001 and the original plant was identified by members of the Botany Department, Aswan Faculty of Science, South Valley University, Egypt. A voucher specimen (No. 10497) has been deposited at the Herbarium of the Faculty of Science, South Valley University, Aswan.

3.3. Extraction and isolation

The dried aerial parts (1.35 kg) of *Oxystelma esculentum* var. *alpini* was exhaustively extracted with EtOH:H_2O (7:3) in a Soxhlet apparatus. The alcoholic extract was condensed under reduced pressure to a syrupy consistency (279.9 g); 129 g from crude extract was dissolved in MeOH:H_2O (2:1) and transferred into a separator funnel. The extract was shaken with hexane, $CHCl_3$ and *n*-BuOH, respectively till exhaustion.

The chloroform fraction (13.4) was loaded on silica gel column. Elution was started with chloroform, and the polarity was gradually increased with addition of MeOH. Fractions eluted with $CHCl_3$–MeOH (8:2) gave compounds **1** (139 mg), **2** (440 mg) and **3** (1.225 g).

3.3.1. Alpinoside A (*1*)

Amorphous powder $[\alpha]_D^{25} = -32.8$ (MeOH; *c* 0.1); ESIMS in negative ion mode (*m*/*z*): 1281 $[M-H]^-$, 1133 $[(M-H)^-$ 148], 1119 [(M−H) −162]$^-$ 957 [(M−H) $-(162\times2)]^-$ 797 $[957-160]^-$, 653 $[957-(160+144)]^-$; HRESIMS *m*/*z* $[M+H]^+$ calcd for $C_{63}H_{94}O_{27}$ 1283.6061, found 1283.6099; ^{1}H NMR of the aglycone moiety (CD_3OD): δ 1.19 (3H, *s*, Me-19), 1.63 (3H, *s*, Me-18), 2.26 (3H, *s*, Me-21), 3.56 (*br m*, H-3), 4.68 (1H, *dd*, *J* = 4.0 and 10.0 Hz, H-12), 5.38 (1H, *m*, H-6), 6.43 (1H, *d*, *J* = 16.0 Hz, H-2′); 7.44 (3H overlapped, H-6′, H-7′, H-8′), 7.65 (3H overlapped, H-3′, H-5′. H-9′); ^{13}C NMR of the aglycone portion (CD_3OD): δ 10.4 (C-18), 18.5 (C-19), 25.4 (C-11), 27.8 (C-21), 30.2 (C-2), 33.3 (C-16), 34.2 (C-15), 35.2 (C-7), 38.1 (C-10), 39.1 (C-1), 39.8 (C-4), 45.1 (C-9), 58.8 (C-13), 74.3 (C-8), 74.9 (C-12), 79.3 (C-3), 89.9 (C-14), 93.0 (C-17), 118.9 (C-2′), 119.7 (C-6), 129.2 (C-5′, C-9′), 130.1 (C-6′, C-8′), 131.6 (C-7′), 135.7 (C-4′), 140.2 (C-5), 146.4 (C-3′), 167.3 (C-1′), 212.2 (C-20); ^{1}H and ^{13}C NMR of the sugar portion: Table 1.

3.3.2. Alpinoside B (*2*)

Amorphous powder $[\alpha]_D^{25} = -14.6$ (MeOH; *c* 0.1); ESIMS in negative ion mode (*m*/*z*): 1119 $[M-H]^-$, 971 [(M−H) −148], 957 [(M−H) −162]$^-$ 957 [(M−H) $-(162\times2)]^-$ 797 $[957-160]^-$, 653 $[957-(160+144)]^-$; HRESIMS *m*/*z* $[M+H]^+$ calc. for $C_{57}H_{84}O_{22}$ 1121.5533, found 1121.5589; ^{1}H and ^{13}C NMR of the aglycone moiety (CD_3OD): super imposable on those reported for **1**; ^{1}H and ^{13}C NMR of the sugar portion: Table 1.

3.3.3. Alpinoside C (*3*)

Amorphous powder $[\alpha]_D^{25} = -26.2$ (MeOH; *c* 0.1); ESIMS in negative ion mode (*m*/*z*): 1265 $[M-H]^-$, 1117 [(M−H) −148], 1103 [(M−H) −162]$^-$ 941 [(M−H) $-(162\times2)]^-$ 797 $[941-144]^-$, 653 $[957-(160+144)]^-$; HRESIMS *m*/*z* $[M+H]^+$ calc. for $C_{63}H_{94}O_{26}$ 1267.6112, found 1283.6197; ^{1}H and ^{13}C NMR of the aglycone moiety (CD_3OD): super imposable on those reported for **1**; ^{1}H and ^{13}C NMR of the sugar portion: Table 1.

3.4. Acid hydrolysis of compounds *1* and *3*

Each compound (80 mg) was heated at 60 °C for 6 h with dioxane (2 ml) and 0.2 N H_2SO_4 (1 ml) to yield kidjolanin and sugars. After hydrolysis, the reaction mixture was diluted with H_2O and extracted with EtOAc. The H_2O layer was neutralized and the eluate was concentrated to dryness. The residue was chromatographed on a silica gel column with $CHCl_3$–MeOH–H_2O (7:1:1.2 bottom layer) system to obtain cymarose (7.2 mg) and thevetose (4 mg) in the case of **1** and cymarose (6.8 mg) and oleandrose (3.2 mg) in the case of **3**.

Cymarose, oleandrose and thevetose were believed to belong to the D series on the basis of their optical rotation values (Abe et al., 1994).

D-Cymarose: $[\alpha]_D^{25} + 54.2$ (*c* = 0.1, 24 h after dissolution in H_2O);
D-Oleandrose: $[\alpha]_D^{25} - 12.2$ (*c* = 0.1, 24 h after dissolution in H_2O);
D-Thevetose: $[\alpha]_D^{25} + 42.3$ (*c* = 0.1, 24 h after dissolution in H_2O).

3.5. GC analysis to determine absolute configuration of glucose

A solution (2 mg each) of compounds **1** and **3** in 1N HCl (0.5 ml) was stirred at 80 °C for 4 h. After cooling, the solution was concentrated by blowing with N_2 . The residue was dissolved in 1-(trimethylsilyl)imidazole and pyridine (0.2 ml), and the solution was stirred at 60 °C for 5 min. After drying the solution with a stream of N_2, the residue was separated by water and CH_2Cl_2 (1 ml, 1:1 v/v). The CH_2Cl_2 layer was analyzed by GC using an 1-Chirasil-Val column (0.32 mm × 25 mm). Temperatures of the injector and detector were 200 °C for both. A temperature gradient system was used for the oven, starting at 100 °C for 1 min and increasing up to 180 °C at a rate of 5 °C/min. The peak of glucose was detected at 14.71 min. Retention time for authentic samples of D-glucose and L-glucose after being treated simultaneously with 1-(trimethylsilyl)imidazole in pyridine

were detected at 14.74 and 14.59 min, respectively (Lee et al., 2000).

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