Three Sesquiterpene Glycosides from *Elsholtzia bodinieri*

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As a species of the genus *Elsholtzia wild* (Labiatae), *E. bodinieri* Van't is an annual herbaceous plant, widely distributed in the mountainous area of the west and southwest district of China (Chinese name "Dongzisu").[1] which has been mainly used as a traditional Chinese folk drug for the treatment of eczema, enteritis, diarrhea, bacillary dysentery and cold, and are also known to have anticancer and antibacterial effects.[2] Regarding the chemical constituents of this plant, the presence of volatile oil, triterpenoids, flavones, β-carotene and phenols in the aerial parts has been previously reported.[3-6] However, as a continuation of our efforts to pursue the active natural products from *E. bodinieri*, three eudesmane-type sesquiterpene glycosides were isolated by repeated column chromatography and preparative TLC from the *n*-BuOH fraction of the methanolic extract of *E. bodinieri* gathered in Gansu province of China. Their structures were elucidated as integrifoside A (**1**),[7] dictamnoside G (**2**)[8] and 3β,5α,11,12,13-pentahydroxy-eudesm-4(15)-ene 3-*O*-β-*D*-apiofuranosyl-(1→4)-α-*L*-rhamnopyranosyl-(1→3)-β-*D*-glucopyranoside (**3**), respectively. Among these, compound **3** was novel, and all compounds were firstly isolated from *E. bodinieri* family. In this paper, we report the isolation and structural elucidation of the novel natural product using chemical and spectroscopic evidence.

Compound **3** was isolated as an amorphous powder from MeOH and responded positively to the Molish test for glycoside. The molecular formula was established as $C_{32}H_{54}O_{18}$ from the HRFAB-MS (positive) ion peak at *m/z* 749.3196 $[M+Na]^+$ ($C_{32}H_{54}O_{18}Na$, calcd. for 749.3208), corresponding to 6 degrees of unsaturation. Its IR spectrum (KBr) indicated the presence of hydroxyl groups (3445-3100 cm^{-1}), exocyclic double bond (1634 cm^{-1}) and glycoside functionalities (1086, 1070 and 1036 cm^{-1}). The positive-ion FAB-MS displayed 595 $[M+H-132]^+$, 449 $[M+H-132-146]^-$ and 287 $[M+H-132-146-162]^-$ fragments, which showed the presence of one terminal pentose, one centre 6-deoxysugar and one inner hexose units in a linear linkage. This was also confirmed by the NMR spectra which showed typical signals of three anomeric protons at δ_H 4.85 (1H, d, J = 8.0 Hz), 6.30 (1H, br s) and 5.66 (1H, d, J = 2.2 Hz), two hydroxymethyl groups at δ_H 4.45 (1H, d, J = 9.5 Hz)/4.29 (1H, d, J = 9.5 Hz) and 4.51 (1H, m)/4.31 (1H, dd, J = 5.5, 11.5 Hz), one epoxymethylene group at δ_H 4.17(1H, d, J = 10.7 Hz)/4.09 (1H, d, J = 10.7 Hz) and one methyl doublet at δ_H 1.70 (1H, d, J = 6.4 Hz), corresponding to carbon signals at δ_C 104.4, 102.6, 111.6, 75.1, 62.5, 65.7 and 18.9. This indicated that three of the unsaturations were due to three sugar rings, and the remaining three should be due to one exocyclic double bond and one fused bicyclic system. The acid hydrolysis of compound **1** yielded *D*-apiose, *D*-glucose and *L*-rhamnose (in the molar ratio of 1 : 1 : 1), respectively, which were compared with authentic samples by *co*-PC and GC analysis.

Besides 17 carbon signals for sugars, 15 additional carbon signals, including one primary methyl (shielded sp^3 carbon), eight secondary methylenes (five alicyclic sp^3, two oxygenated sp^3 and one terminal olefinic carbons), two methines (one alicyclic sp^3 and one diastatic alicyclic sp^3 carbons) and four quaternary carbons (one oxygenated alicyclic sp^3, one oxygenated aliphatic sp^3, one alicyclic sp^3 and one olefinic carbon) were recognized in the broad band decoupled ^{13}C NMR spectrum, suggesting the presence of the bicyclic sesquiterpene moiety in the molecule. The analysis of NMR spectra by the aid of DEPT technique demonstrated the presence of an angular methyl group (δ_H 0.82 and δ_C 20.1) and one exocyclic double bond [δ_H 4.92 (1H, d, J = 2.0 Hz)/4.78 (1H, d, J = 2.0 Hz), corresponding to δ_C 150.3 and 104.7], characteristic of a typical $\Delta^{4(15)}$-eudesmene skeleton.[9,10] The signals at δ_C 85.9 (C-3), 76.4 (C-5), 76.0 (C-11), 64.5 (C-12) and 64.9 (C-13), together with δ_H 4.72 (1H, dd, J = 6.0, 10.0 Hz, H-3), 3.62 (2H, m, H-12) and 3.64 (2H, m, H-13), suggesting that five hydroxyl groups were separately linked at C-3, C-5, C-11, C-12 and C-13, respectively. The *trans*-stereochemistry at the junction of the rings A and B could be deduced from the observed NOE interactions of H-14 with H_{ax}-2/H_{ax}-6/H_{ax}-8 and HO-5 with H-3/H-7/H_{ax}-1/H_{ax}-9 in the NOESY experiments, which also indicated that HO-5 and CH_3-14 were α,β-oriented, respectively. The β-configurations of the substituents attached to C-3 and C-7, were determined from the chemical shifts and coupling constants of H-3 (4.72, dd, J = 6.0, 10.0 Hz) and H-7 (2.17, dd, J = 4.0, 12.0 Hz), and further evidenced by the observed NOE interactions of H-3 with H-1/HO-5 and H-7 with H_{ax}-9/HO-5 in the NOESY spectrum. By comparison of the spectral data of the aglycon with 3β-acetoxy-5α,11,12,13-tetrahydroxy-eudesm-4(15)-ene isolated previously from *Achillea holosericea*,[11] suggested that the aglycon of **1** was 3β,5α,11,12,13-pentahydroxy-eudesm-4(15)-ene.

Comparison of NMR data of the sugar moieties with literature values[12] revealed that the glucose and rhamnose were present in pyranose forms, whereas the apiose was in

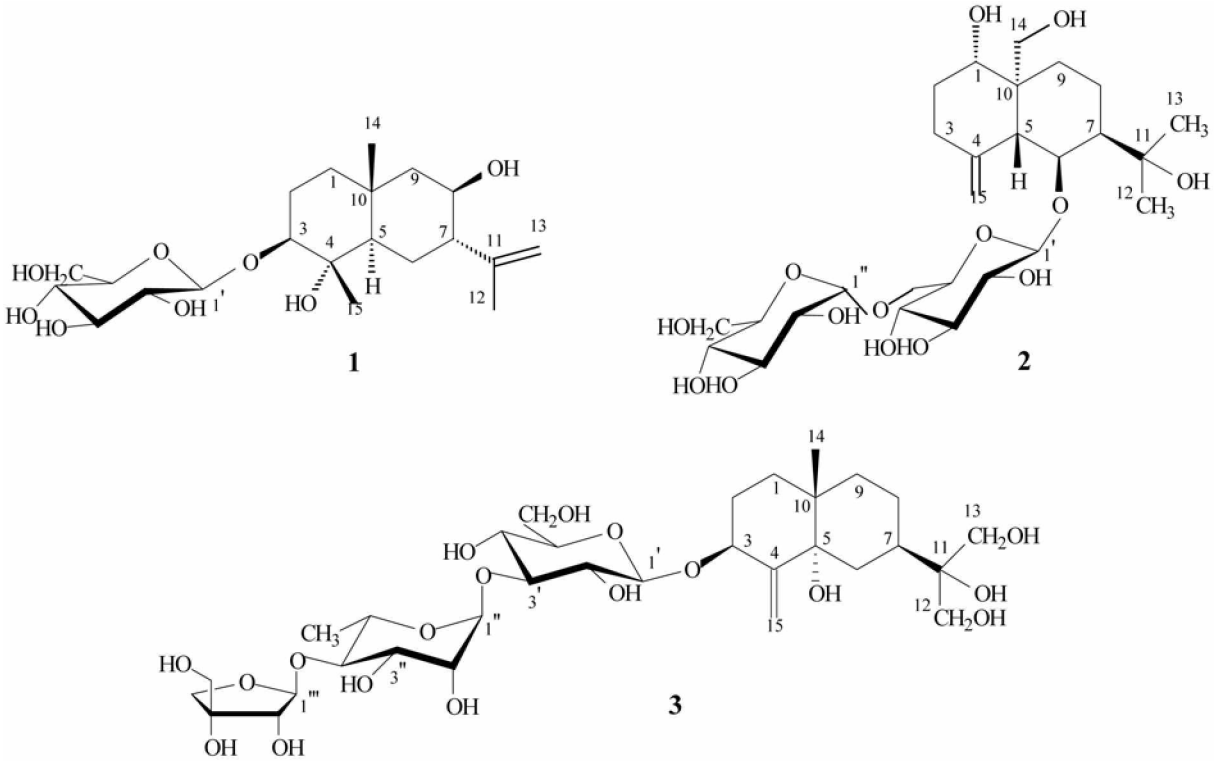

Figure 1. The structures of compound **1**-**3**.

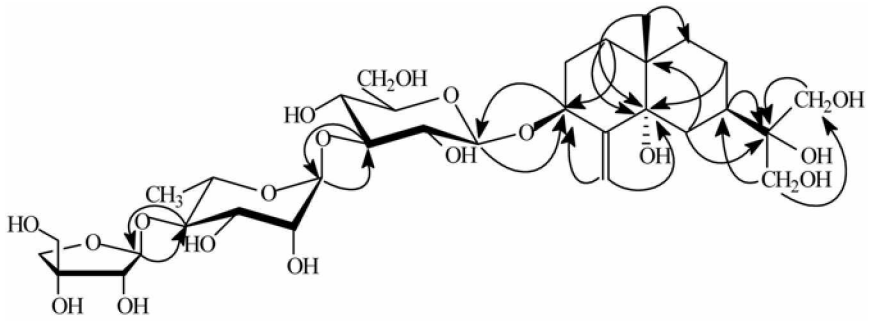

Figure 2. The key HMBC correlations (H⟶C) of compound **3**.

furanose form. The β-anomeric configurations of the apiofuranose and glucopyranose units were determined by their $^3J_{H\text{-}1,H\text{-}2}$ coupling constants (2.2 and 8.0 Hz),[13] and rhamnopyranose unit was determined as the α-configuration by the characteristic broad singlet of its anomeric proton and ^{13}C NMR data.[14] These above conclusions were further confirmed by three strong NOE observed for H-1' with H-3'/H-5' of glucopyranose, H-1" with H-2" of rhamnopyranose and H-1'" with H-5'" of apiofuranose in the NOESY spectrum. The HMBC correlations (Figure 2) between H-1'" of the terminal apiose unit and C-4" of the centre rhamnose unit, H-4" of the centre rhamnose unit and C-1'" of the terminal apiose unit, H-1" of the centre rhamnose unit and C-3' of the inner glucose unit, together with H-3' of the inner glucose unit and C-1' of the centre rhamnose unit, suggested the linkage of β-*D*-apiofuranosyl-(1⟶4)-α-*L*-rhamnopyranosyl-(1⟶3)-β-*D*-glucopyranosyl. Further supporting information came from the observed NOE interactions of H-4"/H-1'" and H-3'/H-1" in the NOESY experiments (Figure 3), together with the FAB-MS ion peaks at *m*/*z* 595 $[M+H-132]^+$ (loss of a terminal apiose unit), 449 $[M+H-132-146]^+$ (loss of a centre rhamnose unit) and 287 $[M+H-132-146-162]^-$ (loss of a inner glucose unit). Facile acid hydrolysis of the glycoside clearly indicated C-O-C type of linkage between aglycon and sugar moiety.[15] The exact position of the trisaccharide chain at C-3 of the aglycon was established from the HMBC correlation between the H-1' (δ_H 4.85) of the inner β-glucose unit and the C-3 (δ_C 85.9) of aglycon, this was also supported by the observed NOE interaction of H-3 with H-1' in the NOESY spectrum. On the basis of all the foregoing statements, the structure of compound **3** was established as 3β,5α,11,12,13-pentahydroxy-eudesm-4(15)-ene 3-*O*-β-*D*-apiofuranosyl-(1⟶4)-α-*L*-rhamnopyranosyl-(1⟶3)-β-*D*-glucopyranoside. To the best of our knowledge, **3** has not been reported previously from any plant source.

The known compounds **1** and **2** were identified by spectral

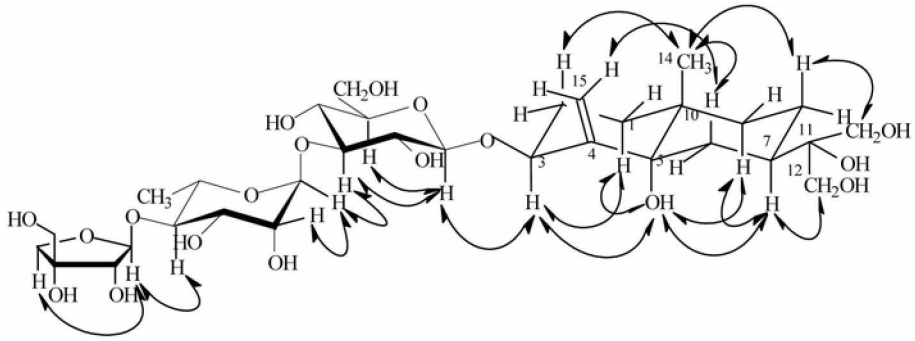

Figure 3. The key NOESY correlations of compound **3**.

analysis and comparison of relevant data with those already reported in the literature.

Experimental Section

General Methods. Melting points were measured on a Chinese X-4 melting point apparatus (uncorrected); Optical rotation was determined on a Perkin-Elmer 341 automatic polarimeter; IR spectra (KBr disks) were obtained on Alpha-Centari FT-IR spectrometer; NMR spectra were scanned on Bruker AM-500 spectrometer (chemical shifts in δ down-field from TMS internal standard) operating 500 and 125 MHz for ^{1}H and ^{13}C NMR respectively, and FAB-MS spectra on VG Autospect 3000 spectrometer; TLC was performed on silica gel GF_{254} plates (0.50 mm thickness); GC on a Hewlett Packard HP 5980 gas chromatography. The spots were visualized under UV light or by exposure to I_2 vapours as well as by spraying (analyt. TLC) with 10% H_2SO_4 in EtOH, followed by heating for a few minute; Separation and purification were performed by column chromatography on silica gel (100-200, 200-300 mesh). All solvents were distilled before use.

Plant Material. The root barks of *E. bodinier* were collected in August 2004 from Ziwuling mountainous area of Gansu Province in China, and identified by Fu-Shun Liu (Department of Biology, Longdong University, China). A voucher specimen (No.107083) of the plant is previously deposited at the Herbarium of the Botany Department, Longdong University, Qingyang, 745000, China.

Extraction and Isolation. The air-dried root barks of *E. bodinier* (2.8 kg) were soxhlet extracted successively with MeOH for 3 days. The extracts were evaporated to dryness. The residue (155 g) was suspended in warm water and extracted with water-saturated *n*-BuOH. The organic layer was concentrated to obtained a residue (80 g) which was redissolved in MeOH (300 mL). Addition of EtOAc gave a flocculent precipitate which was washed with EtOAc (three time), yielded a crude saponin mixture (35 g) which was subjected to silica gel column chromatography (100-200 mesh) with EtOAc-MeOH-H_2O (10 : 4 : 1→100% MeOH, v/v/v) in increasing polarity and combined by monitoring with TLC to give ten fractions (A-M). Fraction F was repeatedly subjected to MCI-gel CHP 20P and silica gel column chromatography (200-300 mesh) to afford **2** (12 mg). Fraction I was further purified by preparative TLC with Me_2CO-MeOH (1 : 8, v/v) as development to provide **1** (15 mg). Fraction L was separated successively over Sephadex LH-20 (MeOH-H_2O 8 : 2, v/v) and silica gel column ($CHCl_3$-MeOH-H_2O 6 : 4 : 1→10 : 5:1, v/v/v) to obtain **3** (11 mg).

Integrifoside A (1) : White crystals from MeOH, $C_{21}H_{36}O_8$, mp. 152-155 °C, $[\alpha]_D^{20}$ −13.5° (c = 4.9, MeOH); HRFAB-MS (positive-ion mode) *m/z*: 439.2314 $[M+Na]^-$ ($C_{21}H_{36}O_8Na$, calcd. for 439.2308); FAB-MS *m/z*: 417 $[M+H]^-$, 255 $[M+H-162]^+$; ^{1}H-NMR (500 MHz, pyridine-d_5): δ_H 1.15/1.41 (2H, m, H-1), 2.01/1.72 (2H, m, H-1), 3.77 (1H, dd, *J* = 2.5, 12.0 Hz, H-3), 1.70 (1H, d, *J* = 13.0 Hz, H-3), 2.44 (1H, d, *J* = 13.0 Hz, H-6α), 2.26 (1H, m, H-6β), 2.78 (1H, br s, H-7), 4.58 (1H, br s, H-8), 1.62 (1H, d, *J* = 12.0 Hz, H-9α), 1.76 (1H, m, H-9β), 1.87 (1H, s, H-12), 5.00 (1H, s, H-13α), 5.09 (1H, s, H-13β), 1.41 (1H, s, H-14), 1.39 (1H, s, H-15), 4.87 (1H, d, *J* = 8.0 Hz, H-1'), 4.01 (1H, m, H-2'), 4.23 (1H, m, H-3'), 4.17 (1H, m, H-4'), 3.98 (1H, m, H-5'), 4.51 (1H, dd, *J* = 5.5, 11.5 Hz, H-6'α), 4.26 (1H, d, *J* = 11.5 Hz, H-6'β); ^{13}C-NMR (125 MHz, pyridine-d_5): δ_C 40.2 (C-1), 26.3 (C-2), 90.2 (C-3), 73.4 (C-4), 46.8 (C-5), 19.1 (C-6), 48.7 (C-7), 68.6 (C-8), 47.3 (C-9), 34.5 (C-10), 146.1 (C-11), 23.5 (C-12), 111.8 (C-13), 21.4 (C-14), 18.5 (C-15), 104.8 (C-1'), 74.6 (C-2'), 78.1 (C-3'), 71.4 (C-4'), 78.1 (C-5'), 62.3 (C-6').

Dictamnoside G (2): White powder from MeOH, $C_{27}H_{46}O_{14}$, mp. 165-167 °C, $[\alpha]_D^{20}$ −8.6° (c = 3.5, MeOH); HRFAB-MS (positive-ion mode) *m/z*: 617.2781 $[M+Na]^+$ ($C_{27}H_{46}O_{14}Na$, calcd. for 617.2785); FAB-MS *m/z*: 595 $[M+H]^+$, 433 $[M+H-162]^-$, 271 $[M+H-162-162]^-$; ^{1}H-NMR (500 MHz, pyridine-d_5): δ_H 3.90 (1H, m, H-1), 2.18/2.05 (2H, m, H-2), 2.38/2.41 (2H, m, H-3), 2.94 (1H, d, *J* = 8.0 Hz, H-5), 4.93 (1H, dd, *J* = 8.0, 6.0 Hz, H-6), 2.40 (1H, m, H-7), 2.04/1.81 (2H, m, H-8), 2.74/1.78 (2H, m, H-9), 1.58 (3H, s, H-12), 1.48 (3H, s, H-13), 4.36/3.98 (2H, m, H-14), 5.24/5.04 (2H, br s, H-15), 5.06 (1H, d, *J* = 8.0 Hz, H-1'), 3.90 (1H, m, H-2'), 4.07 (1H, m, H-3'), 4.09 (1H, m, H-4'), 3.81 (1H, m, H-5'), 4.51 (1H, dd, *J* = 4.5, 10.0 Hz, H-6'α), 4.16 (1H, m, H-6'β), 5.44 (1H, d, *J* = 3.5 Hz, H-1''), 4.09 (1H, m, H-2''), 4.56 (1H, m, H-3''), 4.22 (1H, dd, *J* = 9.5, 9.0 Hz, H-4''), 4.46 (1H, m, H-5''), 4.36/4.43 (1H, m, H-6''); ^{13}C-NMR (125 MHz, pyridine-d_5): δ_C 81.4 (C-1), 33.6 (C-2), 36.1 (C-3), 146.1 (C-4), 50.2 (C-5), 76.3 (C-6), 45.5 (C-7), 20.2 (C-8), 27.7 (C-9), 42.4 (C-10), 72.8 (C-11), 29.8 (C-12), 31.2 (C-13), 63.1 (C-14), 107.9 (C-15), 102.9 (C-1'),

Table 1. ^{1}H and ^{13}C NMR spectral data of compound **3** (500/125 MHz, pyridine-d_5)[a]

No.	δ_H	δ_C (DEPT)	No.	δ_H	δ_C (DEPT)
1	1.94(1H, dd, 10, 9)/1.11(1H, d, 10)	33.3(CH_2)	Glc-1'	4.85(1H, d, 8.0)	104.4(CH)
2	1.90(1H, m)/1.63(1H, m)	26.6(CH_2)	2'	3.97(1H, dd, 8.0, 9.0)	75.7(CH)
3	4.72(1H, dd, 6, 10)	85.9(CH)	3'	4.37(1H, dd, 9.0, 9.0)	83.2(CH)
4	–	150.3(C)	4'	4.23(1H, dd, 9.0, 9.0)	69.5(CH)
5	–	76.4(C)	5'	3.86(1H, m)	78.5(CH)
6	1.68(1H, m)/1.60(1H, m)	30.6(CH_2)	6'	4.51(1H, m)/4.31(1H, dd, 5.5, 11.5)	62.5(CH_2)
7	2.17(1H, dd, 4, 12)	35.9(CH)	Rha-1''	6.30(1H, br s)	102.6(CH)
8	1.49(1H, m)/1.39(1H, m)	20.9(CH_2)	2''	4.70(1H, br s)	72.9(CH)
9	1.73(1H, t, 10)/1.19(1H, t, 10)	33.9(CH_2)	3''	4.66(1H, dd, 3.0, 9.3)	73.0(CH)
10	–	37.9(C)	4''	4.48(1H, m)	80.0(CH)
11	–	76.0(C)	5''	5.01(1H, m)	68.1(CH)
12	3.62(2H, m)	64.5(CH_2)	6''	1.70(1H, d, 6.4)	18.9(CH_3)
13	3.64(2H, m)	64.9(CH_2)	Api-1'''	5.66(1H, d, 2.2)	111.6(CH)
14	0.82(3H, s)	20.1(CH_3)	2'''	4.61(1H, d, 2.2)	78.2(CH)
15	4.92(1H, d, 2)/4.78(1H, d, 2)	104.7(CH_2)	3'''	–	80.6(C)
HO-5	2.84(1H, s)	-	4'''	4.45(1H, d, 9.5)/4.29(1H, d, 9.5)	75.1(CH_2)
3-*O*-Glycosyl moieties			5'''	4.17(1H, d, 10.7)/4.09(1H, d, 10.7)	65.7(CH_2)

[a]The signals are assigned by ^{1}H NMR, ^{1}H-^{1}H COSY, ^{13}C NMR, NOESY, HMBC, 90° and 135° DEPT.

74.7 (C-2'), 78.5 (C-3'), 71.2 (C-4'), 76.5 (C-5'), 68.1 (C-6'), 100.5 (C-1''), 73.8 (C-2''), 75.8 (C-3''), 71.6 (C-4''), 74.1 (C-5''), 62.4 (C-6'').

3β,5α,11,12,13-pentahydroxy-eudesm-4(15)-ene 3-*O*-β-*D*-apiofuranosyl-(1→4)-α-*L*-rhamnopyranosyl-(1→3)-β-*D*-glucopyranoside (3): White powder from MeOH. $C_{32}H_{54}O_{18}$, mp. 211-213 °C, $[\alpha]_D^{20}$ −7.4° (c = 2.8, MeOH); IR (KBr) ν_{max}: 3445-3100, 2965, 2924, 2847, 1634, 1466, 1378, 1260, 1158, 1086, 1070, 1036, 938 cm^{-1}; FAB-MS (positive-ion mode): *m/z* 727 $[M+H]^+$, 595 $[M+H\text{-Api}]^+$, 449 $[M+H\text{-Api-Rha}]^+$ and 287 $[M+H\text{-Api-Rha-Glc}]^+$; ^{1}H and ^{13}C-NMR see Table **1**.

Acid hydrolysis of compound 1: Compound **1** (5 mg) was treated with 10% HCl-MeOH (1 : 1, 0.5 mL) at 90 °C for 4 hr in a water bath. After the completion of the reaction, the mixture was cooled, diluted with 5 mL of H_2O, then extracted twice with EtOAc. The aqueous phase was neutralised with $BaCO_3$ and filtered, the filtrate was passed through an Amberlite IRA-60E column (6 × 60 mm) and the eluate was concentrated in *vacuo*. The residue was examined for sugars against authentic samples by *co*-PC as well as by GC after being converted to their thiazolidine derivatives.[16] *co*-PC conditions and results: using *n*-BuOH : HOAc : H_2O (4 : 1 : 5, v/v/v, top layer) as developing solvent and 0.9% aniline-oxalate solution as color developing reagent, R_f values: *D*-glucose (0.19), *L*-rhamnose (0.37) and *D*-apiose (0.34). GC conditions and results: using column Supelco SPB-TM1 [0.25 mm × 27 m, column temperature (225 °C), carrier gas (N_2)], retention time for derivatives: *D*-glucose (18.4 min), *L*-rhamnose (11.9 min) and *D*-apiose (10.9 min), respectively; retention time for authentic samples: *D*-glucose (18.4 min), *L*-glucose (16.6 min), *D*-rhamnose (11.5 min), *L*-rhamnose (11.9 min), *D*-apiose (10.9 min) and *L*-apiose (10.1 min). From the new glycoside, the glucose and apiose were in the *D*-form, while the rhamnose was in the *L*-form.

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