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Cyclic Triterpenoid Saponins from *Campanula lactiflora*

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Two cyclic natural compounds, 3β-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl]-13α,14α-epoxy-8α,12β,15-trihydroxy-(17*E*,21*E*)-17,21-campanuldien-6′(30)-olide, called lactifloroside A, **1**, and 3β-*O*−[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]-13α,14α-epoxy-8α,12β-dihydroxy-(17*E*,21*E*)-17,21-campanuldien-6′(30)-olide, called lactifloroside B, **2**, were isolated for the first time from *Campanula lactiflora* and their structures deduced by high field 1D and 2D 400 MHz NMR, FT-IR, HPLC, GC-MS, (+/-) LC-MS/MS and (+) FAB-MS spectra. The aglycones of the 2 saponins were named 13α,14α-epoxy-3β,8α,12β,15-tetrahydroxy-(17*E*,21*E*)-17,21-campanuldien-30-oic acid and 13α,14α-epoxy-3β,8α,12β-trihydroxy-(17*E*,21*E*)-17,21-campanuldien-30-oic acid, and designated as campanuloic acid and 15-deoxycampanuloic acid, respectively.

Key Words: *Campanula lactiflora*, lactifloroside A and B, campanuloic acid, 15-deoxycampanuloic acid, cyclic bisdemoside.

Introduction

The genus *Campanula* L. belongs to the family *Campanulaceae*[1]. One *Campanula* species, *Campanula lactiflora* Bieb., was naturalized in northern Turkey[1]. Previous phytochemical studies on *C. lactiflora* have shown the presence of luteolin 7-β-D-glucopyranoside[2], luteolin[3] and 4′-O-(p-hydroxybenzoyl)-isorhamnetin-3,7-di-O-β-D-glucopyranoside, sitosterol β-D-glucoside, p-hydroxybenzoic acid and ethyl docosanoate[4], and triterpenes[5]. In our continuing phytochemical investigation of the mixture of chloroform and methanol extracts of air-dried leaves of *C. lactiflora*, 2 new cyclic triterpene saponins (**1, 2**) were isolated. This paper reports the isolation and characterization of 2 new cyclic natural products, designated as lactifloroside A (**1**) and lactifloroside B (**2**), through spectral analyses. Some cyclic triterpene saponins were reported earlier and

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named "cyclic bisdemosides" in the literature[6,7]. Labdane-type structures are well known and characterized by spectral analyses[8].

Experimental

General and Instrumentation

NMR spectra were recorded on a Varian NMR at 400 MHz instrument in C_5D_5N. (+) FABS were recorded on a ZabSpec MS instrument and (+/-) LC-MS/MS were carried out on a Micromass Quattro spectrometer. Infrared spectra were obtained with a Perkin-Elmer 1600 FT-IR (4000-400 cm^{-1}) spectrophotometer. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. Mps were obtained using a Kofler hot stage apparatus and are uncorrected. Flash CC was performed on silica gel (230-400 mesh) and reversed-phase silica gel RP-18, and PTLC was performed with precoated reversed-phase silica gel RP-18 $F_{254}S$. Analytical HPLC was carried out on an Agilent 1100 series using a RI detector and the column was Zorbax Carbohydrate (4.6 mm ID x 250 mm, 5 μm) with CH_3CN/H_2O (75:25), flow 0.3 mL/min.

GC-MS analysis was performed using an Agilent-5973 Network. A mass spectrometer with an ion trap detector in full scan under electron impact ionization (70 eV) was used. The chromatographic column for the analysis was a DB-1701P capillary column (25 m x 0.32 mm i.d., film thickness 0.25 μm). The carrier gas used was helium at a flow rate of 1 mL/min. The injection was performed in split mode (ratio 20:1) at 230 ^{o}C. Then a 1 μL crude fraction was injected and analyzed with the column held initially at 100 ^{o}C for 1 min and then increased to 300 ^{o}C with a 10 ^{o}C/min heating ramp and subsequently kept at 300 ^{o}C for 15 min.

Plant Material. A whole *C. lactiflora* Bieb plant was collected in August 1996 at Arpalı, Trabzon Hills (∼2000 m), Turkey. A voucher specimen (Yaylı 9-1996 KTUK and KATO 12654-1996) has been deposited in the Department of Chemistry and Faculty of Forestry at Karadeniz Technical University, Turkey. The species was identified according to the Flora of Turkey[1].

Extraction and Isolation. The extraction procedure was as described previously[4].

Fraction CL7 (230 mg) was again purified by flash CC on silica gel (60 g, 230-400 mesh). The column was eluted with $CHCl_3$ (60 mL) followed by a discontinuous gradient with $CHCl_3$-MeOH (2:0.5, 100 mL; 2:1, 50 mL, 1:1, 50 mL, 1:2, 50 mL), then with MeOH (50 mL) and finally with MeOH-H_2O (10:0.5, 50 mL) to give 33 fractions (∼ 8-10 mL each). After TLC analysis, fractions 7-23 (mixture), 24-28 (84 mg) (saponin mixture called CL72) and 29-33 (mixture) were combined. CL72 was rechecked by reverse-phase-18 TLC in a MeOH-H_2O (1.5:1) solvent system and found to be a mixture. It was rechromatographed by RP-18 (15 g) flash column chromatography (2 x 60 cm). The column was eluted by a discontinuous gradient with acetone-H_2O (1:1, 20 mL; 1:0.8, 20 mL, 1:0.6, 30 mL, 1:0.5, 30 mL, 1:0.2, 30 mL) and finally with acetone (30 mL) to give 32 fractions (∼ 5-6 mL each). After TLC analysis, fractions 20-21 (32 mg) were combined and rechromatographed by RP-18 PTLC (0.25 mm, 20 x 20 cm, 4 plates) using MeOH-H_2O (1.5:0.5) to give 5 bands; CL722C: **1**, 14.3 mg, R_f = 0.40; CL722D: **2**, 9 mg, R_f = 0.26; CL722A, CL722B and CL722E contained minor unidentified compounds, R_f = 0.63, R_f = 0.46, R_f = 0.16, respectively.

3β-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl]-13α,14α-epoxy-8α,12β,15-trihydroxy-(17*E*,21*E*)-17,21-campanuldien-6′(30)-olide, (1): Amorphous powder, mp 143-146 ^{o}C; $[\alpha]^D$ +12.28^o (MeOH; c 1.14 x 10^{-3}); ^{1}H NMR (C_5D_5N, 400 MHz) and ^{13}C NMR (C_5D_5N, 100

MHz) δ (ppm) see Table 1; FAB-MS m/z (%); m/z = 835(48) $[M+Na]^+$, 851(13) $[M+K]^+$, 550(12), 522(10), 393(43), 369(24), 322(75), 253(32), 193(55), 179(48) and 149(32). (+) LC-MS/MS m/z (%); m/z = 835(100) $[M+Na]^+$, 851(28) $[M+K]^+$; (-) LC-MS/MS m/z (%); m/z = 811(45) $[M-H]^+$, 812(21) $[M]^+$, 491(25%), 254(48%), 126(100%); FT-IR cm^{-1}: 3427, 2942, 1699, 1646, 1451, 1388, 1074, 1032.

3β-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]-13α,14α-epoxy-8α,12β-dihydroxy-(17*E*,21*E*)-17,21-campanuldien-6′(30)-olide, (2): Amorphous powder, mp 158-162 ^oC; $[\alpha]^D$ $+9.61^o$ (MeOH; c 5.2 x 10^{-4}); ^1H NMR (C_5D_5N, 400 MHz) and ^{13}C NMR (C_5D_5N, 100 MHz) δ (ppm) see Table 1; FAB-MS m/z (%); m/z = 835(25) $[M+Na]^+$, 851(13) $[M+K]^+$, 552(3), 513(5), 391(13), 345(28), 253(32), 192(100) and 179(16); (+) LC-MS/MS m/z (%); m/z = 835(100) $[M+Na]^+$, 851(62) $[M+K]^+$; (-) LC-MS/MS m/z (%); m/z = 811(55) $[M-H]^+$, 812(25) $[M]^+$, 325(28%), 182(62%), 126(100%); FT-IR cm^{-1}: 3435, 2940, 1695, 1645, 1456, 1386, 1275, 1124, 1075.

Acid Hydrolysis of 1: Compound **1** (5 mg) was refluxed with 2N HCl (1 mL) in MeOH (1 mL) for 8 h. The reaction mixture was then concentrated under reduced pressure to remove MeOH. It was then diluted with H_2O (3 mL) and aglycone extracted with $CHCl_3$. LC-MS/MS showed its $[M+H]^+$ peak at 523 (18%) The aqueous layer was adjusted to pH 7 with NaOH and filtered. The supernatant was concentrated and compared with reference sugars on TLC (silica gel, CH_2Cl_2:MeOH:H_2O, 15:9:1). The sugars were detected on a silica gel plate by spraying of a solution of 50% H_2SO_4 in EtOH. The sugar part was identified as glucose and rhamnose, which were also determined by HPLC and GC-MS (trimethylsilyl derivatives of sugars) analysis.

Acid Hydrolysis of 2: The acid hydrolysis of compound **2** (3 mg) was performed in the same way as compound **1**. For compound **2**, glucose was determined by HPLC, GC-MS (trimethylsilyl derivatives of sugars) analysis and TLC analysis with an authentic sugar as well.

Results and Discussion

The mixture of chloroform and methanol extracts of the air-dried leaves of *C. lactiflora* was subjected to silica gel and reversed phase RP-18 chromatographies (CC, TLC and PTLC) to give 2 new cyclic compounds, **1** and **2**.

Conventional ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectra combined with DEPT data yielded the structure of the molecule **1** consisting of a triterpenoid aglycone $(C_{30}H_{50}O_7)$[9−12] and a disaccharide (C_{12}) sugar moiety. The COSY, TOCSY, HMQC, HMBC and NOESY maps afforded a comprehensive description of through-bond and through-space proton-proton and proton-carbon connectivities of **1**.

Compound **1** showed its $[M+Na]^+$ and $[M+K]^+$ peaks at m/z 835 (48% and 100%) and 851 (13% and 38%) in the positive FAB and (+) LC-MS/MS spectra, respectively, and (-) LC-MS/MS gave a $[M-H]^+$ peak at m/z 811 (45%), and a $[M]^+$ peak at 812 (21%). These results indicate the molecular formula $C_{42}H_{68}O_{15}$. The FT-IR spectrum of compound **1** showed absorption bands for a hydroxyl group (3427 cm^{-1}), double bonds (1646 cm^{-1}) and a conjugated ester carbonyl (1699 cm^{-1}).

The sequential assignments of individual aglycone were readily available from ^1H chemical shift correlations (H_3 to H_1; H_5 to H_7; H_9 to H_{13}, H_{15} to H_{28}; and H_{19} to H_{29}) in ^1H COSY and TOCSY spectra of **1**. All correlated parts together gave aglycone of compound **1**, which is a new example of labdane-containing triterpenoids[8] and obeys the isoprene rule. The ^1H NMR spectrum of compound **1** in C_5D_5N displayed the

Table 1. ^{13}C and ^{1}H NMR spectral data for lactifloroside A and B (**1**, **2**) in C_5D_5N.

	1[ab]			**2**[a,b]	
C/H	^{13}C (δ, ppm)	^{1}H (δ, ppm)	C/H	^{13}C (δ, ppm)	^{1}H (δ, ppm)
1	37.79	1.40[c], 2.01[c], m	1	38.09	1.42[c], 2.02[c], m
2	24.90	2.06[c], 2.00[c]	2	25.33	2.12[c], m
3	89.00	3.35, t, J = 8.4 Hz	3	89.45	3.40, dd, J = 4.2, 8.5 Hz
4	38.63	-	4	38.81	-
5	55.27	0.91, d, J = 12 Hz	5	55.60	0.97, dd, J = 9.3, 11.1 Hz
6	18.34	1.90[c], 1.98[c], m	6	18.49	1.90[c], 2.15[c], m
7	43.10	1.52[c], 1.98[c], m	7	43.18	1.50[c], 1.96[c], m
8	71.70	-	8	71.77	-
9	51.31	1.82, bd, J = 8.0 Hz	9	51.25	1.80, bd, J = 8.0 Hz
10	38.12	-	10	38.33	-
11	27.81	1.85[c], 2.18[c], m	11	27.97	1.86[c], 2.10[c], m
12	75.81	4.18, dd, J = 3.2, 7.6 Hz	12	77.36	4.03, dd, J = 3.6, 7.9 Hz
13	64.40	3.74, d, J = 7.6 Hz	13	69.46	3.23, d, J = 7.9 Hz
14	61.25	-	14	59.60	-
15	70.57	4.13, dd, J = 1.6, 6.8 Hz	15	39.95	2.14[c], m
16	30.60	2.42[c], 2.69[c], m	16	23.96	2.10[c], 2.24[c], m
17	118.55	5.73, t, J = 6.8 Hz	17	124.11	5.46[d], bs
18	135.38	-	18	135.17	-
19	37.44	1.46[c], 2.32[c]	19	37.63	1.45[c], 2.30[c], m
20	28.22	1.90[c], 2.20[c], m	20	28.39	1.85[c], 2.12[c], m
21	141.66	6.95, t, J = 6.8 Hz	21	141.61	7.00, t, J = 6.7 Hz
22	128.06	-	22	128.40	-
23	29.06	1.30, s	23	28.72	1.32, s
24	16.96	1.01, s	24	17.20	1.04, s
25	16.23	1.32, s	25	16.19	1.34, s
26	31.54	1.43, s	26	31.56	1.39, s
27	16.75	1.45, s	27	17.70	1.42, s
28	16.96	1.72, s	28	16.19	1.61, s
29	12.18	1.84, s	29	12.39	1.95, s
30	167.67	-	30	167.83	-
Gluc 1′	101.53	4.63, d, J = 8.0 Hz	Gluc I 1′	102.38	4.71, d, J = 7. 8 Hz
2′	76.31	4.42, t, J = 8.0 Hz	2′	80.50	4.60, dd, J = 7.7, 8.0 Hz
3′	70.57	4.20[c]	3′	70.05	4.31[c]
4′	70.68	4.26[c]	4′	74.81	4.34[c]
5′	73.28	3.97, bd, J = 9.2 Hz	5′	76.39	4.06, t, J = 8.0 Hz
6′	65.14	4.56[c]	6′	65.21	4.64[c]
		4.98, dd, J = 9.6, 11.6 Hz			5.05, dd, J = 9.9, 10.6 Hz
Rha 1″	101.24	6.23, bs	Gluc II 1″	105.30	5.20, d, J = 7.6 Hz
2″	71.69	4.82, dd, J = 1.6, 3.2 Hz	2″	73.35	4.03, dd, J = 7.6, 8.0 Hz
3″	71.76	4.63[c]	3″	71.55	4.13, t, J = 9.1 Hz
4″	71.70	4.26, t, J = 9.6 Hz	4″	70.97	4.20, t, J = 9.5 Hz
5″	69.34	4.57[c]	5″	77.79	3.72[c]
6″	18.27	1.52, d, J = 6.4 Hz	6″	62.04	4.33[c], 4.36[c]

[a] Chemical shifts (ppm) are relative to C_5D_5N.

[b] Assignments based on ^{1}H, ^{13}C, DEPT, 2D-COSY, TOCSY, HMQC, HMBC and NOESY spectra.

[c] Signal patterns unclear due to overlapping.

[d] Beside the HDO peak.

presence of 7 methyl singlets at δ 1.01, 1.30, 1.32, 1.43, 1.45, 1.72 and 1.84[9−13], and 1 methyl doublet at δ 1.52 (J = 6.4 Hz) typical of a rhamnose -CH_3. In addition, there were peaks at δ 5.73 (1H, H_{17}, t, J = 6.8 Hz) and 6.95 (1H, H_{21}, t, J = 6.8 Hz) for 2 olefinic protons, at δ 4.13, (H_{15}, dd, J = 1.6, 6.8 Hz) and 4.18, (H_{12}, dd, J = 3.2, 7.6 Hz) for 2 hydroxyl substituted methine protons, δ 3.74 (H_{13}, d, J = 7.6 Hz) for an epoxy substituted methine proton, and a triplet at δ 3.35 (J = 8.4 Hz) due to H_3.

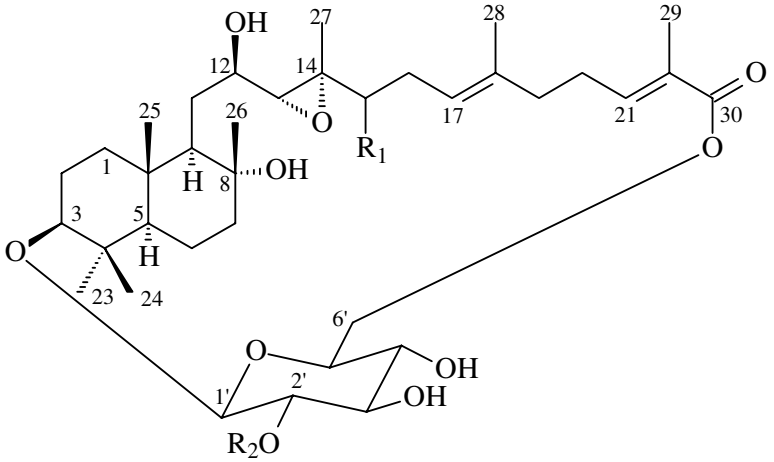
The ^{13}C resonances for the aglycone in the downfield area showed that there were 6 oxygenated sp^3 carbon resonances (C_3: δ 89.00, C_8: δ 71.70, C_{12}: δ 75.81, C_{13}: δ 64.40, C_{14}: δ 61.25, C_{15}: δ 70.57) and the remaining oxygenated carbon resonances were accounted for by the 2 sugars. The 30 carbon aglycone was shown by DEPT spectra to have 4 quaternary carbons (δ 38.12, 38.63 61.25, 71.70), 6 methines (δ 51.31, 55.27, 64.40, 70.57, 75.81, 89.00), 8 methylenes (δ 18.34, 24.90, 27.81, 28.22, 30.60, 37.44, 37.79, 43.10), 7 methyl groups (δ 12.18, 16.23, 16.75, 16.96 (2 peaks interpreted from HMBC and HMQC), 29.06, 31.54), and 5 sp^2 carbons (δ 118.55 (CH), 141.66 (CH), 128.06 (C), 135.38 (C), 167.67 (O-C=O)). Thus, the structure of the aglycone of compound **1** had a new cyclic triterpene feature, having 2 double bonds in *E* configuration proved by carbon chemical shifts (δ 118.55, 135.38, 141.66, 128.06 for C_{17}, C_{18}, C_{21}, C_{22}, respectively), 4 hydroxyls (3β, 8α, 12β, 15), an epoxy (13α, 14α) and a C_{30} carboxylic ester group. Some of the important HMBC correlations for compound **1** are listed in Table 2. Analysis of the NMR data of compound **1** showed that the aglycone had unfused C/D and D/E ring systems.

Table 2. Some of the important HMBC correlations for lactifloroside A and B (**1**, **2**).

1		**2**	
Proton	Carbon	Proton	Carbon
H_3	C_2, C_4,C_{24}	H_3	C_2, C_4
H_{12}	C_{12}	H_{13}	C_{14}
H_{13}	C_{12}, C_{14}, C_{15}	H_{21}	C_{22}, C_{29}, C_{30}
H_{15}	C_{13}, C_{14}, C_{16}, C_{17}, C_{27}	H_{23}	C_3, C_4, C_5,C_{24}
H_{16}	C_{14}, C_{15}, C_{17}, C_{18}	H_{24}	C_3, C_4, C_5, C_{23}
H_{17}	C_{16},C_{19}, C_{28}	H_{25}	C_5, C_9, C_{10}, C_{25}
H_{21}	C_{19}, C_{20}, C_{22}, C_{29}, C_{30}	H_{26}	C_7, C_8, C_9
H_{23}	C_3, C_5, C_{24}	H_{27}	C_{13},C_{14}, C_{15},
H_{24}	C_3, C_5, C_{23}	H_{28}	C_{17},C_{18}, C_{19},
H_{25}	C_1, C_5, C_9, C_{10}	H_{29}	C_{21}, C_{22}, C_{30}
H_{26}	C_8, C_9, C_{11}	Gluc I H_1	C_3
H_{27}	C_{13}, C_{14}, C_{15}	Gluc I H_2	Gluc II C_1, Gluc I C_3
H_{28}	C_{17}, C_{18}, C_{19}	Gluc I H_{6a}/H_{6b}	C_{30}, Gluc I C_5
H_{29}	C_{21}, C_{22}, C_{30}	Gluc II H_1	Gluc I C_2
Gluc H_1	C_3, Gluc C_2		
Gluc H_2	Rham C_1, Gluc C_2,Gluc C_4		
Gluc H_{6a}/H_{6b}	C_{30}, Gluc C_5		
Rha H_1	Gluc C_2, Rham C_2, Rham C_3		

The NOESY data for the aglycone revealed that the relative stereochemistries at the common centers in ring A and B were identical to those of similar reported diterpenes[8] and triterpenes[13,14]. For example, a NOESY experiment with **1** showed the presence of cross-peak correlations of $H_{12\alpha}$ (δ 4.18) with $H_{9\alpha}$ (δ 1.82), H_{25} (δ 1.32) with H_{26} (δ 1.43), H_9 (δ 1.82) with H_5(δ 0.91), and H_3 (δ 3.35) with H_5(δ 0.91). The H_{12} and H_{13} were axial-axial oriented due to the coupling constant (7.6 Hz). Therefore, the hydroxyl at C_{12}

and an epoxy at C_{13}/C_{14} were assigned to be β and α configurations, respectively, but the stereochemistry at C_{15} is not certain.



1 R_1= -OH, R_2= -Rhamnose (lactifloroside A)

2 R_1= -H, R_2= -Glucose (lactifloroside B)

Acid hydrolysis of compound **1** afforded aglycone, which showed its $[M+H]^+$ peak at 523 (22%), $[M+H_2O+H]^+$ peak at 541 (39%), $[M+2H_2O+H]^+$ peak at 559 (18%) and $[M\text{-}OH]^+$ peak at 505 (22%) in the (+) LC-MS/MS spectrum.

The individual proton spin systems for the each sugar residue of the glycone of compound **1** were derived from ^{1}H COSY and TOCSY spectra. The HMQC spectrum allowed for assignments of carbon resonances in the sugar moiety of compound **1**. The ^{1}H NMR spectrum of **1** showed 2 anomeric proton signals at δ 4.63 ($H_{1'}$, d, J = 8.0 Hz) and δ 6.23 ($H_{1''}$, bs) ppm, indicating the presence of 2 monosaccharides. Two anomeric protons at δ 4.63 and 6.23 ppm correlating with carbon signals at δ 101.53 and 101.24 ppm were assigned to β-D-glucose and α-L-rhamnose, respectively[13−18]. Compound **1** afforded a mixture of D-glucose and L-rhamnose (1:1) on acid hydrolysis as determined by HPLC and GC-MS (trimethylsilyl derivatives of sugars) and on TLC analysis with authentic sugars. Formation of a glycosidic bond was indicated by a 3 to 6 ppm downfield shift of the resonance (δ_C 76.31 (Gluc C_2) and 65.14 (Gluc C_6)) due to the carbon atoms involved in the glycosidic linkage[5,13−17], a fact clearly reflected by the ^{13}C chemical shift data of lactifloroside A. Two interglycosidic linkages, δ 65.14 (Gluc C_6) and 76.31 (Gluc C_2) ppm, were present in the glucose moiety of compound **1** (HMBC, NOESY) (Tables 1 and 2). The HMBC cross signals for glucose H_1/C_3 (δ 4.63/89.00) showed the glycosidation with glucopyranose in position C_3 (Table 2). In addition the glycosidation of the position C_{30} was indicated by the HMBC cross peak of the ^{3}J coupling between glucose H_6/C_{30} (δ 4.56, 4.98/167.67). These HMBC data showed that glucopyranose was linked from glucose H_1 to C_3 and glucose H_{6a}-H_{6b} to C_{30}through a glycosidic and an ester linkage, respectively.

The sequence of the sugars and the aglycone part of compound **1** were also established through positive ion FAB-MS, which exhibited molecular ion peaks at m/z = 835 $[M+Na]^+$, 851 $[M+K]^+$ and fragment ions m/z = 550 $[M\text{-}292\text{-}O+Na]^+$, 522 $[Aglycone]^+$, 369 $[(Gluc\text{-}O\text{-}Rham)\text{-}OH+Na]^+$, 322 $[(Gluc\text{-}O\text{-}Rham)\text{-}O+2Na]^+$, 193 $[Rha+2Na]^+$, $[Rha+2Na]^+$and 149 $[Rha+2H]^+$, respectively.

Based upon the above observations, the structure of compound **1** was established as 3β-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl]-13α,14α-epoxy-8α,12β,15-trihydroxy-(17*E*,21*E*)-17,21-campanuldien-6′(30)-olide, a new cyclic natural saponin isolated for the first time from *C. lactiflora*.

The second compound (**2**) exhibited its $[M+Na]^+$ and $[M+K]^+$ peak at m/z 835 (23% and 100%) and 851 (13% and 62%) in the positive FAB and (+) LC-MS/MS spectra, respectively, and (-) LC-MS/MS gave a $[M-H]^+$ peak at m/z 811 (55%), and a $[M]^+$ peak at 812 (25%). These data indicate the molecular formula $C_{42}H_{68}O_{15}$, which was the same molecular formula as compound **1**, but it had 2 significant differences in the NMR data of compound **2**: the signal of the H_{15}(-OH substituted) at δ 4.13 was missing and, instead of rhamnose, another terminal glucose unit was present (Table 1). The FT-IR spectrum of compound **2** showed absorption bands for a hydroxyl group (3435 cm^{-1}), double bonds (1645 cm^{-1}) and a conjugated ester carbonyl (1695 cm^{-1}).

The ^1H NMR spectrum of compound **2** revealed the presence of 7 methyl singlets at δ 1.04, 1.32, 1.34, 1.39, 1.42, 1.61 and 1.95. There were also peaks at δ 7.00 (H_{21}, t, J = 6.7 Hz) and 5.46 (H_{17}, bs beside HDO peak, COSY, HMQC) for 2 olefinic protons, at δ 4.03, (H_{12}, dd, J = 3.6, 7.9 Hz) for 1 methine proton (CHOH), 3.23, (H_{13}, d, J = 7.9 Hz) for an epoxy methine proton and at δ 3.40 (dd, J = 4.2, 8.5 Hz) due to H_3.

After the analyses of the NMR spectra of compound **2** (^1H, ^{13}C, DEPT, H-COSY, TOCSY, HMQC, HMBC and NOESY) (Table 1), a comparison of the NMR data (Table 1) of **2** with those of **1** showed disaccharide sugar moieties as **1** except that terminal rhamnose was replaced by another glucose unit, which showed a downfield shift of its carbon at δ 105.30 (Gluc II C_1). Compound **2** gave D-glucose on acid hydrolysis as determined by HPLC and GC-MS (trimethylsillyl derivatives of sugars) and on TLC analysis with an authentic sugar. The ^1H NMR spectrum of **2** exhibited signals for 2 anomeric protons at δ 4.71 (Gluc I H_1, d, J = 7.8 Hz) and δ 5.20 (Gluc II H_1, d, J = 7.6 Hz), which correlated with carbon signals at δ 102.38 and 105.30, assigned to 2 glucose units[14,16,18]. The HMBC cross signals for glucose I H_1/C_3 (δ 4.71/89.45) and glucose I H_{6a}-H_{6b}/C_{30} (δ 4.64, 5.05/167.83) showed the glycosidation between glucopyranose I and aglycone at C_3 and C_{30} in compound **2** as in compound **1**. These HMBC data showed that linkage and lactonization were the same as in compound **1** (Table 2). Some of the important HMBC correlations for compound **2** are listed in Table 2. Analysis of the NMR data of compound **2** showed that the aglycone of compound **2** had the same unfused C/D and D/E ring systems as in compound **1**, but showed no hydroxyl at C_{15}. Thus, the structure of aglycone of compound **2** features 2 double bonds (Δ^{17} and Δ^{21}), 3 hydroxyls (3β, 8α and 12β), an epoxy (13α and 4α) and a C_{30} carboxylic ester. Acid hydrolysis of compound **2** afforded aglycone, which showed its $[M+H]^+$peak at 507 (24%), $[M+H_2O+H]^+$ peak at 525 (13%) and $[M+2H_2O+H]^+$ peak at 543 (7%) in the (+) LC-MS/MS spectrum.

The sequence of the sugars and the aglycone part of compound **2** were also established through positive ion FAB-MS, which exhibited the molecular ion peaks at m/z = 835 $[M+Na]^+$, 851 $[M+K]^+$ and fragment ions m/z = 552 [Aglycone+2Na]$^+$, 391 [Glc II-O-Glc I-OCO(368)+Na]$^+$and 179 [Glc II-O]$^+$, respectively.

From the above evidence, the structure of compound **2** was proved to be 3β-O−[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]-13α,14α-epoxy-8α,12β-dihydroxy-(17*E*,21*E*)-17,21-campanuldien-6′(30)-olide, a new cyclic natural compound isolated for the first time from *C. lactiflora*.

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References

1. J. Damboldt, Campanula L. In: P.H. Davis, "**Flora of Turkey and the East Aegean Islands**", Vol 6, University Press, Edinburgh. p. 2-65, 1977.
2. S.F. Dzhumyrko, E.T. Oganesyan and A.L. Shinkarenko, **Khim. Prir. Soedin 5**, 440-441 (1969).
3. S.F. Dzhumyrko, **Khim. Prir. Soedin 2**, 273-274 (1973).
4. N. Yaylı, N. Yıldırım, A. Usta, S. Özkurt and V. Akgün, **Turk. J. Chem. 27**, 749-755 (2003).
5. N. Yaylı, N. Yıldırım, N. Doğan, A. Usta and L. Altun, **J. Asian Nat. Prod. Res. 7**, 771-775 (2005).
6. F.H. Kong, D.Y. Zhu, R.S. Xu, Z.C. Fu and L.Y. Zhou, **Tetrahedron Letters 27**, 5765-5768 (1986).
7. R. Kasai, M. Miyakoshi, R.L. Nie, J. Zhou, K. Matsumoto, T. Morita, M. Nishi, K. Miyahara and O. Tanaka, **Phytochemistry 27**, 1439-1446 (1988).
8. A.F. Barrero and J. Altarejos, **Mag. Reson. Chem. 31**, 299-308 (1993).
9. S.B. Mahato and S. Sen, **Phytochemistry 44**, 1185-1236 (1997).
10. S.B. Mahato, S.K. Sarkar and G. Poddar, **Phytochemistry 27**, 3037-3067 (1988).
11. S.B. Mahato, A.K. Nandy and G. Roy, **Phytochemistry 31**, 2199-2249 (1992).
12. S.B. Mahato and A.P. Kundu, **Phytochemistry 37**, 1517-1575 (1994).
13. T. Baykal, T. Panayır, D. Taşdemir, O. Sticher and İ. Çalış, **Phytochemistry 48**, 867-873 (1998).
14. M. Konishi, Y. Hano, M. Takayama, T. Nomura, A.S. Hamzah, R.B. Ahmad and H. Jasmani, **Phytochemistry 48**, 525-528 (1998).
15. S.S. Yu, Z.Y. Xiao, P. Cai, T.Y. Jiang and J.K. Snyder, **Tetrahedron 50**, 11601-11612 (1994).
16. N.P. Sahu, **Phytochemistry 41**, 883-886 (1996).
17. Z. Jia, K. Koike, M. Kudo, H. Li and T. Nikaido, **Phytochemistry 48**, 529-536 (1998).
18. P.K. Agrawal, **Phytochemistry 31**, 3307-3330 (1992).