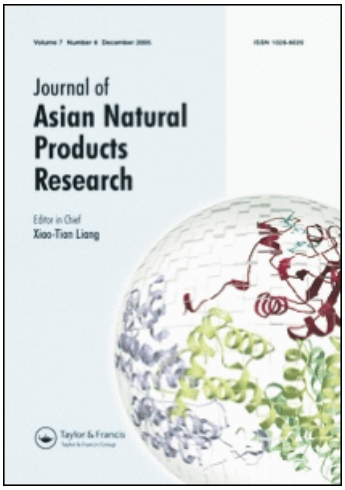

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Triterpenes from *Campanula lactiflora*

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Note

Triterpenes from *Campanula lactiflora*

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One new triterpene compound, 3β-acetoxylup-20(30)-en-29-al (**1**) and two known 3-acetylptiloepoxide (**2**) and 3β-acetoxylup-20(29)-ene (**3**) were isolated for the first time from the leaves of *Campanula lactiflora* and their structures were deduced by high field 1D and 2D 400 MHz NMR, EI-MS and (+) LC-MS/MS spectra.

Keywords: *Campanula lactiflora*; *Campanulaceae*; 3β-Acetoxy-lup-20(30)-en-29-al; Triterpene

1. Introduction

The genus *Campanula* L. belongs to the Campanulaceae family [1]. Previous phytochemical studies on the *Campanula* genus have shown the presence of many different natural compounds such as flavonoids, anthocyanins, triterpenoids, coumarins, inositol, etc. [2–16]. One of the species of the family, *Campanula lactiflora* Bieb, is naturalised in northern Turkey [1]. Previous phytochemical studies on the *C. lactiflora* led the isolation of luteolin 7-β-D-glucopyranoside [7] and luteolin [3], 4′-*O*-(*p*-hydroxybenzoyl)-isorhamnetin-3,7-di-*O*-β-D-glucopyranoside, sitosterol β-D-glucoside, *p*-hydroxybenzoic acid and ethyl docosanoate [17]. In continuation of our phytochemical investigation of a chloroform and methanol extracts of air dried leaves of *C. lactiflora,* one new triterpene (**1**), and two known triterpenes (**2**, **3**) were isolated for the first time from this plant. This paper reports the isolation and characterisation of one new natural product, designated as 3β-acetoxylup-20(30)-en-29-al (**1**), and two known products, 3-acetyptiloepoxide (**2**) and 3β-acetoxylup-20(29)-ene (**3**), through spectral analyses.

2. Results and discussion

The chloroform and methanol extracts of the air-dried leaves of *Campanula lactiflora* were subjected to column chromatography and preparative TLC to separate compounds **1–3**.

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Compound **1** showed a molecular ion peak at *m/z* 482 $[M]^+$ in its EIMS and the $[M + Na]^+$ at *m/z* 505 in its LC-MS/MS corresponding to the molecular formula $C_{32}H_{50}O_3$. This was corroborated by the ^{13}C NMR spectrum, which showed signals for the 32 carbons of the molecule (two of those were superimposed. HMBC, HMQC). The C-20 peak was very weak in the ^{13}C NMR spectrum, but C-20 exhibited very strong $^2J_{H-C}$ correlation signals with H-30 and H-29 in the HMBC experiment.

The ^1H NMR spectrum of **1** showed seven tertiary methyls (δ_H 0.86 (9H, s), 0.83, 0.93, 1.03, 2.05 (each 3H, s)), one aldehyde proton (δ_H 9.52, 1H, bs, H-29), two olefinic singlets at δ 6.31(1H, bs, H-30) and 5.93 (1H, bs, H-30) [18]. The facile loss of 55 mass units from the molecular ions in the mass spectrum of **1** indicated the presence of oxygenated isopropenyl side chain in the molecule. This side chain was also seen in other natural compound named skimmial [18]. In the ^1H NMR spectrum of **1**, the characteristic H-3 signal was exhibited at δ 4.46 (1H, m) and in its ^{13}C NMR spectrum (table 1), the presence of C-3 signals at δ 80.93 agreed with acetyl substitution [19,20]. A peak in the ^1H NMR spectrum also indicated the presence of one acetoxyl group in the molecule at δ 2.05 appearing as a singlet and integrating for three protons and in the ^{13}C NMR spectrum two peaks at δ 171.03

Table 1. ^1H and ^{13}C NMR spectral data of the triterpene compounds **1–3** in $CDCl_3$.

C No.	*1*[*,†] *^1H, δ*	*1*[*,†] *^{13}C, δ*	*3*[*,†] *^{13}C, δ*	*2*[*,†] *^{13}C, δ*	*2*[*,†] *^1H, δ*
1	1.43, 1.26, m	38.36	38.37	38.39	1.76, 1.70, m
2	1.62, 0.94, m	23.67	23.70	23.66	1.63, 1.60, m
3	4.46, m	80.93	80.96	80.92	4.50, dd, $J = 5.6, 5.6$ Hz
4	–	37.38	37.78	37.78	–
5	0.82, m	55.35	55.36	55.34	1.38, m
6	1.72, 1.53, m	18.17	18.19	18.15	1.58, 1.42, m
7	1.54, 1.38, m	34.19	34.20	34.02	1.41, 1.27, m
8	–	40.77	40.83	40.99	–
9	1.28, m	50.13	50.32	50.27	1.35, m
10	–	37.03	37.07	37.01	–
11	2.16, 1.43, m	20.93	20.93	21.42	1.59, 1.24, m
12	2.76, 1.22, m	27.63	25.08	26.15	1.66, 1.28, m
13	1.40, m	37.68	38.03	37.90	1.65, m
14	–	42.66	42.48	42.23	–
15	1.46, 1.38, m	27.30	27.42	26.51	1.80, 1.29, m
16	1.68, 1.42, m	35.36	35.55	33.62	1.79, 1.42, m
17	–	43.26	42.99	36.28	–
18	1.41, m	47.80	48.27	42.13	1.45, m
19	1.64, m	50.13	47.99	36.15	2.02, p, $J = 6.8$ Hz
20	–	157.40	150.95	151.38	–
21	1.41, 1.39, m	27.63	29.82	56.05	3.47, d, $J = 4.4$ Hz
22	1.47, 1.39, m	39.92	39.88	63.98	2.93, d, $J = 4.4$ Hz
23	0.86, s	27.93	27.94	27.94	0.87, s
24	0.86, s	16.48	16.49	16.50	0.86, s
25	0.86, s	16.12	16.17	16.31	0.89, s
26	1.03, s	15.92	15.96	15.96	1.04, s
27	0.83, s	14.35	14.49	14.73	0.97, s
28	0.93, s	17.77	17.99	15.12	0.83, s
29	9.52, bs	195.11	109.34	27.23	1.06, d, $J = 6.4$ Hz
30	6.31, bs, 5.93, bs	132.17	19.27	111.96	5.07, br s, 4.88, br s
31	–	171.03	171.00	171.00	–
32	2.05, s	21.31	21.32	21.32	2.06, s

* Spectra recorded in $CDCl_3$.
† Assignments based on ^1H NMR, ^{13}C NMR, DEPT, 2D-COSY, TOCSY, HMQC, HMBC and NOESY spectra.

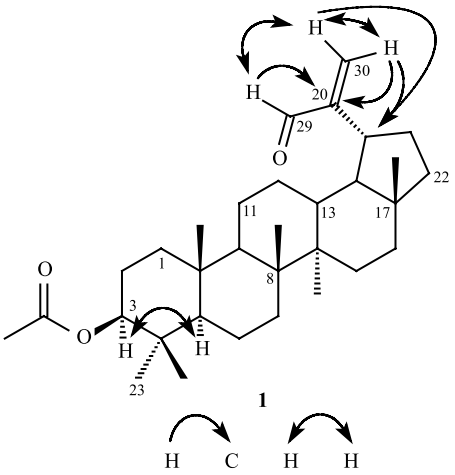

Figure 1. Important HMBC (H → C) and NOESY (H ← → H) correlations of compound **1**.

(ester C═O) and 21.31 (acetyl —CH_3). In addition, the position of the acetoxyl group on C-3 was confirmed to be β oriented, since a cross NOE correlation were seen from H-3 to H-5 in the NOESY spectrum of compound **1**. This acetoxyl group was placed on C-3 (δ 80.93) based on HMBC and NOESY correlations. Some of the important HMBC and NOESY correlations can be seen in figure 1.

These spectral data of the compound **1** resembled those of lupeol derivatives with predictable differences. The ^{13}C NMR spectra of the compound **1** showed the presence of an α,β-unsaturated oxygenated function which deshielded C-20 and C-30 by 7 and 22 ppm (HMBC), in case of the C-29 aldehyde as compared to lupenyl acetate and lupeol, respectively [18–20]. The C-12 signal was also deshielded by 2.55 ppm due to the preferred conformation of C-29 oxygen function with its lone pair of electrons lying in close proximity to C-12. This deshielding effect also was seen in H-12 in ^{1}H NMR spectrum at δ 2.76 ppm.

Concerted application of one-dimensional (^{1}H, ^{13}C and DEPT) and two-dimensional (COSY, TOCSY, NOESY, HMQC and HMBC) experiments and literature data [18–20] indicated that compound **1** was 3β-acetoxylup-20(30)-en-29-al, which is a new naturally occurring compound.

Compound **2** exhibited the $[M]^+$ at *m/z* 482 in its EI–MS and the $[M + Na]^+$at *m/z* 505 in its LC–MS/MS corresponding to the molecular formula $C_{32}H_{50}O_3$. Analyses of the NMR spectra (^{1}H, ^{13}C, DEPT, H-COSY, HMQC and NOESY) showed that compound **2** was 3-acetylptiloepoxide [20,21]. Ptiloepoxide has been isolated from *Ptilostemmon gnaphaloides* as a natural product, but its acetyl derivative was prepared by Menichini et al. [21]. Therefore, compound **2** was first time isolated and identified from *C. lactiflora* as a natural product.

Compound **3** showed the $[M]^+$ at *m/z* 468 in its EIMS corresponding to the molecular formula $C_{32}H_{52}O_2$. Analyses of NMR spectra of compound **3** (^{1}H, ^{13}C, DEPT, H- COSY and HMQC) (table 1, figure 1) showed that compound **3** was identified as lupenyl acetate [18,20–22].

3. Experimental

3.1 General experimental procedures

Melting points were obtained using a Kofler hot-stage apparatus and are uncorrected. NMR spectra were recorded on a Varian NMR at 400 MHz instrument in $CDCl_3$. EI–MS spectra

were recorded on a Kratos MS-50 and Micromass Quattro LC-MS/MS instrument. Infrared spectra were obtained with a Perkin-Elmer 1600 FT-IR (4000–400 cm^{-1}) spectrometer. Flash column chromatography was performed on silica gel (230–400 mesh); preparative TLC was performed with pre-coated normal phase silica gel.

3.2 Plant material

Whole plants of *Campanula lactiflora* Bieb were collected in August 1996 at Arpali, Trabzon Hills (~2000 m), Turkey. A voucher specimen has been deposited at the Department of Chemistry of Karadeniz Technical University. The identification of this species was according to Flora of Turkey [1].

3.3 Extraction and isolation

Air-dried leaves of *C. lactiflora* (388 g) were successively extracted first with $CHCl_3$ then with MeOH. The $CHCl_3$ and MeOH extracts were concentrated in vacuo at 30–35°C to give a brown viscous residue (4.2 g, 11.6 g wet weight, respectively). After the TLC analysis, crude mixtures were found to be similar then combined. Crude mixture (10.2 g) was subjected to flash column chromatography on silica gel. The column was eluted with *n*-hexane followed by a discontinuous gradient with *n*-hexane-$CHCl_3$, $CHCl_3$, $CHCl_3$—MeOH, MeOH, $CHCl_3$-MeOH-H_2O and finally with H_2O to give 37 fractions. After TLC analysis, fractions 1–3 (called CL1), 4 (called CL2), 5 (called CL3), 6–13 (called CL4), 14–18 (CL5), 19–22 (called CL6) and 23–37 (called CL7) were combined [17].

Fraction CL2 was again purified by flash column chromatography on silica gel (30 g, 230–400 mesh). The column was eluted with *n*-hexane (50 ml) followed by a discontinuous gradient with *n*-hexane-$CHCl_3$ (2.5:0.5, 200 ml; 1:1, 50 ml) and finally with $CHCl_3$ (50 ml) to give 36 fractions (~5–10 ml each). After TLC analysis, fraction 13 gave compound **3** (19 mg, CH_2Cl_2-*n*-hexane (1:2)). The rest of the fractions were found to be mixtures.

Fraction CL4 was purified by small silica gel preparative TLC using *n*-hexane-EtOAc (2.5:0.5) to give two bands (CL4A, 28 mg; CL4B, 135 mg). The first band (CL4A) was again checked on TLC in CH_2Cl_2 solvent system and found to be a mixture, and then it was purified by small silica gel preparative TLC using CH_2Cl_2 to give compound **2** (CL4AB, 13 mg).

Fraction CL5 was again purified by flash column chromatography on silica gel eluted with *n*-hexane followed by a discontinuous gradient with *n*-hexane-CH_2Cl_2 and finally with *n*-hexane-CH_2Cl_2—$CHCl_3$ to give 23 fractions. After TLC analysis, fractions 5–7 were combined and purified by small silica gel preparative TLC using n-hexane-EtOAc (3:1) to give compound **1** (9 mg).

3.3.1 3β-Acetoxylup-20(30)-en-29-al (1). Oily compound, $[\alpha]_D + 26$ ($CHCl_3$; *c* 5×10^{-2}); ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm), see table 1; EI–MS *m/z* (%): *m/z* = 482(9) $[M]^+$, 468(39), 451(2), 438(2), 422(18), 407(18), 394(4), 381(16), 297(14), 215(22), 189(85), 135(72), 107(80), 96(74), 83(76), 60(78), 55(82), 43(100), 28(48). LC–MS/MS *m/z* (%): *m/z* = 505(10) $[M + Na]^+$, 482(3) $[M]^+$, 413(12), 379(15), 365(30), 304(38), 153(100), 102(36). FT-IR–cm^{-1}: 3045, 2939, 2839, 1758, 1715, 1690, 1230.

3.3.2 3-Acetylptiloepoxide (2). White amorphous crystals, mp 242–246°C (dec); $[\alpha]_D + 80$ ($CHCl_3$; *c* 2×10^{-2}); ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm), see table 1; EI–MS *m/z* (%): *m/z* = 482(6) $[M]^+$, 466(7), 422(13), 407(15), 380(10), 339(9), 289(5), 249(12), 203(28), 189(100), 175(32), 135(63), 122(61), 108(58), 96(54), 83(48), 68(51), 55(42), 43(75). LC-MS/MS *m/z* (%): *m/z* = 505(52) $[M + Na]^+$, 489(65) $[M + Na\text{-}16]^+$, 423(32), 365(28), 153(100), 102(86).

3.3.3 3β-Acetoxylup-20(29)-ene (3). ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm), 4.70 (1H, d, J = 2.4 Hz), 4.58 (1H, dq, J = 1.2 and 2.4 Hz), 4.49 (1H, m), 2.06, 1.70, 1.05, 0.95, 0.87, 0.86, 0.85, 0.80 (s, 3H each) and ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm), see table 1; EI-MS m/z (%): m/z = 468(5) $[M]^+$, 453(4) 408(6), 365(4), 356(3), 296(6), 256(3), 249(5), 189(55), 175(18), 147(22), 121(36), 107(33), 69(28), 43(39), 41(13), 32(31).

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