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New premyrsinane-type diterpene polyesters from *Euphorbia falcata*

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ABSTRACT

Four new premyrsinane-type diterpenes (**1**–**4**) were isolated from the methanol extract of the whole, undried plant of *Euphorbia falcata*. Their structures were determined by a combination of 1D and 2D NMR (COSY, HMBC, HSQC and NOESY) techniques and mass spectral data as di-, tetra-, penta- and hexaesters of diterpene polyols, esterified with acetic, benzoic, propanoic, isobutanoic and *n*-hexanoic acids. One of the compounds contains a rare hemiacetal moiety. This type of diterpenes was previously detected only in four *Euphorbia* species (*Euphorbia aleppica*, *Euphorbia decipiens*, *Euphorbia macroclada* and *Euphorbia pithyusa* subsp. *cupani*).

1. Introduction

Plants in the spurge family (Euphorbiaceae) are well known for the chemical diversity of their isoprenoid (sesqui-, di- and triterpenes) constituents. The genus *Euphorbia* is the largest in this family, comprising more than 2000 species, and diterpenoids are amongst their characteristic compounds. Many secondary metabolites based on specific types of diterpene skeletons (e.g., jatrophane, lathyrane, tigliane, daphnane, ingenane, myrsinane, etc.) have been isolated from different parts (leaves, aerial parts, milky latex, roots and seeds) of plants of the *Euphorbia* species,[1] and are of chemotaxonomical significance. Moreover, *Euphorbia* diterpenes possess a number of interesting biological activities, such as skin-irritant and tumour-promoting activities (due to their protein kinase C-activating effects), and also antiproliferative, antiviral and multidrug resistance-reversing activities.[2–5]

We have previously investigated the bioactive compounds of Hungarian *Euphorbia* species (*Euphorbia esula*, *Euphorbia peplus*, *Euphorbia lathyris*, *Euphorbia salicifolia*, *Euphorbia serrulata*, *Euphorbia platyphyllos*, *Euphorbia villosa* and *Euphorbia pannonica*) for diterpene constituents.[6–12] In a continuation of this programme, the present paper reports on a phytochemical investigation of *Euphorbia falcata* L, an annual herb widely distributed in garbage tips, cropland, and fallow areas. The diterpene content of this plant has not been described previously.

From the MeOH extract of *E. falcata*, four new pentacyclic premyrsinane-type diterpene polyesters (**1**–**4**) were isolated. Compound **4** contains a rare hemiacetal moiety. Premyrsinane diterpenes occur rarely; less then twenty compounds have previously been isolated from four *Euphorbia* species (*Euphorbia decipiens*, *Euphorbia aleppica*, *Euphorbia macroclada* and *Euphorbia pithyusa* subsp. *cupani*).[13–18]

2. Results and discussion

Four new diterpenes (**1**–**4**) were isolated from the $CHCl_3$ phase of the MeOH extract prepared from the whole plant of *E. falcata* by a combination of different chromatographic methods, including CC, VLC, CPC and preparative TLC. The structure elucidation was carried out by extensive spectroscopic analysis, including 1D and 2D NMR (^1H–^1H COSY, HSQC and HMBC) and HRESIMS experiments. The stereochemistry was studied by means of NOESY measurements.

Compound **1** was isolated as white crystalline form with $[\alpha]_D^{28.5}$ −68 (*c* 0.1, $CHCl_3$). It was shown by HRESIMS to have the molecular formula $C_{28}H_{44}O_7$ through the presence of *m*/*z* 515.2985 $[M+Na]^+$ (calcd for $C_{28}H_{44}O_7Na$ 515.2984). The ^1H and ^{13}C NMR spectra of **1** revealed the presence of one acetate group [δ_H 2.15 s; δ_C 170.2 (*C*O) and 21.1 (*C*H$_3$)] and one *n*-hexanoate group [δ_H 2.41 m (2H), 1.67 m (2H), 1.31 m (4H) and 0.89 t (3H); δ_C 175.8, 34.6, 31.3, 24.9, 22.3 and 13.9] (Table 1). Additionally, the ^1H NMR spectrum exhibited signals attributed to four methyls (1.18 s, 1.05 s, 0.98 d and 0.96 s). The ^{13}C and JMOD spectra suggested that the skeleton consisted of 20 carbons: four methyls, four methylenes, eight methines and four quaternary carbons. A quaternary carbon (δ_C 18.3) in the ^{13}C NMR

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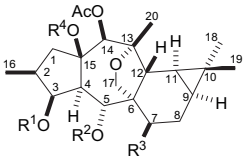

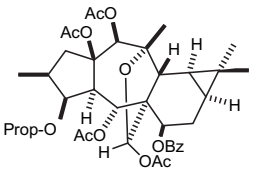

4

Compound	R^1	R^2	R^3	R^4
1	Hex	H	H	H
2	Prop	Ac	OBz	Ac
3	iBu	H	OBz	Ac

Ac = acetate, iBu = isobutanoate, Bz = benzoate, Hex = *n*-hexanoate, Prop = propanoate

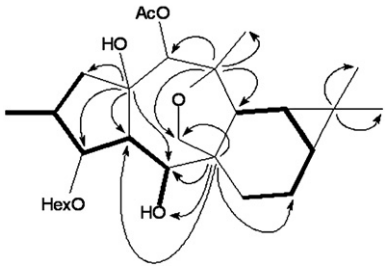

Fig. 1. ^{1}H–^{1}H COSY (–) and key HMBC (C→H) correlations of **1**.

spectrum together with the signals at δ_H 0.70 m, 0.51 t, 1.05 s, 0.96 s and δ_C 25.5, 19.0, 28.5 and 15.2 indicated the presence of a *gem*-dimethyl-substituted cyclopropane ring.[14] From the HSQC spectrum, the chemical shifts of the protonated carbons were assigned, and the proton–proton connectivities were then studied. The ^{1}H–^{1}H COSY spectrum defined two structural fragments with correlated protons: –CH_2–CH(CH_3)–CHR–CH–CHR– (A) (δ_H 2.40, 1.50, 2.18, 0.98, 5.28, 2.60 and 3.45) and –CH_2–CH_2–CH–CH–CH– (B) (δ_H 2.08, 1.70, 0.72, 0.70, 0.51 and 2.69) (Fig. 1). Their connectivities were determined from the long-range C–H correlations observed in the HMBC spectrum (Table 1). The two- and three-bond correlations of the quaternary carbons (C-6, C-10, C-13 and C-15) with protons of the structural fragments A and B established a pentacyclic premyrsinane diterpene with *O*-functionalities on C-3, C-5, C-14 and C-15 (Fig. 1). Moreover, the heteronuclear long-range coupling between H-17a and C-13, and between C-5 and H-12 and H-17, established a saturated furan ring through C-17 and C-13, which is characteristic for many myrsinane, cyclomyrsinane and premyrsinane esters.[19]

The positions of the ester groups were established via the HMBC experiment. The correlations of the carbonyl signal at δ_C 175.8 (*n*-hexanoyl *C*O) with the proton signals at δ_H 5.28 (H-3) and δ_H 2.41 (the methylene signal of *n*-hexanoyl) indicated the presence of the *n*-hexanoyl group on C-3. Similarly, the HMBC cross-peak of the carbonyl carbon signal at δ_C 170.2 with the proton signal at δ_H 4.88 (H-14) and the acetyl methyl signal at δ_H 2.15 demonstrated the presence of the acetyl group on C-14. Two proton signals, at δ_H 3.75 d and 1.58 s, which showed no correlations to any carbon in the HSQC spectrum, were assigned to two hydroxyl groups. These hydroxyl groups were situated on C-5 and C-15 with regard to the coupling constant $J_{5,5\text{-OH}}$=2.8 Hz, and the NOESY correlation between H-5 and the 15-hydroxyl group.

The stereochemistry and relative configuration of **1** were studied by means of a NOESY experiment (Table 1). The relative configuration of **1** was deduced by starting from the α-orientation of H-4, characteristic of many types of Euphorbiaceae diterpenes.[1]

Table 1
NMR data of compound **1** [$CDCl_3$, 500 MHz (^{1}H), 125 MHz (^{13}C), δ (ppm)]

Atom	^{1}H	^{13}C	HMBC (H No.)	NOESY (H No.)
1α	2.40–2.44 m	51.0	3, 14	1β, 2, 14
1β	1.50 dd (14.1, 10.3)			1α, 16
2	2.17–2.20 m	35.8	1α, 1β, 16	1α, 3, 4
3	5.28 t (3.0)	81.9	15, 16	2, 4, 5-OH
4	2.60 dd (10.3, 3.0)	54.2	1α, 1β, 5	2, 3, 14, 17a
5	3.45 dd (10.2, 2.8)	68.0	4, 12, 17a, 17b, 5-OH	7, 8β, 12, 15-OH
6	—	52.3	4, 5, 8α, 17a, 17b, 5-OH	—
7	2.05–2.10 m (2H)	28.8	5, 9	5
8α	1.64–1.71 m	17.9		9
8β	0.67–0.75 m			5, 19
9	0.67–0.75 m	25.5	7, 11, 18, 19	11, 18
10	—	18.3	18, 19	—
11	0.51 t (7.8)	19.0	14, 18, 19, 20	9, 17b
12	2.69 d (7.8)	36.7	14, 20	5, 19
13	—	85.9	12, 14, 17a, 20	—
14	4.88 s	82.5	1α, 1β, 4, 12, 14-COMe, 20	1α, 4
15	—	80.3	1α, 1β, 3, 4, 5	—
16	0.98 d (6.8)	14.5	1β, 2	1β
17a	4.14 d (8.5)	71.9	5, 12	4
17b	3.34 d (8.5)			11
18	1.05 s	28.5	19	9
19	0.96 s	15.2	18	8β, 12
20	1.18 s	23.2	14	—
14-*O*-Acetyl	2.15 s	21.1	—	—
		170.2	14, 14-CO*Me*	—
3-*O*-*n*-Hexanoyl				
1′	—	175.8	3	—
2′	2.40–2.44 m (2H)	34.6	3′	3′
3′	1.64–1.71 m (2H)	24.9	2′	4′
4′	1.31–1.33 m (2H)	31.3	2′, 3′, 6′	3′, 5′
5′	1.31–1.33 m (2H)	22.3	2′, 3′, 6′	4′, 6′
6′	0.89 t (6.5)	13.9	4′, 5′	5′
5-OH	3.75 d (2.8)	—	—	3
15-OH	1.58 s	—	—	5

Cross-peaks between H-4/H-3 and H-4/H-14 proved the β-orientation of the hexanoyl group on C-3 and the acetyl group on C-14. The NOE interaction observed between the 5-hydroxyl group and H-3 indicated the presence of an α-hydroxyl group on C-5. Moreover, the nuclear Overhauser effect between H-4 and H-2 indicated the β-orientation of the methyl group on C-2. The NOE interactions of H-5 and H-8β, H-5 and H-12, H-5 and the 15-hydroxyl group and H-12 and one of the geminal methyl groups on C-10 dictate the β-orientation of H-8b, H-12, the 15-hydroxyl group and the 19-methyl group. The NOE interactions of the other geminal methyl group with H-11, and other NOEs between H-11/H-9, H-11/H-17b and H-2/H-1α indicated the α-orientation of all these protons. All of the above evidence confirmed the structure of this compound as depicted in structural formula **1**.

Compound **2** was isolated as a colourless amorphous solid with $[\alpha]_D^{28.5}$ −4 (*c* 0.1, $CHCl_3$). It has the molecular formula $C_{36}H_{46}O_{11}$, as determined from HRESIMS and NMR analyses. From the ^1H and JMOD spectra, three acetyl [δ_H 2.16, 2.07 and 1.43; δ_C 170.5, 169.3 and 168.5 (*C*O), and 22.7, 21.2 and 21.1 (*C*H$_3$)] one benzoyl (δ_H 7.98, 7.41 and 7.54; δ_C 166.3, 133.0, 130.4, 130.0 and 128.3) and one propanoyl group [δ_H 2.25, 2.21 and 1.03; δ_C 174.1, 27.7 and 9.0] were identified (Table 2). Additionally, the JMOD spectra exhibited resonances for four methyls, three methylenes, nine methines and four quaternary carbons. The ^1H NMR and ^1H–^1H COSY spectra revealed the structural elements –CH_2–CH(CH_3)–CHR–CH–CHR– (C-1–C-2(C-16)–C-3–C-4–C-5) and –CHR–CH_2–CH–CH–CH– (C-7–C-12). The connection of these partial structures was carried out on the basis of two- and three-bond long-range correlations detected in the HMBC spectrum, and led to the conclusion that **2** is also a premyrsinane derivative. The ^1H and ^{13}C chemical shifts and coupling constants of **1** and **2** were very similar, except for the signals of C-7 and H-7. For **2**, the $\delta_{H\text{-}7}$ 4.85 and $\delta_{C\text{-}7}$ 73.7 signals clearly demonstrated O-substitution on C-7.

Table 2
NMR data of compound **2** [$CDCl_3$, 500 MHz (^1H), 125 MHz (^{13}C), δ (ppm)]

Atom	^1H	^{13}C	HMBC (H No.)	NOESY (H No.)
1β	2.71 dd (16.0, 10.4)	43.9	3, 14, 16	16
1α	2.50 dd (16.0, 9.2)			2, 14
2	2.08–2.10 m	36.1	1β, 16	1α, 3, 4
3	5.14 t (3.5)	76.7	1α, 16	2, 4
4	3.15 dd (10.7, 3.5)	52.7	5, 6	3, 14, 17a
5	5.82 d (10.7)	68.2	4, 7, 12, 17a, 17b	8β, 12
6	—	52.7	7, 17a	—
7	4.85 dd (8.0, 3.8)	73.7	8β	8α, 9, 17b
8α	2.08–2.10 m	24.4	7	7, 9
8β	1.76 ddd (14.8, 7.6, 5.4)			5, 19
9	0.88 dt (9.3, 7.5)	22.9	7, 18, 19	7, 8α, 11, 18
10	—	18.1		—
11	0.72 dd (9.3, 5.9)	17.5	12, 18, 19	9
12	2.73 d (5.9)	38.4	14, 17a, 20	5, 19
13	—	86.6	11, 14, 17, 20	—
14	5.00 s	80.8	4, 12, 15, 20	1α, 4
15	—	89.9	1β, 3, 14	—
16	0.77 d (6.7)	14.2		1β
17a	4.29 d (9.2)	71.4	5, 7, 12	4
17b	3.81 d (9.2)			7
18	1.09 s	28.9	11, 19	9
19	1.14 s	15.7	18	8β, 12
20	1.26 s	24.4	14	—
5-*O*-Acetyl	1.43 s	21.2	—	—
	—	169.3	5, 5-CO*Me*	—
14-*O*-Acetyl	2.07 s	21.1		—
		170.5	14, 14-CO*Me*	—
15-*O*-Acetyl	2.16 s	22.7	—	—
		168.5	—	—
3-*O*-Propanoyl				
1′	—	174.1	3, 3′, 2′	
2′	2.25 dq (16.3, 7.6) 2.21 dq (16.3, 7.6)	27.7	3′	
3′	1.03 t (7.6)	9.0	2′	
7-*O*-Benzoyl	—	166.3	2″, 6″	
1″	—	130.4	—	
2″, 6″	7.98 d (7.4)	130.0	2″–6″	5, 19, 15-OAc
3″, 5″	7.41 t (7.6)	128.3	3″, 5″	
4″	7.54 t (7.3)	133.0	2″, 6″	

Table 3
NMR data of compounds **3** and **4** [$CDCl_3$, 500 MHz (^1H), 125 MHz (^{13}C), δ (ppm)]

Atom	^1H	^{13}C	^1H	^{13}C
1β	2.81 dd (15.9, 10.4)	44.2	2.72 dd (15.9, 10.0)	44.1
1α	2.53 dd (15.9, 9.2)		2.46 dd (15.9, 9.6)	
2	2.12–2.17 m	35.7	2.09–2.11 m	35.7
3	5.20 t (3.5)	79.9	5.08 t (3.6)	76.7
4	2.96 dd (10.2, 3.5)	55.3	3.07 dd (10.5, 3.6)	53.6
5	4.07 dd (10.2, 3.0)	65.8	5.83 d (10.5)	67.3
6	—	53.3	—	56.0
7	5.19 dd (7.2, 3.4)	71.4	5.37 dd (8.4, 2.5)	73.1
8α	2.12–2.17 m	27.1	2.09–2.11 m	23.4
8β	1.60–1.64 m		1.61 ddd (14.5, 8.8, 4.5)	
9	0.81–0.84 m	18.9	1.00 dt (8.7, 8.6)	19.3
10	—	17.8	—	18.3
11	0.72 dd (9.7, 5.6)	20.7	0.80 dd (8.7, 6.9)	23.6
12	2.65 d (5.6)	37.3	2.69 d (6.9)	38.7
13	—	86.6	—	87.6
14	4.97 s	81.5	5.03 s	80.1
15	—	90.2	—	89.8
16	0.89 d (6.7)	14.4	0.75 d (6.7)	14.7
17	4.34 d (8.6), 3.57 d (8.6)	69.9	6.43 s	97.6
18	1.16 s	15.9	1.13 s	15.9
19	1.10 s	29.5	1.13 s	28.3
20	1.24 s	24.8	1.30 s	25.0
5-*O*-Acetyl	—	—	1.27 s	21.2
	—	—	—	169.4
14-*O*-Acetyl	2.07 s	21.2[a]	2.05 s	20.9
	—	170.4	—	170.0
15-*O*-Acetyl	2.07 s	22.6[a]	2.11 s	22.9
	—	168.3	—	168.3
17-*O*-Acetyl	—	—	2.21 s	21.2
	—	—		169.9
3-*O*-Isobutanoyl			3-*O*-Propanoyl	
1′	—	178.4	—	174.0
2′	2.44 sept (7.0)	34.2	2.33 dq (16.5, 7.5)	
2.25 dq (16.5, 7.5)	23.6			
3′	1.07 d (7.0)	19.7	1.09 t (7.6)	8.9
4′	1.01 d (7.0)	19.3	—	—
7-*O*-Benzoyl CO	—	165.6	—	166.2
1″	—	132.7	—	132.9
2″,6″	7.96 d (7.3)	129.5	7.95 d (7.5)	130.4
3″,5″	7.43 t (7.5)	128.7	7.41 t (7.6)	128.4
4″	7.56 t (7.5)	133.2	7.53 t (7.2)	133.2
5-OH	3.79 d (3.0)	—	—	—

[a] Signals may be interchanged.

The locations of the ester groups were established via HMBC. The correlation of the carbonyl signal at δ_C 174.1 (propanoyl *C*O) with the proton signals at δ_H 5.14 (H-3) and δ_H 1.03 (the methyl signal of propanoyl) indicated the presence of the propanoyl group on C-3. Similarly, the long-range couplings of the carbonyl carbon signals at δ_C 169.3 and 170.5 with the proton signals at δ_H 5.82 (H-5) and 5.00 (H-14) demonstrated the presence of acetyl groups on C-5 and C-14. The correlation of the carbonyl signal at δ_C 166.3 (benzoyl *C*O) with the proton signal at δ_H 4.85 (H-7) indicated the benzoyl moiety on C-7. The remaining acetyl group, with no long-range correlations, was located on quaternary carbon C-15, as indicated by the downfield-shifted C-15 signal ($\delta_{C\text{-}15}$ 89.9) as compared with that of **1** ($\delta_{C\text{-}15}$ 80.3).

A careful comparison of the NOESY spectra of **1** and **2** suggested the same stereochemistry for **2** and **1** (Tables 1 and 2). As concerns the chiral centre C-7, the NOESY cross-peaks between H-7/H-9, H-7/H-8α and H-7/H-17b proved the β-orientation of the benzoyl group on C-7.

Compound **3** was isolated as a colourless amorphous solid with $[\alpha]_D^{28.5}$ −31 (*c* 0.05, $CHCl_3$). Its HRESIMS displayed a quasimolecular ion peak at *m*/*z* 649.2989 $[M+Na]^+$, indicating a molecular composition of $C_{35}H_{46}O_{10}$. The ^1H NMR and JMOD spectra of **3** revealed two acetate, one isobutanoate and one benzoate groups. Additionally, the spectra exhibited resonances closely related to those of **2**. After the ^1H and ^{13}C NMR data on **3** had been assigned by analysis of its $^1H-^1H$ COSY, HSQC and HMBC spectra, it was obvious that compounds **2** and **3** are based on the same parent system and differ only in the substitution on C-3 and C-5. The absence of one acetate and the propanoate signals and the appearance of signals of an isobutanoate and a hydroxyl group indicated the replacement of propanoate residue with an isobutanoate group and an acetyl group with a hydroxyl group.

The position of the acyl residue was corroborated by the HMBC cross-peak between δ_H 5.20 (H-3) and the carbon signal at δ_C 178.4 (isobutanoyl *C*O). A careful comparison of the NOESY spectra of **2** and **3** indicated the same stereochemistry for **3** and **2**.

Compound **4** was isolated as a colourless amorphous solid with $[\alpha]_D^{28.5}$ −35 (*c* 0.1, $CHCl_3$). The HRESIMS (*m*/*z* 735.2993 $[M+Na]^+$) and NMR analyses indicated the molecular formula of $C_{38}H_{48}O_{13}$. The ^1H NMR and JMOD spectra exhibited typical signals for one propanoyl (δ_H 2.33 dq, 2.25 dq and 1.09 t; δ_C 174.0, 23.6 and 8.9), one benzoyl (δ_H 7.95 d, 7.53 t and 7.41 t; δ_C 166.2, 133.2, 132.9, 130.4 and 128.4) and four acetyl groups [δ_H 2.21 s, 2.11 s, 2.05 s and 1.27 s; δ_C 170.0, 169.9, 169.4 and 168.3 (*C*O) and 22.9, 2×21.2 and 20.9 (*C*H_3)]. After the ^1H and ^{13}C NMR data on **4** had been assigned by analysis of its $^1H-^1H$ COSY, HSQC and HMBC spectra, it was concluded that **4** has a similar parent system to that of **1**–**3**. The spectroscopic data were especially similar to those of **3**, the main difference between the two compounds being the presence of a further *O*-functionality in **4**, as suggested by the NMR signals at δ_H 6.43 s and δ_C 97.6. This methine, substituted with an acetyl group, was assigned to C-17 in view of the HMBC correlations between C-17/H-5, C-17/H-7, C-17/H-12 and H-17/acetyl *C*O. A careful comparison of the NOESY spectra of **4** and the other compounds indicated the same stereochemistry for **4**. As concerns the chiral centre C-17, the strong NOESY cross-peak between H-4/H-17 proved the β-orientation of the acetyl group on C-17.

In summary, the isolated compounds were identified as di-, tetra-, penta- and hexaesters of the pentacyclic premyrsinane polyol acylated with acetic, propionic, isobutanoic, *n*-hexanoic and benzoic acids, all these compounds being newly identified natural products. Compound **4** additionally contains a rare hemiacetal moiety, such diterpenes being very rare in Euphorbiaceae family; they have been isolated previously only from *Euphorbia aleppica* and *E. decipiens*. Moreover, compounds **1**–**4** are the first known premyrsinane-type diterpenes containing an acyl moiety, instead of a keto group on C-14. Biogenetically, premyrsinanes can be derived from epoxylathyranes by intramolecular cyclization; these compounds can be regarded as the precursors of polycyclic diterpenes, such as myrsinanes and cyclomyrsinanes.[1]

3. Experimental section

3.1. General procedures

Column chromatography (CC) was carried out on polyamide (ICN); vacuum liquid chromatography (VLC) on silica gel G (15 μm, Merck); preparative thin-layer chromatography (preparative TLC) on silica gel 60 F_{254} and RP-18 F_{254} plates (Merck); and centrifugal planar chromatography (CPC) on silica gel 60 GF_{254} with a Chromatotron instrument (Harrison Research). NMR spectra were recorded in $CDCl_3$ on a Bruker Avance DRX 500 spectrometer at 500 MHz (^1H) and 125 MHz (^{13}C), with TMS as internal standard. Two-dimensional data were acquired and processed with standard Bruker software. In the COSY, HSQC and HMBC experiments, gradient-enhanced versions were used. High-resolution MS data were recorded on a Waters-Micromass Q-TOF Premier mass spectrometer equipped with an electrospray source. The resolution was over 1 ppm. The data were acquired and processed with MassLynx software.

3.2. Plant material

E. falcata was collected in September 2008 in Mosonmagyaróvár (Hungary). The plant material was identified by Dr. Gyula Pinke (Department of Botany, Faculty of Agricultural and Food Sciences, University of West Hungary, Mosonmagyaróvár, Hungary). A voucher specimen (No. 775) has been deposited at the Herbarium of the Department of Pharmacognosy, University of Szeged, Szeged, Hungary.

3.3. Extraction and isolation

The fresh plant material (20 kg), which was stored at −20 °C before processing, was crushed in a blender and then percolated with MeOH (178 L) at room temperature. The crude extract was concentrated in vacuo and subjected to solvent–solvent partitioning with $CHCl_3$ (30 L). On evaporation, an organic phase residue of 344 g was obtained, which was chromatographed over a polyamide column (1100 g) with mixtures of H_2O–MeOH (3:2, 2:3 and 1:4) as eluents. The fractions obtained with H_2O–MeOH (3:2 and 2:3) were combined and subjected to silica gel VLC, using a gradient system of cyclohexane–EtOAc–MeOH (from 8:2:0 to 0:0:1). The CC fractions were combined into six fractions according to the TLC monitoring. From fraction 3, obtained with cyclohexane–EtOAc–MeOH (7:3:0), compound **1** was crystallized. It was further purified on NP-TLC (normal phase), using *n*-hexane–acetone (7:3), to yield 24.8 mg of **1**. The mother liquor of compound **1** was subjected to CPC, eluted with cyclohexane–CH_2Cl_2–MeOH of increasing polarity (80:20:1, 70:20:1, 60:30:2 and 60:50:3). Fraction 3/5, eluted with cyclohexane–CH_2Cl_2–MeOH (60:30:2), was further separated on RP-VLC (reverse phase) with MeOH–H_2O (1:1, 7:3, 4:1 and 85:15). The fraction obtained with MeOH–H_2O (4:1) was purified by preparative NP-TLC, with *n*-hexane–acetone (3:1) as developing system, and finally by preparative NP-TLC with $CHCl_3$–acetone (49:1), to yield compounds **2** (3.7 mg), **3** (3.2 mg) and **4** (3.5 mg).

*3.3.1. Compound **1**.* White crystalline form (mp 165–167 °C); $[\alpha]_D^{28.5}$ −68 (*c* 0.1, $CHCl_3$); ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m*/*z* 515.2985 $[M+Na]^+$ (calcd for $C_{28}H_{44}O_7Na$ 515.2984).

*3.3.2. Compound **2**.* An amorphous solid; $[\alpha]_D^{28.5}$ −4 (*c* 0.1, $CHCl_3$); ^1H and ^{13}C NMR data, see Table 2; HRESIMS *m*/*z* 677.2938 $[M+Na]^+$ (calcd for $C_{36}H_{46}O_{11}Na$ 677.2935).

*3.3.3. Compound **3**.* An amorphous solid; $[\alpha]_D^{28.5}$ −31 (*c* 0.05, $CHCl_3$); ^1H and ^{13}C NMR data, see Table 3; HRESIMS *m*/*z* 649.2989 $[M+Na]^+$ (calcd for $C_{35}H_{46}O_{10}Na$ 649.2999).

*3.3.4. Compound **4**.* An amorphous solid; $[\alpha]_D^{28.5}$ −35 (*c* 0.1, $CHCl_3$); ^1H and ^{13}C NMR data, see Table 3; HRESIMS *m*/*z* 735.2993 $[M+Na]^+$ (calcd for $C_{38}H_{48}O_{13}Na$ 735.2989).

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