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Salicifoline and salicinolide, new diterpene polyesters from *Euphorbia salicifolia*

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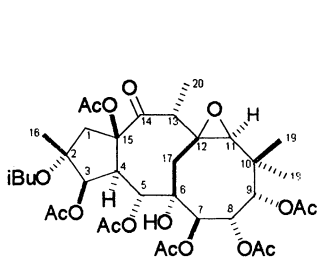
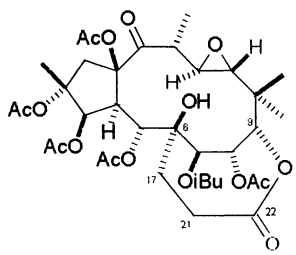
Abstract—Two new diterpenes (**1** and **2**) were isolated from a dichloromethane extract of fresh, whole plants of *Euphorbia salicifolia*: salicifoline (**1**) is the first representative of a new type of tricyclic diterpenes involving a novel 5-8-8 fused ring system, and salicinolide (**2**) is a bishomoditerpene lactone based on the jatrophane skeleton. The carbon skeleton of **1** is formally derived from jatrophane via transannular intramolecular C–C bond formation between C-12 and C-17. © 2001 Elsevier Science Ltd. All rights reserved.

Plants of the genus *Euphorbia* have been the subject of many chemical and pharmacological studies. The biological activities including antitumor,[1] antiviral, cytotoxic,[2] protein kinase C activating[3] and vanilloid receptor stimulating properties[4] and different vascular effects[5] are generally attributed to the presence of specific types of diterpenes: both macrocyclic and polycyclic derivatives. Our previous investigations on fresh, whole plants of *Euphorbia salicifolia* Host. yielded three jatrophane diterpenes and euphosalicine, a compound with a new bicyclic skeleton and exhibiting multidrug resistance reversing activity.[6] In a continuation of our study on the same plant, we now report on the isolation and structure elucidation of two uncommon diterpene polyesters (**1** and **2**). Salicifoline (**1**) represents a new class of cembrene cation-derived 'Euphorbiaceae' diterpenes possessing a new tricyclic skeleton, while salicinolide (**2**) is a bishomojatrophane, including a C-6–C-9 bonded eight-membered lactone ring as a new structural feature.

The fresh plant material of *E. salicifolia*, collected in Budapest (Pesthidegkút), Hungary, was processed according to the procedure recently described.[6] Fractions 19–37 obtained on silica gel flash chromatography were subjected to DCCC with the *n*-hexane–EtOAc–MeCN–MeOH (8:2:2:3) solvent system, and then to preparative TLC, to afford compounds **1** and **2** in 0.0001 and 0.0004% yields, respectively.



1



2

The HRFABMS suggested the molecular formula $C_{36}H_{50}O_{17}$ for salicifoline (**1**) {$[\alpha]_D^{24}$ −33 (*c* 0.5, $CHCl_3$)}, with the *m*/*z* 777.2897 $(M+Na)^+$ ion (calcd *m*/*z* 777.2946, Δ −6.3 ppm). The molecular formula pointed to a degree of unsaturation of 12 for the compound. Its ^1H NMR spectrum in $CDCl_3$ at ambient temperature contained several broad and overlapped signals; these became sharper in C_6D_6 at 335 K, and all NMR spectra were therefore recorded under these latter conditions. From the ^1H NMR and JMOD (*J*-modulated spin echo experiment) spectra of **1**, seven ester residues were easily identified as six acetate and one isobutanoate groups (Table 1). Additionally, the JMOD spectrum, supported by HSQC and HMBC correlations, revealed the existence of a C_{20} carbon-containing diterpene core, composed of one carbonyl group, five sp^3 quaternary carbons, eight sp^3 methines, two methylenes, three ter-

Keywords: *Euphorbia salicifolia*; Euphorbiaceae; diterpene polyester.
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Table 1. NMR data on salicifoline (**1**) [500 MHz (^{1}H), 125 MHz (^{13}C), C_6D_6, 335 K, δ (ppm) (J = Hz)]

Atom	^{1}H[a]	^{13}C[b]	HMBC (H no.)	NOESY (H no.)
1α	4.03 d (16.8)	50.1	3, 16	1β
1β	2.19 d (16.8)			1α, 16
2	–	86.9	1α, 3, 16	–
3	5.55 d (3.2)	82.4	1α, 16, Ac δ 1.75[c]	4, 16, 3′
4	3.03 dd (10.4, 3.2)	50.8	1α, 3, 5	3
5	5.89 d (10.4)	75.2	17b, Ac δ 1.80[c]	17b
6	–	76.3	5, 17b	–
7	5.52 brd (7.6)	72.2	5, 17b	18
8	6.00 brd (7.6)	71.3	9	9
9	5.45 brs	72.2	19, Ac δ 1.75[c]	8, 17a, 19
10	–	38.2	9, 11, 18, 19	–
11	3.10 s	65.7	9, 13, 17b, 18, 19	18, 20
12	–	63.5	11, 13, 17b, 20	–
13	3.26 q (6.9)	49.0	17a, 17b, 20	17b
14	–	213.2	1α, 13, 20	–
15	–	92.2	1α, 1β, 3, 5, Ac δ 1.69[c]	–
16	1.42 s	19.5	1α	1β, 3, 3′, 4′, 15-Ac
17a	2.72 d (16.7)	48.6	5, 13	9
17b	2.62 d (16.7)			5, 13
18	1.35 s	25.0	–	7, 11
19	1.10 s	25.7	–	9
20	1.10 d (6.9)	12.3	13	11
*i*Butanoyl				
1′	–	175.3	3′, 4′	–
2′	2.49 sept (7.1)	35.3	3′, 4′	3′, 4′
3′	1.23 d (7.1)	19.6	2′, 4′	3, 16
4′	1.13 d (7.1)	19.6	2′, 3′	3-Ac, 16

[a] ^{1}H NMR signals of acetyl groups: 9-OAc: 1.75 s; 15-OAc: 1.69 s; 3, 5, 7, 8-OAc: 2.20 s, 2×1.80 s, 1.75 s.
[b] ^{13}C NMR signals: 9-OAc: 21.2, 169.8; 15-OAc: 21.4, 170.8; 3, 5, 7, 8-OAc: 22.7, 21.5, 21.2, 20.7, 170.7, 170.5, 170.2, 170.1.
[c] $^4J_{C-H}$ couplings.

tiary methyl groups and one secondary methyl group. The ^{1}H–^{1}H connectivities detected in the ^{1}H–^{1}H COSY spectrum indicated only short sequences of correlated protons, –CH(OR)–CH–CH(OR)– (A), –CH(OR)–CH(OR)–CH(OR)– (B) and CH_3–CH– (C) (R = acyl), and two isolated methylenes (H-1 and H-17). Moreover, weak W-type ^4J couplings were observed in the ^{1}H–^{1}H COSY spectrum between methylene and adjacent methine protons (H-1α/H-3 and H-17b/H-11). These structural fragments, together with six quaternary carbons, were assembled by analysis of the long-range C–H correlations gleaned from an HMBC experiment (Fig. 1). The correlations of the quaternary carbons at δ_C 86.9 (C-2) and δ_C 92.2 (C-15) with the proton signals at δ_H 4.03, 2.19 (H-1), 5.55 (H-3), 1.42 (H-16) and 5.89 (H-5) indicated that structural element A and one methylene group form a methyl-substituted five-membered ring, present in many types of Euphorbiaceae diterpenes. The HMBC cross-peaks between the carbon signals at δ_C 76.3 (C-6) and 72.2 (C-7) and the proton signals at δ_H 2.62 (H-17b) and 5.89 (H-5) showed the connection of partial structures B and C through a quaternary carbon (C-6).

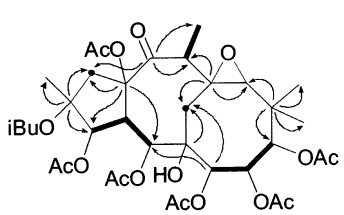

Figure 1. Connection of partial structures of salicifoline (**1**) via HMBC correlations (C→H).

The $^2J_{C-H}$ and $^3J_{C-H}$ correlations of the quaternary carbons at δ_C 38.2 (C-10), 63.5 (C-12) and 213.2 (C-14) permitted assignment of the molecule as a modified tricyclic jatrophane structure. In the skeleton of **1**, a methylene bridge is formed by the coupling of C-17 to C-12. Similar C-17 bridging in Euphorbiaceae diterpenes are known in the literature, e.g. in segetane and euphoperfoliane, which are products of other intramolecular cyclizations of jatrophane.[7,8]

The substitution pattern and positions of the ester groups were determined via HMBC measurements. The HMBC spectrum recorded at an evolution time of 65 ms proved the location of acetyl groups at C-3, C-5 and C-9 by $^3J_{C-H}$ couplings of oxymethine protons and carbonyl carbons. On the basis of the $^4J_{C-H}$ coupling observed in the spectrum recorded at an evolution time of 120 ms between C-15 and one of the acetyl methyl protons (δ_H 1.69), the presence of an acetyl group at C-15 was evident. Unfortunately, the positions of three acyl groups cannot be determined by HMBC experiments. As concerns the location of the isobutanoyl group, interpretation of the NOESY correlations was informative: NOE-enhanced signals detected between the isobutanoyl methyl protons and H-3 and H-16

clearly indicated the position of an isobutanoyloxy group at C-2. The remaining two acetyl groups were placed of necessity at C-7 and C-8. In view of the molecular formula and degree of unsaturation, an epoxy and a hydroxy group were also concluded. Considering the ^{1}H and ^{13}C chemical shift values, the epoxy group must be present at C-11–C-12,[9,10] similarly as in jatrophane diterpenes isolated previously from this plant, and the hydroxy group must be at C-6.[11]

The stereochemistry of salicifoline (**1**) was assessed by analysing the NOESY spectrum. Starting from the α-position of the proton at the ring junction (H-4), it was found that a β-oriented methyl group at C-2 and acetyl groups at C-3 and C-15 are present with regard to the diagnostic enhancements between H-4/H-3, H-3/H-1α, H-1β/H-16 and H-16/15-OAc. The NOESY correlation between 15-OAc and H-13 indicated the β-position of H-13, while the correlation between H-13 and H-17b revealed a β-oriented 17-methylene bridge. Further important NOEs were observed between H-17b/H-5, H-17a/H-9, H-9/H-8 and H-9/H-19, indicating the β orientation for these protons, and between H-18/H-7, H-18/H-11 and H-20/H-11, suggesting α-oriented hydrogens and methyls. On the basis of the above evidence, it was assumed that the structure of salicifoline is **1**. The relative configuration elucidated here is in accordance with those of diterpenes isolated earlier from *E. salicifolia*.[6]

Compound **2**, an amorphous solid {$[\alpha]_D^{25}$ +5 (*c* 0.1, $CHCl_3$)}, has the molecular formula $C_{34}H_{50}O_{16}$, determined via the quasimolecular ion peak at *m/z* 737.3009 $(M+Na)^+$ (calcd *m/z* 737.2997, Δ +1.6 ppm) in the positive HRFABMS and supported by proton and carbon counts in the NMR spectra. The molecular formula suggested a degree of unsaturation of 10. The ^{1}H NMR and JMOD spectra indicated a polyester with five acetate and one isobutanoate groups (Table 2). Apart from the signals of the ester moieties, the spectra contained resonances for skeletal carbons and protons, which were assigned by interpretation of the two-dimensional ^{1}H–^{1}H COSY, TOCSY, HSQC and HMBC spectra. The detected proton and carbon connectivities demonstrated a C_{22} carbon-containing bishomoditerpene core, consisting of six quaternary carbons, nine sp^3 methines, three methylenes, three tertiary methyl groups and one secondary methyl group. Vicinal proton relationships extracted from the ^{1}H–^{1}H COSY spectrum confirmed five structural fragments: $–CH_2–$ (A), –CH(OR)–CH–CH(OR)– (B), –CH(OR)–CH(OR)–CH(OR)– (C), –CH–CH–$CH(CH_3)$–

Table 2. NMR data on salicinolide (**2**) [500 MHz (^{1}H), 125 MHz (^{13}C), $CDCl_3$, δ (ppm) (*J* = Hz)]

Atom	^{1}H[a]	^{13}C[b]	HMBC (H no.)	NOESY (H no.)
1α	3.37 d (16.8)	47.8	3, 16	4
1β	2.29 d (16.8)			16
2	–	88.9	1α, 3, 16, Ac δ 2.05[c]	–
3	5.76 d (3.0)	80.7	1α, 4, 5, 16, Ac δ 2.12[c]	4, 6-OH, 17b/21b
4	3.08 d (3.0)	51.1	1α, 1β, 5	1α, 3, 5, 17a, 17b, 21a, 21b
5	5.50 s	71.6	4, 7, 6-OH, 17a, 17b	4, 6-OH, 8, 11
6	–	78.4	4, 5, 7, 8, 6-OH, 17b/21b	–
7	4.97 d (10.1)	78.0	5, 8	8, 9, 17a
8	5.66 dd (10.1, 9.2)	70.0	7, 9, Ac δ 1.95[c]	5, 7, 9, 11, 18
9	4.78 d (9.2)	79.9	7, 8, 18, 19	7, 8, 18, 19
10	–	38.6	8, 9, 11, 18, 19	–
11	3.84 d (1.8)	62.3	9, 13, 18, 19	5, 8, 13, 18
12	2.85 dd (8.4, 1.8)	58.9	11, 13, 20	13, 19, 20
13	2.68 dq (8.4, 1.8)	44.1	11, 12, 20	11, 12, 20, 15-Ac
14	–	216.7	1α, 1β, 4, 13, 20	–
15	–	94.6	1α, 1β, 3, 4, 5, Ac δ 2.10[c]	–
16	1.49 s	19.5	1β	1β, 3-Ac
17a	2.40 m	39.8	7, 6-OH, 21a, 21b	4, 7
17b	2.32 m			3, 4
18	0.99 s	22.7	11	8, 9, 11, 8-Ac
19	0.73 s	19.3	9, 11	9, 12
20	1.27 d (6.6)	18.2	13	12, 13
21a	2.47 m	31.2	6-OH, 17b	4
21b	2.30 m			3, 4
22	–	172.1	9, 21a, 21b	–
6-OH	3.36 s	–	–	3, 5, 3′, 4′
*i*Butanoyl				
1′	–	175.3	2′, 3′, 4′, 7	–
2′	2.48 sept (7.0)	34.8	–	3′, 4′
3′	1.16 d (7.0)	19.6	–	2′, 6-OH
4′	1.13 d (7.0)	19.3	–	2′, 6-OH

[a] ^{1}H NMR signals of acetyl groups: 2-OAc: 2.05 s; 3-OAc: 2.12 s; 5-OAc: 2.22 s; 15-OAc: 2.10 s.
[b] ^{13}C NMR signals: 2-OAc: 22.7, 170.1; 3-OAc: 22.1, 169.6; 5-OAc: 22.1, 168.8; 9-OAc: 21.7, 170.0; 15-OAc: 22.1, 170.3.
[c] $^{4}J_{C-H}$ couplings.

(D) and $-CH_2-CH_2-$ (E). On the basis of the HMBC correlations, these elements and the quaternary carbons were incorporated into a jatrophane skeleton bearing a two-carbon segment, which is connected to C-17. These two carbon atom formed a lactone ring between C-17 and C-9, as indicated by $^2J_{C-H}$ and $^3J_{C-H}$ couplings between C-21/H-17b, C-22/H-21 and C-22/H-9. Structurally close C_{22} diterpenes based on the 17-ethyljatrophane skeleton were isolated from *E. terracina* by Marco et al., but in terracinolides and isoterracinolides the lactone is formed with C-5-OH and C-3-OH, respectively.[12,13] The acyl residues in salicinolide (**2**) were located unambiguously through three- and four-bond long-range correlations, as in **1**. The hydroxy group at δ_H 3.36 was placed at C-6, in accordance with its HMBC cross-peak with C-6. An epoxy group, deduced from the molecular formula, was located at C-11–C-12, as confirmed by the chemical shifts values of C-11, C-12 and H-11 and H-12.[6]

The stereochemistry of all stereogenic centers was investigated by means of a NOESY experiment. Relevant NOE interactions were observed between H-4/H-3, H-4/H-1α, H-4/H-21, H-4/H-17 and H-17/H-7, indicating that these protons are all in the α-position, and the lactone bridge is oriented below the plane of the macrocycle. The Overhauser effect between H-1β and H-16 points to a β-oriented methyl group at C-2. The NOE interactions between 6-OH/H-5, H-5/H-8, H-8/H-18, H-8/H-11, H-8/H-9, H-11/H-13 and H-13/15-OAc are consistent with β-oriented protons at C-5, C-8, C-9, C-11 and C-13 and with 15β-OAc group. Moreover, the NOESY cross-peak between H-19 and H-12 established the orientation of the epoxy group as *trans*. All of the above data indicate structure **2** for salicinolide.

Salicifoline (**1**) exhibited pronounced multidrug resistance reversing activity on mouse lymphoma cells;[6] a fluorescence activity ratio (R) of 8.09 was measured at a concentration of 2 μg/ml. The positive control verapamil was less potent; it gave $R=6.65$ at a concentration of 5 μg/ml.

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