SUPPLEMENTARY MATERIAL

Analysis of diterpenoid compounds from the latex of two Euphorbiaceae by liquid chromatography-electrospray ionisation mass spectrometry

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Abstract: The latex of *Euphorbia papillosa* A.St.-Hil. and *Sapium glandulosum* (L.) Morong was phytochemically investigated by liquid chromatography/mass spectrometry. The results reveal the presence of tiglane-type diterpenoid compounds, particularly 12-deoxyphorbol esters, in both species. In addition, ingenane-type diterpene esters were found in the latex of *E. papillosa*. The latex of *S. glandulosum* showed only tiglane-type diterpenes, confirming the literature data on this genus. According to mass data, we are proposing the presence of 10 compounds derived from 12-deoxyphorbol monoesters- and diesters, from one 12-deoxyphorbaldehyde, from two ingenol derivatives, and from one nitrogen-containing phorbol ester in the latex of the analysed species. Considering that 12-deoxy tiglane diterpenes are described as anti-tumour and antiviral agents, these results indicate a pharmacological potential for these two Euphorbiaceae species.

Keywords: *Euphorbia papillosa*, ingenane, phorbol esters, *Sapium glandulosum*, tiglane.

Experimental

Plant material

Euphorbia papillosa A.St.-Hil. was collected in Passo Fundo, southern Brazil, identified and deposited in the herbarium of the University of Passo Fundo (RSPF 12647). *Sapium glandulosum* (L.) Morong was collected in Guaíba, southern Brazil, identified and deposited in the herbarium of the Federal University of Rio Grande do Sul (ICN 164089). Both plants were collected in December 2011. The crude latex was extracted by cutting through the leaves and stems of the plants, placed in glass tubes, and lyophilised.

Preparation of the samples

For reversed-phase high-performance liquid chromatography analysis, the lyophilised latex was suspended in methanol:water (1:1, v/v) with a concentration of 50 mg/ 3 mL, sonicated for 15 min and centrifuged. The supernatant was placed into a solid-phase extraction (SPE) cartridge (Chromabond C18, 3 mL/ 500 mg) and eluted with 6 mL portions of 30%, 50%, 70% aqueous methanol and a 6 mL portion of methanol. These aliquots were collected, concentrated under reduced pressure at 50°C in a rotatory evaporator and analysed by HPLC. The methanol aliquot of both samples was selected for further investigations.

HPLC and MS analysis

The HPLC-MS from the methanol aliquot was performed in an Agilent 6210-System, consisting of an analytical Agilent 1100 HPLC-System with an diode array detector (DAD) and an ESI-MSD TOF by Agilent Technologies. The extracts were analysed in the positive ion mode; capillary voltage, 4kV; fragmentor voltage, 200 V; scan range, m/z 50 to 2,000. A C18 reversed-phase cartridge column (Ultrasep ES Pharm, 125 x 3 mm, 5 μ m) was used as the stationary phase. Compounds were detected by UV-absorption at 210 nm in an acetonitrile (eluent A)/water (eluent B) gradient, as follows: 0-5 min (20% B); 10-20 min (5% B); 25-30 min (0% B); 35 min (5% B); 40-45 min (20% B). The flow rate was 0.5 mL/min at room temperature and the injected sample volume was 20 μ L. Solvents were HPLC grade, and deionised water was obtained using a Millipore Milli-Q water system. Standard TPA (12-*O*-tetradecanoylphorbol-13-acetate) was purchased from EMD chemicals, USA.

Figure S1. Positive ESI-MS profiles from the latex of *E. papillosa* (a) and *S. glandulosum* (b) at 16.02 min. Main peak: m/z 441.44

Figure S2. Structures of compounds 1-13. (a) tygliane-type diterpenes; (b) ingenane type diterpenes; (c) nitrogen-containing phorbol esters.

Figure S3. Positive ESI-MS from *E. papillosa*. Time 4.09 min. Peaks: m/z 313; 295, typical fragmentation of ingenol and deoxyphorbol.

Table S1. HPLC-ESI-MS data of suggested compounds, and literature review.

(a) Results of the latex analysis of both species.

	Rt (min)	(<i>m/z</i>)	Suggested compound	Isolated from (ref.)
1	11.4	455.42 [M + Na] ⁺	12-deoxyphorbol-13(2-methylbutyrate) (C ₂₅ H ₃₆ O ₆)	<i>E. poisonii</i> , <i>E. unispina</i> (5), <i>E. ledienii</i> , <i>E. coerulescens</i> (4)
2	13.1	391.38 [M + H] ⁺	12-deoxyphorbol-13-acetate (prostratin) (C ₂₂ H ₃₀ O ₆)	<i>E. fischeriana</i> (2), <i>E. cornigera</i> (13)
3	13.5	389.44 [M + H] ⁺	12-deoxyphorbolaldehyde-13-acetate (C ₂₂ H ₂₈ O ₆)	<i>E. fischeriana</i> (3)
4	15.4	483.44 [M + Na] ⁺	12-deoxyphorbol-13-isobutyrate-20-acetate (C ₂₆ H ₃₆ O ₇)	<i>E. poisonii</i> (5), <i>E. ledienii</i> , <i>E. triangularis</i> (4), <i>E. resinifera</i> (1), <i>E. bothae</i> (6)
5	16.0	441.44 [M + Na] ⁺	12-deoxyphorbol-13-isobutyrate (C ₂₄ H ₃₄ O ₆)	<i>E. poisonii</i> (5), <i>E. ledienii</i> , <i>E. triangularis</i> (4)
6	17.1	475.46 [M + H] ⁺	12-deoxyphorbol-13(2-methylbutyrate)-20-acetate (C ₂₇ H ₃₈ O ₇)	<i>E. poisonii</i> , <i>E. unispina</i> (5), <i>E. ledienii</i> , <i>E. coerulescens</i> , <i>E. triangularis</i> (4), <i>E. bothae</i> (6)
7	18.6	489.46 [M + Na] ⁺	12-deoxyphorbol-13-phenylacetate (DPP) (C ₂₈ H ₃₄ O ₆)	<i>E. poisonii</i> (7)
8	25.5	531.44 [M + Na] ⁺	12-deoxyphorbol-13-phenylacetate-20-acetate (C ₃₀ H ₃₆ O ₇)	<i>E. poisonii</i> (7)

(b) Results of the latex analysis of *E. papillosa*.

	Rt (min)	(<i>m/z</i>)	Suggested compound	Isolated from (ref.)
9	2.8	611.30 [M + Na] ⁺	3- <i>O</i> -(2,3-dimethylbutanoyl)-13- <i>O</i> -octanoylingenol (C ₃₄ H ₅₂ O ₈)	<i>E. esula</i> (10)
10	4.1	473.42 [M + H] ⁺	a) 12-deoxyphorbol-13-acetate-20-angelate (ref. 5 and 9) b) 12-deoxyphorbol-13-angelate-20-acetate (ref. 1) (C ₂₇ H ₃₆ O ₇)	<i>E. poisonii</i> , <i>E. unispina</i> (5), <i>E. cauducifolia</i> (9), <i>E. resinifera</i> (1)
11	15.3	573.48 [M + Na] ⁺	17-benzoyloxy-3- <i>O</i> -(2,3-dimethylbutanoyl)-20-deoxyingenol (C ₃₃ H ₄₂ O ₇)	<i>E. esula</i> (10)
12	23.3	453.44 [M + H] ⁺	12-deoxyphorbol-13-benzoate (C ₂₇ H ₃₂ O ₆)	<i>S. sebiferum</i> (8)

(c) Results of the latex analysis of *S. glandulosum*.

	Rt (min)	(<i>m/z</i>)	Suggested compound	Isolated from (ref.)
13	25.5	562.47 [M + Na] ⁺	a) 12- <i>O</i> -[2-methylaminobenzoyl]-4-deoxy-5-hydroxyphorbol-13-acetate (sapintoxin B) b) 12- <i>O</i> -[2-methylaminobenzoyl]-phorbol-13-acetate (sapintoxin D) (C ₃₀ H ₃₇ NO ₈)	<i>S. indicum</i> (11, 12)

Ref. (1) Fattorusso et al. 2002; (2) Ma et al. 1997; (3) Wang et al. 2006; (4) Sosath et al. 1988; (5) Schmidt & Evans 1977; (6) Popplewell et al. 2010; (7) Evans & Schmidt 1976; (8) Brooks et al. 1987; (9) Baloch et al. 2010; (10) Lu et al. 2008; (11) Taylor et al. 1981a; (12) Taylor et al. 1981b; (13) Miana et al. 1985.

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