INVESTIGATION OF DITERPENE ESTERS IN THE LATEX OF TWO SPECIES OF EUPHORBIACEAE

SOBOTTKA, A.M. 1*, BERTOL, C.D. 1; GÖRICK, C. 2; MELZIG, M.F. 2

1 UPF-Universidade de Passo Fundo, Passo Fundo, RS, Brasil. E-mail: sobottka@upf.br
2 Freie Universität Berlin, Germany

Keywords: Euphorbia papillosa, Sapium glandulosum, deoxyphorbol esters, ingenol, HPLC

The genera Sapium and Euphorbia belong to the Euphorbiaceae and are composed essentially of latex-bearing species. Studies with both genera have showed the presence of toxic tetracyclic diterpenoids, namely phorbol esters, that possess activities as skin irritant and tumor promoting agents, and have demonstrated also activity as tumor inhibitors and antileukemic agents. Euphorbia papillosa A. St.-Hil. and Sapium glandulosum (L.) Morong are latex-bearing species native from Brazil. The aim of this work was the investigation of the latex from these plants concerning the presence of diterpenoic compounds. E. papillosa was collected in Passo Fundo and S. glandulosum in Guaíba, Rio Grande do Sul, Brazil. The crude latex was extracted and lyophilized. The lyophilisate (50mg) was suspended in 3mL methanol:water (1:1, v/v), sonicated for 15min and centrifuged. The supernatant was injected in a solid-phase extraction (SPE) cartridge and eluted with 6ml portions of 30%, 50%, 70% aqueous methanol and 100% methanol. Aliquots were collected, concentrated and analyzed by HPLC, using a C18 reversed-phase column (125 x 3mm, 5µm), and 0,5 ml/min flow rate. Compounds were detected at 210nm in an acetonitrile (eluent A)/water (eluent B) gradient, as follows: 0/5min –20% B; 10/20min – 5% B; 25/30min – 0% B; 35min – 5% B; 40/45min – 20% B. The methanol aliquot seemed interesting and was analyzed by Reverse-phase HPLC-ESI-MS. Spectrum from the standard phorbol ester TPA (12-O-tetradecanoyl-phorbol-13-acetate) was also obtained and showed a typical fragmentation from a diterpene ester of the tigliane type (phorbol ester), with peaks at m/z 311, 389. This fragmentation was not found in the spectra obtained from the latex of the species studied. They showed the typical fragmentation from diterpene of ingenane type and from esters of deoxyphorbol (m/z 313 and 295). A comparison with the literature suggest the presence of 12-deoxyphorbol-13(2-methyl-butyrate); 12-deoxyphorbol-13-acetate; 12-deoxyphorbol-13-isobutyrate-20-acetate; 12-deoxyphorbol-13-isobutyrate; 12-deoxyphorbol-13(2-methyl-butyrate)-20-acetate; 12-deoxyphorbol-13-phenylacetate; 12-deoxyphorbol-13-phenylacetate-20-acetate. The MS spectrum from both plants showed a similar profile. All possible compounds found are esters of 12-deoxyphorbol. No evidence for the presence of diterpene ester of the tigliane type was obtained.