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CONSTITUENTS OF *Phagnalon sordidum*

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Phagnalon sordidum (L.) Reichenb. (*Compositae*) is a perennial weed widespread in the entire Mediterranean region. As a medicinal one, this plant is used alone or mixed with *Lippia citriodora* and/or *Malva sylvestris* to cure renal calculosis in the medicinal folk traditions of the Balearic Islands [1]. No other therapeutic applications nor phytochemical studies about this plant are cited in the literature. In the present communication we report the results obtained on the qualitative and quantitative analysis of secondary metabolites extracted from *P. sordidum* grown in central Italy.

The plant was collected in September 1999 in Spello and Ferentillo (Umbria, Italy) and air dried. Two hundred grams of finely triturated plant was extracted for 24 h with methanol (500 mL) in a Soxhlet apparatus. The methanolic extract was concentrated to about 50 mL and partitioned twice with *n*-hexane (100 mL); while carrying out this step a white precipate formed and was immediately separated by filtration under reduced pressure. The two organic phases were separated and evaporated to dryness from which were obtained, 1.30 and 2.2 g from the *n*-hexane and methanol portions, respectively.

The *n*-hexane extract was purified by silica gel column chromatography eluting with dichloromethane: two main fractions were obtained and each was analyzed by ^{1}H NMR, ^{13}C NMR, JMODXH, IR, and GC/MS spectrometry. The less polar fraction (0.94 g, 0.47 % of the dry weight) consisted of a mixture of long-chain hydrocarbons. ^{1}H NMR ($CDCl_3$) spectrum of the more polar fractions eluted from the column showed six singlets (each integrating for 3 H) in the range 0.70–0.95 ppm, another singlet (also integrating for 3 H) at 1.62 ppm, a multiplet (integrating for 1 H) centered at 2.32 ppm, a doublet of doublets (integrating for 1 H and J = 3.4 and 6.9 Hz) centered at 3.13 ppm, and two multiplets (each integrating for 1 H) centered at 4.49 and 4.63 ppm, respectively; in the ^{13}C NMR and JMODXH spectra ($CDCl_3$) the diagnostically significant signals were at 150.9 and 109.8 ppm (indicating an exomethylene olefinic moiety) and 80.3 ppm (indicating a secondary hydroxyl function). The recorded data were in full agreement with those reported in the literature for lupeol (**1**, 280 mg, 0.16% of the dry weight) [2].

The methanolic extract was purified by silica gel column chromatography eluting with a dichloromethane/methanol 95:5 mixture. Three main fractions were obtained and each analyzed as described above. The NMR spectra of two of these fractions were similar to those obtained for lupeol, suggesting that these two metabolites also belong to the lupane series. In the ^{1}H NMR spectrum ($CDCl_3$) of the less polar fraction the only difference was the presence of an AB system (integrating for 2H) in the range 3.72–4.28 ppm, indicating a primary hydroxyl function confirmed by a signal at 62.7 ppm in the ^{13}C NMR and JMODXH spectra ($CDCl_3$); in the ^{1}H NMR spectrum ($CDCl_3$) of the more polar fraction the difference was the lack of a methyl signal around 1.00 ppm and the presence of a quaternary carbon at 172.1 ppm, indicating a carboxylic acid moiety. These analytical data were in full agreement for those reported for betulin (**2** , 60 mg, 0.03% of the dry weight) and betulinic acid (**3**, 40 mg, 0.02% of the dry weight); the latter compound is one of the most effective apoptosis inducer in melanoma, and neuroectodermal and malignant brain tumor cells with no toxicity for normal cells [3].

The last fraction revealed, after comparison of NMR data recorded with those reported in the literature for the same compound [4], the presence of *β*-sitosterol in the form of *β-D*-glucopyranoside (**4**, 140 mg, 0.07% of the dry weight).

Finally, the white precipitate formed at the beginning of the work-up procedure was washed with a few mililiters of methanol and analyzed as described above. ^{1}H NMR, ^{13}C NMR spectra (D_2O), GC-MS (carried out on the hexacetylated derivative), and optical rotation values of our compound in comparison with those of a commercial sample confirmed unambiguously the presence of *L*-(-)-chiroinositol (**5**, 160 mg, 0.08% of the dry weight).

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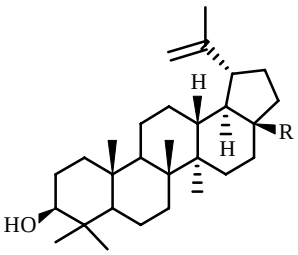
This is the first time that lupane triterpenes and *L*-(-)-chiroinositol were isolated from a plant belonging to the genus *Phagnalon*.



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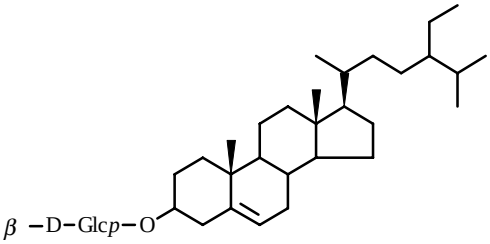
1: R = CH_3
2: R = CH_2OH
3: R = COOH



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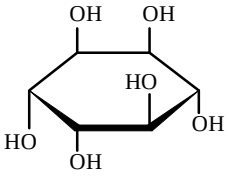

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