Triterpenes and a Novel Natural Xanthone as Lipophilic Glandular Products in *Hypericum balearicum*

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Leaves and young twigs of *Hypericum balearicum* L. exhibit lipophilic glandular products that can be recovered by rinsing green aerial parts with acetone. Major components of the resinous material are the triterpenes lupeone and lupeol. The intensely yellow colour of the acetone solution is due to a novel natural product, identified as 1,2,5-trihydroxyxanthone.

Introduction

Hypericum balearicum L. is a shrub native to the Balearic Islands, growing up to 1.20 m in dry forests and stony places (Krüssmann, 1977). Thanks to the attention of a gardener our interest was drawn to the fact that leaves and young twigs of *Hypericum balearicum* bear glandular structures and exhibit an aromatic scent. During our search for plants producing exudate flavonoid (Wollenweber, 1990) we examined it, therefore, for the presence of such products.

Materials and Methods

Hypericum balearicum is cultivated in the Botanical Garden at Darmstadt. Twigs of two-year-old plants were cut and rinsed with acetone to dissolve the lipophilic material. The intensely yellow solution was concentrated *i.v.* to yield a dark-brown resin (*ca.* 1.1% of fresh weight). This was dissolved in a small amount of boiling methanol, cooled to room temperature, then to –10 °C for 15 min and centrifuged, thereby eliminating *ca.* 35% of "fatty" material. The supernatant solution was passed over a Sephadex LH-20 column eluted with methanol to separate the phenolic from the terpenoid portion. A two component mixture crystallized from the concentrated acetone/methanol solution of the terpenoids and was chromatographed over silica to yield two pure substances. The relatively polar phenolic portion was further chromatographed over acetylated polyamide. Some combined fractions deposited yellow crystals which were minimally soluble in acetone. This major phenolic component was further studied by spectroscopic methods, while several other constituents had to be neglected, due to lack of material. No TLC spot likely to correspond to a flavonoid was detected.

Sephadex as well as silica columns were eluted with toluene and increasing amounts of methylethyl ketone and methanol. Thin layer chromatography was performed on silica plates with solvents A (toluene–methylethyl ketone 9:1) and B (toluene–dioxane–glacial acetic acid 18:5:1) and on polyamide DC-11 with solvents C (toluene–dioxane–methanol 8:1:1) and D (toluene–methylethyl ketone–methanol 12:5:3). Chromatograms were viewed in UV_{366} before and after spraying with Naturstoffreagenz A. Terpenoids were visualized by spraying silica plates with $MnCl_2$ reagent, followed by heating (Jork *et al.*, 1989). Mass spectra were recorded on a Varian MAT 311 at 70 eV by direct inlet. ^1H and ^{13}C NMR spectra were recorded in DMSO-d_6 on a Nicolet NT-WB FT spectrometer at 200 and at 50 MHz, respectively (triterpenes) and on a Bruker ARX 400 instrument at 400 and at 100 MHz (xanthone).

Results and Discussion

The acetone leaf wash of *Hypericum balearicum* was intensely yellow and afforded a brown resin on evaporation. Two major products crystallized from the terpenoid portion of this material. Direct chromatographic comparisons with markers suggested their structures which were confirmed by their MS and NMR spectra.

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Lupenone: Colourless crystals, m.p. 165 °C (Lit. 168–169 °C). M^+ at m/z 424, corresponding to $C_{30}H_{48}O$.

Lupeol: Colourless crystals, m.p. 181 °C (Lit. 212–215 °C). M^+ at m/z 426, corresponding to $C_{30}H_{50}O$. The melting point depression is due to traces of waxes, as indicated by the relevant NMR signals.

From the phenolic portion, a xanthone deposited in fine crystals.

1,2,5-Trihydroxyxanthone: Yellow solid, m.p. not determined due to paucity of material; MS, m/z (rel. int.): M^+ 244 (100), 216 (11, M^+–C=O), 215 (19, M^+–HC=O), 121 (13, $C_7H_5O_2$). UV, λ_{max} (MeOH): 400, 320(sh), 264, 246; NaOMe 442, 354, 276; $AlCl_3$ 474, 320, 278, 250, unchanged after addition of HCl. – ^1H NMR in DMSO-d_6 (ppm δ downfield from TMS): 12.52, br s, 1-OH; 10.52, br s, OH; 9.38, br s, OH; 7.60, dd, J 1.6, 7.9 Hz, H-8; 7.35, d, J 8.9 Hz, H-4; 7.34, dd, J 1.6, 7.9 Hz, H-6; 7.26, t, J 7.9 Hz, H-7; 7.00, d, J 8.9 Hz, H-3. – ^{13}C NMR: 147.2 (C-1), 140.0 (C-2), 124.5 (C-3), 106.3 (C-4), 148.1 (C-4a), 145.5 (C-4b), 146.4 (C-5), 120.6 (C-6), 123.8 (C-7), 114.5 (C-8), 120.3 (C-8a), 108.5 (C-8b), 182.5 (C-9). ^{13}C NMR resonances were assigned after comparison with published spectra for several xanthones which served as models (Frahn and Choudhuri, 1979).

Lupeol is a very widespread triterpene, known since 1889 when it was isolated from the seed shell of *Lupinus luteus.* Lupenone is far less widely distributed; it was first isolated in 1961 from the bark of *Alnus incana* (Karrer *et al.,* 1977). The presence of both products in the material under study appears rather trivial, although these substances have not previously been reported for *Hypericum* (Mahato *et al.,* 1992).

Guttiferae and Gentianaceae are the two families which have thus far yielded most of the known xanthones, followed by Moraceae, Polygalaceae and a few other families (Sultanbawa, 1980). The genus *Hypericum* is well known for the production of xanthone derivatives such as the dimeric hypericin (Hegnauer, 1966). The production, by *Hypericum balearicum,* of a trihydroxyxanthone is hence not surprising. It must be stressed, however, that this is the first time a xanthone with 1,2,5-oxygenation has been isolated, and hence our compound is a new natural product. All of the known trihydroxyxanthones have 1,3,5-, 1,3,7-, 1,5,6-, 1,6,7- or 2,3,4-tri-O-substitution (Sultanbawa, 1980; Schaufelberger, 1986). Furthermore this is only the second time that the occurrence of a free xanthone in a plant excretion is reported. The only other plant so far known is *Orphium frutescens* in the Gentianaceae, where glandular trichomes on leaves and stems produce three tetraoxygenated xanthones (Roitman *et al.,* 1992). In the case of *Hypericum balearicum* we are not certain that the lipophilic products reported herein are truly external; they might be extracted from minute dark sessile glands on rinsing with acetone.

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