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Sesquiterpenes from *Onopordum illyricum* and their Antifeedant Activity

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Phytochemical investigation of the acetone extract of the aerial parts of *Onopordum illyricum* L. afforded five known sesquiterpenoids: compounds **3** and **4** already isolated from *O. illirycum*, and 8α-[4'-hydroxymethacryloyloxy]-sonchucarpolide (**1**), 8α-[4'-hydroxymethacryloyloxy]-4-epi-sonchucarpolide (**2**) and 8-(4'-hydroxymethacryloyl)-dehydromelitensin (**5**), not previously detected in this species. Compounds **4** and **5** showed moderate antifeedant activity against larvae of *Spodoptera littoralis*.

Keywords: Asteraceae, *Onopordum illyricum*, Germacrane, Elemanes, Eudesmanes, Antifeedant activity.

The genus *Onopordum* L. includes about 50 species belonging to the tribe *Cardueae* (Asteraceae family) [1a]. The metabolites isolated from *Onopordum* species have been recently reviewed [1b]. *O. illyricum* (Illyrian thistle or Illyrian cottonthistle) is used as a vegetable in Lucania [1c] and Sardinia [1d]. In folk medicine, either a decoction or a tea of the whole plant is used as a digestive, cough sedative and in biliary diseases. The decoction or infusion of flowering tops is allegedly used for the treatment of malarial fever as an antipyretic, and for washing exanthematic skin [1b,2a]. Furthermore, the leave juice is used for cancer problems and to treat skin ulcers [2b]. Caffeoylquinic acids isolated from *O. illyricum* [3] have been implicated in the inhibition of HIV integrase, a key player in HIV replication and its insertion into host DNA [4].

Previous phytochemical studies of this species showed different sesquiterpene profiles. A sample collected in eastern Sicily, Italy [5] was very rich in different germacranes, elemanes and eudesmanes, including compounds **3** and **4**, whereas the plant material collected in Poland [6a] was shown to contain just the germacrane, onopordopicrin (**6**), and that collected in Sardinia [3], both onopordopicrin (**6**) and the germacrane **3**. In the frame of our ongoing researches on plants of the Asteraceae family [6b,7a,7b], in the present paper we report the isolation from a sample of *O. illyricum* collected at Mt. Pellegrino, Palermo, western Sicily, of several sesquiterpenes and on their antifeedant activity against the pest *Spodoptera littoralis*.

The aerial parts of the plant were extracted with acetone and the extract, after repeated column chromatography on silica gel, yielded in order of increasing polarity the following guaianolides: 8α-[4'-hydroxymethacryloyloxy]-sonchucarpolide (**1**) [8a], 8α-[4'-hydroxy methacryloyloxy]-4-epi-sonchucarpolide (**2**) [8a], compound **3** [8b], compound **4** [8c] and 8-(4'-hydroxymethacryloyl)-dehydro-melitensin (**5**) [9]. The structures of the isolated compounds were readily identified by comparing their physical and spectral data

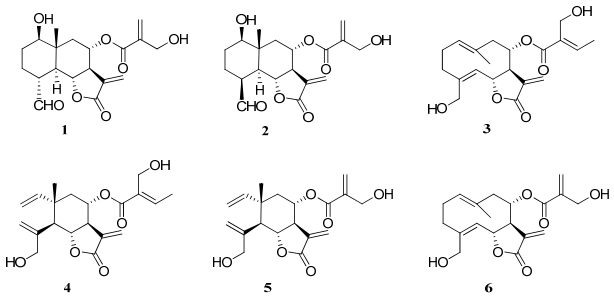

Figure 1: Structures of compounds **1**-**6**.

with those reported in the literature [1b]. Various biological properties of these sesquiterpene lactones have been reported.

The *in vitro* cytotoxic activity of compound **5** was tested against P388, A549, and HT29 cancer cells and compared with those of other sesquiterpene lactones. As expected for compounds with an α-methylene-γ-lactone group, they have cytotoxic activity that is increased by the presence of an additional α,β-unsaturated ester group (**5**). On the other hand, **5** did not show good antimicrobial activity against nine different microorganisms [10a]. Furthermore, compound **5**, when examined for its cytotoxic/cytostatic activity against five human cell lines (DLD1, SF268, MCF7, H460 and OVCAR3), exhibited a growth inhibiting effect against most of them [10b].

Also compounds **1-3** and **5** were tested *in vitro* against nine fungal species, using the micro-dilution method. The results showed that compound **3** had the lowest minimum inhibitory concentrations for the fungal growth. The results supported the hypothesis of an inverse relationship between polarity and antifungal activity [11]. We tested the sesquiterpenes **1**, **2**, **4** and **5** against larvae of *Spodoptera littoralis* at a concentration of 100 ppm (Table 2). Two of them (**4** and **5**) showed moderate antifeedant activity. The two

active compounds have an elemane skeleton, whereas those compounds with an eudesmane skeleton (**1** and **2**) were inactive.

From a chemotaxonomic point of view it is important that among the numerous sequiterpenes isolated from *O. illyricum* collected in different geographical areas [3,5,6a] only compounds **3** and **4** were isolated from our sample. On the other hand, compounds **1**, **2** and **5**, present in several species of *Onopordum*, *Centaurea* and *Cheirolophus* [1b], were not detected. Furthermore, the absence of onopordopicrin is noteworthy, as this metabolite is present in almost all the species of *Onopordum* studied so far, and had been considered a chemical marker of the genus [1b].

Table 2: Effect of sesquiterpenoids from *Onopordum illyricum* on feeding behavior of larvae of *Spodoptera littoralis*.

Compound	100 ppm ± sem[a]
1	0.7 ± 3.8
2	6.2 ± 2.0
4	42.3 ± 4.0
5	30.0 ± 3.9

[a] Feeding Index ((C - T)/(C + T))% when the compounds were tested at 100 ppm (n = 10), sem = standard error of the mean, $**p < 0.01$, $*p < 0.05$ Wilcoxon matched-pairs test.

Experimental

General experimental procedures. Optical rotations, JASCO P-1010 digital polarimeter; NMR, Bruker Avance series 300 MHz spectrometer; ESI-MS, Applied Biosystem API-2000 mass spectrometer; IR, Shimadzu FTIR-8300 spectrophotometer. Merck silica gel (70-230 mesh), deactivated with 15% H_2O, was used for column chromatography.

Plant material: The aerial parts of *Onopordum illyricum* L. were collected in June 2010 on Mt. Pellegrino, Palermo, in Sicily, Italy and voucher specimens (PAL 10-718) were deposited in the Herbarium of the Botanical Garden of Palermo, Italy.

Extraction and isolation: Fine, dried, aerial parts of *O. illyricum* (3 Kg) were extracted 3 times with acetone (3x3L) at room temperature. After removal of the solvent under reduced pressure, a gum (260 g.) was obtained. This was fractionated by CC on Si gel (silica gel deactivated with 15% of water) using gradient elution from pure light petroleum to pure ethyl acetate (EtOAc). The fraction eluted with EtOAc was subjected to several purifications by Si gel CC using a mixture of DCM/MeOH 20:1 to give, in order of polarity, compounds: **1** (4 mg) [8a], **2** (12 mg) [8a], **3** (3 mg) [8b], **4** (42 mg) [8c] and **5** (112 mg) [9].

Antifeedant bioassay: A binary choice bioassay using sucrose treated glass-fiber discs (Whatman 2.1 cm diameter) was used to investigate whether compounds influenced the feeding behavior of final stage larvae of *S. littoralis* (Lepidoptera) [12]. Single larvae were placed in a Petri dish with a control disc (C) and a disc treated with the test compound (T). The respective amounts eaten of each disc were used to calculate the Feeding Index (FI) ((C-T)/(C + T))%. Antifeedant activity is represented by positive values. The compounds were each tested at 3–4 concentrations (50, 100, 250 and 500 ppm) to calculate the concentration required to give a FI of 50%. Each concentration was tested against from 10 to 15 different larvae. The Wilcoxon matched-pairs test was used to analyze the data from testing the compounds at 100 ppm. Regression analysis was used to calculate the concentration required to give a FI of 50% (FI50).

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