Germacrane Derivatives from *Santolina pinnata* subsp. *neapolitana*

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From the aerial parts of *Santolina pinnata* subsp. *neapolitana*, one new and four known germacrane derivatives were isolated. The new compound was characterized as 1α,10β-epoxy-7αH-germacr-4(15)-ene-2β,5α,6β-triol by spectral methods.

Key words: Santolina pinnata subsp. *neapolitana*, Sesquiterpenoids, Germacranes

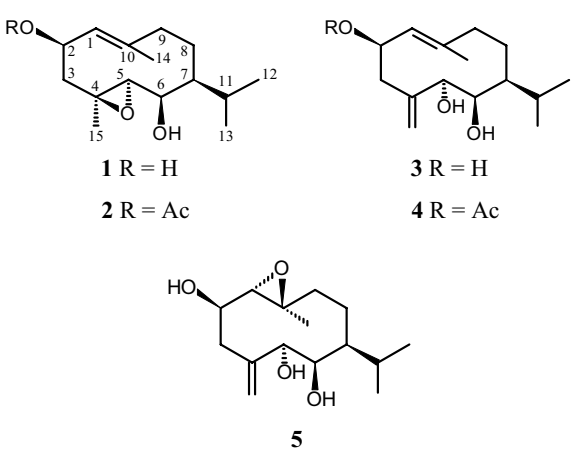
Introduction

Plants of the genus *Santolina* (Asteraceae, tribe Anthemideae) grow in South Europe and North Africa. Several species of this taxon have been investigated chemically yielding a number of mono- and sesquiterpenoids along with some other secondary metabolites (Barrero *et al.*, 2000, 1999, 1998; Marco *et al.*, 1993 and ref. cited herein). Oxygenated germacrane derivatives seem to be characteristic constituents of *S. chamaecyparissus* subsp. *squarrosa* (Barrero *et al.*, 1998; Marco *et al.*, 1993; Sanz *et al.*, 1991) and have also been found in *S. rosmarinifolia* subsp. *canescens* (Barrero *et al.*, 1999). The former species, which grows abundantly in the Spanish Mediterranean coast, has been used widely in traditional medicine for its analgesic, antispasmodic, anti-inflammatory, digestive and antimicrobial properties (Giner *et al.*, 1988). The reputed properties of *S. chamaecyparissus* subsp. *squarrosa* were confirmed in pharmacological studies. The plant extracts produced a significant reduction of the spontaneous activity in mice and showed an analgesic effect in the thermic and mechanical tests (Giner *et al.*, 1988). They inhibited isolated smooth muscle contractions induced by different agonists, including histamine and serotonin, and were anti-inflammatory in the carrageenan paw oedema assay in rats (Giner *et al.*, 1989). Moreover, the plant appeared to be a good source of compounds inhibiting the phospholipase A_2 activity both *in vitro* and *in vivo* (Sala *et al.*, 2000).

The present paper deals with the composition of sesquiterpenoids in aerial parts of *S. pinnata* Viv. subsp. *neapolitana* (Jord. et Fourr.) Guinea (syn. *S. neapolitana* Jord. et Fourr.), which reportedly contains acetylenic compounds (Christensen, 1992). From the plant material, the known oxygenated germacranes **1**–**4** (Fig. 1), first reported from *S. chamaecyparissus* subsp. *squarrosa* (Marco *et al.*, 1993; Sanz *et al.*, 1991), have been isolated, together with the new closely related compound **5**.

Results and Discussion

The aerial parts of the title plant were extracted with methanol and the extract, after purification and successive fractionation on silica gel, gave, in



1 R = H
2 R = Ac
3 R = H
4 R = Ac
5

Fig. 1. Chemical structures of 4β,5α-epoxy-7αH-germacr-1(10)*E*-ene-2β,6β-diol (**1**), its 2-acetate **2**, 7αH-germacra-1(10)*E*,4(15)-diene-2β,5α,6β-triol (**3**), its 2-acetate **4** and 1α,10β-epoxy-7αH-germacr-4(15)ene-2β,5α,6β-triol (**5**).

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Table I. ^{1}H NMR data (500.13 MHz) of **3**, **4** and **5** in $CDCl_3$[a].

H	**3**, δ_H (J [Hz])	**4**, δ_H (J [Hz])	**5**, δ_H (J [Hz])
1	5.25 dq (10.2, 1.3)	5.23 dq (10.3, 1.3)	2.95 d (9.5)
2α	4.56 ddd (10.2, 9.8, 6.8)	5.51 ddd (10.3, 10.3, 6.8)	3.57 ddd (10.3, 9.5, 6.3)
3α	2.81 dd (12.0, 6.8)	2.81 dd (12.1, 6.8)	2.87 dd (12.8, 6.3)
3β	2.19 dd (12.0, 9.8)	2.27 dd (12.1, 10.3)	2.34 br dd (12.8, 10.3)
5β	3.79 d (9.6)	3.78 br d (9.6)	3.92 d (9.7)
6α	3.92 d (9.6)	3.93 d (9.6)	4.02 d (9.7)
7α	1.09 br dd (8.6, 6.0)	1.10 br dd (9.2, 6.4)	1.42 m[b]
8α	1.44 m	1.44 m	1.42 m[b]
8β	1.68 m[b]	1.70 m[b]	1.95 br ddd (13.5, 11.5, 5.6)
9α	1.68 m[b]	1.70 m[b]	1.65 br dd (13.9, 5.6)
9β	2.42 br d (7.5)	2.43 br d (8.2)	1.60 br d (6.3)
11	1.76 m	1.76 m	1.79 m
12	0.98 d (6.7)	0.99 d (6.8)	1.02 d (6.7)[c]
13	0.98 d (6.7)	0.99 d (6.8)	1.01 d (6.7)[c]
14	1.69 d (1.3)	1.76 d (1.3)	1.36 s
15	4.93 br s	4.98 br s	5.12 br s
15′	5.01 br s	5.10 br s	5.20 br s
-OAc	–	2.04 s	–

[a] The assignments were confirmed by ^{1}H-^{1}H COSY and NOESY correlations.
[b] Signals fully or partially overlapped.
[c] Values interchangeable.

addition to **5**, 4β,5α-epoxy-7αH-germacr-1(10)*E*-ene-2β,6β-diol (**1**), its 2-acetate (**2**), 7αH-germacra-1(10)*E*,4(15)-diene-2β,5α,6β-triol (**3**) and its 2-acetate (**4**). Compounds **1**–**4** were easily identified by comparison of their spectral and physical properties with those in the literature (Marco *et al.*, 1993; Sanz *et al.*, 1991). The identity of **3** was further confirmed by direct comparison of its hitherto unreported NMR data in $CDCl_3$ with those of **4** (Tables I and II). The ^{13}C NMR data of **4** are compatible with those reported previously (Marco *et al.*, 1993), but some carbon chemical shift values seem to be interchanged as given in Table II.

Structure **5** for the new natural product was readily established when its mass, 1D and 2D ^{1}H NMR and ^{13}C NMR spectral data were directly compared with those of **3**. From this comparison, it became apparent that **5** differed from **3** in that an epoxy group was present in the C-1, C-10 positions in **5**. This assignment was in accord with the upfield chemical shifts of the C-14 methyl, H-1 and H-2 signals observed in the ^{1}H NMR spectrum of **5**. The H-1 signal at δ 2.95 appeared as a doublet (J = 9.5 Hz) which was coupled with the H-2 signal at δ 3.57 as shown by ^{1}H-^{1}H COSY spectrum. The spectrum also supported the remaining proton signal assignments and the structural skeleton of **5** was in agreement with the molecular formula $C_{15}H_{26}O_4$ confirmed by ESIMS (m/z = 271 $[M + H]^+$, 253 $[M + H - H_2O]^+$, 235 $[M + H - 2H_2O]^+$). Relative positions of H-2, H-5, H-6 and H-7 were assigned as in **3** and **4** on the basis

Table II. ^{13}C NMR data (125.76 MHz) of **3**, **4** and **5** in $CDCl_3$.

C	**3**, δ_C	**4**, δ_C	**5**, δ_C
1	127.91	124.04	63.60
2	70.38	72.06	71.04
3	44.71	41.05	41.24[c]
4	145.72	145.02	143.89
5	a	a	a
6	73.21	73.22	73.07
7	42.49	41.99	41.72[c]
8	28.92	28.86	24.89
9	35.68	35.81	36.88
10	140.19	142.20	63.60
11	31.72	31.78	31.32
12[b]	21.42	21.40	21.43
13[b]	21.15	21.14	20.55
14	22.72	22.73	23.88
15	114.45	115.49	116.82
MeCO–	–	170.44	–
MeCO–	–	21.26	–

[a] Obscured by the solvent signal (77.02).
[b,c] Values interchangeable.

of close similarity of coupling constants of the corresponding proton signals. Thus, the large coupling constant of the H-1 doublet indicated that H-1 was β-oriented. In order to establish the configuration of the epoxide ring and preferred conformation of **5** in solution, NOESY spectra of **3**, **4** and **5** were compared. In the spectra H-2α correlated with H-3α and H-14, H-3α correlated with H-2α and H-15′, H-6α with H-7α, H-15 and H-12 (H-13), while H-1 correlated with H-3β, H-5β and H-9β, H-5β with H-1 and H-3β. Thus, the epoxy group was proven to be *trans* (1α,10β) and the relative stereochemistry of **5** was confirmed. Accordingly, compound **5** was presumed to be 1α,10β-epoxy derivative of **3**. The values of the coupling constants observed in the ^{1}H NMR spectra of **3**, **4** and **5**, and the results of the NOESY correlation analysis suggested the same preferred conformation (C/α,α/N) in solution which corresponds to the crossed-boat type with both methyl (H-14) and methylene groups (H-15) below the mean plane of the ring (Barrero *et al.*, 1999; Marco *et al.*, 1993; Ugliengo *et al.*, 1990).

All germacrane derivatives isolated from the plant material possess 2β-hydroxy or 2β-acetoxy groups. Compounds **1** and **3** were found among major constituents of the anti-phospholipase A_2 fraction of *S. chamaecyparissus* (Sala *et al.*, 2000). Moreover, compound **1** showed significant antitumour activity against several human cell lines tested (Barrero *et al.*, 1999).

Experimental

Plant material

Aerial parts of *S. pinnata* subsp. *neapolitana* were collected in October 2001 from plants growing in the Garden of Medicinal Plants of the University of Medical Sciences in Poznań, where a voucher specimen (No 176/1999) is deposited. Seeds of the plant were provided by the Botanical Garden in Vacratot, Hungary.

Extraction and isolation

The dried plant material (140 g) was ground and exhaustively extracted with MeOH at room temperature providing a residue (10 g) which was dissolved in a H_2O/EtOH (15:1, v/v) mixture (400 ml), treated with a saturated solution of $Pb(OAc)_2$ in H_2O, left overnight and filtered. The filtrate was exhaustively extracted with $CHCl_3$ and the extract was dried over anhydrous sodium sulphate. A crude mixture (18 mg) of compounds **1** and **3** (by TLC) which crystallized from the $CHCl_3$ extract was separated and subjected to column chromatography on silica gel (Merck, Art. 7729) eluted with hexane/$CHCl_3$/EtOAc (1:1:3, v/v/v) to give fractions containing pure **1** (3.4 mg, *m.p.* 134–136 °C) and **3** (1.6 mg, *m.p.* 197–199 °C). The $CHCl_3$ supernatant was evaporated *in vacuo* and the residue (3.7 g) was chromatographed on a silica gel (Merck, Art. 7734) column using a $CHCl_3$/EtOAc gradient solvent system. Fractions from $CHCl_3$/EtOAc (7:1, v/v) elution yielded **2** (1.7 mg), while fractions eluted with $CHCl_3$/EtOAc, 1:1 and 1:4, v/v, gave **4** (3.6 mg) and **5** (4.5 mg), respectively, after purification of **2** and **4** by column chromatography (Merck, Art. 7729) using hexane/EtOAc (5:1, v/v) and hexane/$CHCl_3$/EtOAc (1:2:1, v/v/v), respectively.

1α,10β-Epoxy-7αH-germacr-4(15)ene-2β,5α,6β-triol (**5**)

Needles, *m.p.* 189–190 °C. $[\alpha]_D^{25}$ – 5.5° ($CHCl_3$, *c* 0.5). – ESIMS (positive mode): *m/z* = 271 $[M + H]^+$, 253 $[M + H - H_2O]^+$, 235 $[M + H - 2H_2O]^+$. – ^{1}H NMR: Table I. – ^{13}C NMR: Table II.

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