# Terpenoids from *Achillea setacea*

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*Achillea setacea*, Guaianolides, Monoterpene Triol

The aerial parts of *Achillea setacea* afforded, in addition of a rare monoterpene triol and 12 known sesquiterpene lactones, a new guaianolide containing an endoperoxide ring. Its structure was elucidated by spectral methods.

## Introduction

*Achillea setacea* Waldst. & Kit. is described as a white flowering diploid taxon occurring in central and south-east Europe, extending to western Asia (Richardson, 1976). This species belongs to the *Achillea millefolium* group (Richardson, 1976) which has received much attention for its medical properties. Thus, three antitumor sesquiterpenoids have been recently found in *A. millefolium* from Japanese origin (Tozyo *et al.*, 1994). A number of sesquiterpene lactones , mainly of guaianolide type are isolated from different taxa of the *A. millefolium* group and they have been proved to be responsible for the anti-inflammatory activity of the plant infusions (Kastner *et al.*, 1991; Kastner *et al.*, 1993). So far, 11,13-dihydrodeacetylmatricarin, rupicolin-A and rupicolin-B are the only sesquiterpene lactones found in *A. setacea* (Zitterl-Eglseer *et al.*, 1991). In the course of our study on the Bulgarian *Achillea* species used in the traditional medicine we investigated wild growing *A. setacea* and report now the isolation and identification of a rare monoterpene triol and 13 sesquiterpene lactones, among which the new endoperoxide guaianolide **1**.

## Experimental

### *Plant material*

The aerial parts of *A. setacea* were collected in flowering stage in July 1998 from the following locations: Vitosha mountain (sample 1), Pirin mountain (sample 2) and Golo bardo (sample 3). Voucher specimens (SOM 154209, SOM 154212 and SOM 154207) were deposited in the Herbarium of the Institute of Botany, Bulgarian Academy of Sciences, Sofia.

### *Extraction and isolation*

The air-dried above ground parts of sample 1 (150 g) were extracted with $CH_2Cl_2$ (2x500 ml) at room temperature. After evaporation of the solvent *in vacuo* and working up as described earlier (Todorova *et al.*, 1998) a crude lactone fraction (2.7 g) was obtained . The latter was separated by column chromatography on silica gel (90 g) using solvent mixtures ($CHCl_3$-MeOH) with increasing polarity. Selected fractions (IR control) were additionally purified by CC and prep. TLC on silica gel to give: sintenin (5 mg), 1β,10α-epoxy-3β, 9β- diacetoxy-11α,13-dihydrocostunolide (3 mg), rupicolin-A (10 mg) and rupicolin-B (12 mg), 1-desoxy-1α-peroxy-rupicolin-A (4 mg) and 1-desoxy-1α-peroxy-rupicolin-B (3 mg), 3α,4α-epoxyrupicolin-A (4 mg) and 3α,4α-epoxyrupicolin-B (5 mg), rupin A (8 mg), desacetyl-1α,4α-dihydroxybishopsolicepolide (7 mg), desacetyl-1α,4β-dihydroxybishopsolicepolide (4 mg), 11,13-dehydrodesacetylmatricarin (5 mg), arteludovicinolide A (5 mg), 1S,2R,4S, trihydroxy-p-menthane (4 mg) and 8α-hydroxy-tanaparthin-α-peroxide (5 mg).

Crude lactone fractions (200 mg and 550 mg) were obtained from samples 2 (23 g) and 3 (600 g), respectively, using the procedure described above. TLC analyses were performed on TLC aluminum sheets, Silicagel 60, F254, Merck using $CHCl_3$-MeOH (15:1 v/v) as a solvent system.

### *8α-Hydroxy-tanaparthin-α-peroxide* (**1**)

Gum, CIMS (NH3) *m/z* (rel. int.): 312 $[M+NH_4]^+$ (100), 294 $[M]^+$ (8). EIMS (70 eV) *m/z* (rel.int.): 294 $[M]^+$ (0.5), 276 $[M-H_2O]^+$(0.5), 262

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$[M-O_2]^+$ (17), 244 $[262-H_2O]^+$ (8), 229 $[244-CH_3]^+$ (6), 226 $[244-H_2O]^+$ (4), 219 (10), 201 (14) 122 (67), 111 (55), 43 (100). $^1H$ NMR (250 MHz, $CDCl_3$) δ: 6.24 (1H, d, 5.7, H-2), 6.33 (1H, d, 5.7, H-3), 2.73 (1H, d, 10.3, H-5), 3.70 (1H, t, 10.3, H-6), 3.35 (1H, dddd, 10.3, 8.3, 3.3, 3.6, H-7), 3.93 (1H, dddd, 11.6, 8.3, 5.2, 1.7, H-8), 1.99 (1H, ddd, 16.2, 5.2, 2.3, H-9), 2.22 (1H, dd, 16.2, 1.7, H-9'), 5.99 (1H, d, 3.3. H-13), 6.22 (1H, d, 3.6, H-13'), 1.45 (3H, s, H-14), 1.72 (3H, s, H-15), 2.99 (1H, d, 11.6, OH-8), 2.70 (1H, d, 2.3, OH-10).

*1S, 2R, 4S-Trihydroxy-p-mentane* (**3**)

Amorphous powder. EIMS (70 eV) *m/z* (rel. int.): 170 $[M-H_2O]^+$ (20), 152 $[170-H_2O]^+$ (29), 134 $[152-H_2O]^+$ (18). $^1H$ and $^{13}C$ NMR: in Table I.

## Results and Discussion

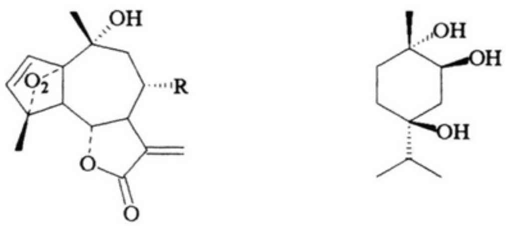
Air-dried plant material (sample 1, see Experimental) was extracted with dichloromethane and the crude extract was subjected to column chromatography separation to give the lactone containing fractions (IR control). Further rechromatography and purification afforded 14 individual compounds. By analogy of their spectral data to those reported, twelve were assigned as the following sesquiterpene lactones: sintenin (Goren *et al.*, 1988), 1β,10α-epoxy-3β, 9β- diacetoxy-11α,13-dihydro-costunolide (Milosavljevic *et al.*, 1991), rupicolin-A and rupicolin-B (Irwin and Geissman, 1973), 1-desoxy-1α-peroxy-rupicolin-A and 1-desoxy-1α-peroxy-rupicolin-B (Bohlmann *et al.*, 1980), 3α,4α-epoxyrupicolin-A and 3α,4α-epoxyrupicolin-B (Todorova *et al.*, 1998), rupin A (Irwin *and* Geissman, 1973), desacetyl-1α,4α-dihydroxy-bishopsolicepolide (Jakupovic *et al.*, 1988), desacetyl-1α,4β-dihydroxy bishop-solicepolide (Milosavljevic *et al.*, 1994), 11,13-dehydrodesacetylmatricarin (Ohno *et al.*, 1980; Ognyanov and Todorova, 1983) and arteludovicinolide A (Bohlmann and Zdero, 1982).

Compound **1** was isolated as colourless gum. The mass spectrum (EI) displayed a molecular ion peak at *m/z* 294 with very low intensity which corresponded to a molecular formula $C_{15}H_{18}O_6$. The other informative fragments were *m/z* 262 $[M-O_2]^+$, 244 $[262 - H_2O]^+$ and 226 $[244 - H_2O]^+$. The $^1H$ NMR spectrum (see Experimental) gave the clue to the structure of this compound, as it was very similar to that of tanaparthin-α-peroxide **2** (Bohlmann and Zdero, 1982), the stereochemistry of which was determined unambiguously (Jakupovic *et al.*, 1986). The observed chemical shifts of the signals for H-2, H-3, H-5 and H-6 (δ 6.24, 6.33, 2.73 and 3.70, respectively) are consistant with the α-orientation of the endoperoxide ring and the OH group at C-10. However, the presence of an additional OH group in **1** was required by both the MS and $^1H$ NMR data. The location of this OH group at C-8 and its α-orientation followed from the multiplicity of the H-8 signal (dddd) and the magnitude of the coupling constant $J_{8,7}$ = 8.3 Hz, as well as the downfield shift of the H-13' signal to δ 5.99. Thus, the relative stereochemistry of the chiral centers in **1** was the same as that in **2**. Therefore, compound **1** was identified as 8α-hydroxy-tanaparthin-α-peroxide.

Besides the sesquiterpene lactones described above, the polyoxygenated monoterpene **3** was

Table I. NMR data of (**3**) in $CDCl_3$ (500/125.3 MHz).

| Position | H | C | HMQC |
|---|---|---|---|
| 1 | – | 71.62 s | H-5, H-6, H-10, H-2 |
| 2 | 3.5 brs | 74.84 d | H-6, H-3', H-10 |
| 3 | 2.01 dd (14.5, 3.3) | 33.77 t | H-7 |
| 3' | 1.79 ddd (14.5, 3.0, 3.0) | | |
| 4 | – | 75.31 s | H-5', H-3', H-7, H-8, H-9 |
| 5 | 2.07 ddd (13.8, 13.8, 4.0) | 29.58 t | H-7 |
| 5' | 1.87 ddd (13.8, 13.8, 3.0) | | |
| 6 | 1.35–1.50 m | 29.16 t | H-5, H-10 |
| 7 | 1.65 qui (6.8) | 38.46 d | H-8, H-9 |
| 8 | 0.93 d (6.8) | 16.62 q | H-7 |
| 9 | 0.93 d (6.8) | 16.64 q | H-7 |
| 10 | 1.35 s | 27.69 q | H-6 |



**1** R=OH
**2** R=H
**3**

Fig. 1. **1:** 8α-hydroxy-tanaparthin-α-peroxide;
**2:** Tanaparthin-α-peroxide;
**3:** 1*S*, 2*R*, 4*S*-*t*rihydroxy-*p*-menthane.

also isolated as an amorphous powder. Its structure and relative stereochemistry was elucidated by MS and NMR data ($^1$H-, $^{13}$C-NMR, COSY and NOESY). To the best of our knowledge, the monoterpene triol 1*S*,2*R*,4*S*-trihydroxy-*p*-menthane was isolated for the first time from an Indian plant belonging to the Rutacea family (Thappa *et al.*, 1976). As only selected NMR data are reported in this early publication, we present in Table I the thorough NMR properties of the rare monoterpene triol **3**.

Finally, comparative analyses by TLC were performed on the crude lactone fractions obtained from the aerial parts of *A. setacea* collected from two other localities (samples 2 and 3, see Experimental) using the isolated terpenoids from sample 1 as references. The results revealed no differences in the composition of the investigated samples regarding the sesquiterpene lactones, thus demonstrating the homogeneity of the studied species.

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