Achillea collina, Asteraceae, Sesquiterpene Lactones

The species of genus Achillea, particularly those belonging to the Achillea millefolium group have received a lot of attention so far, due to their use in traditional medicine. Among the numerous chemical compounds isolated from the A. millefolium species, the sesquiterpene lactones are usually one of the major constituents (Seaman, 1982). Continuing our chemotaxonomic examination of Bulgarian Achillea taxa, we have undertaken an investigation of the chloroform extract of Achillea collina Becker, a tetraploid species of the A. millefolium group. Previous chemical studies on this species revealed the presence of lactones belonging to the guaiane type only. The main lactones were reported to be artabsin derivatives (Verzar-Petri et al., 1980; Kastner et al., 1991a; Kastner et al., 1991b; Kubelka et al., 1994), although a matricin derivative (Verzar-Petri et al., 1980) and a matricarin derivative (Glasl et al., 1994) were also isolated. In contrast, we could not detect any other lactones but germacranolides and eudesmanolides. In the present paper, we wish to report the isolation of three new sesquiterpene lactones, 2, 4 and 5, in addition to the known lactones 1, 3, 6, 7 and 8.

Introduction

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Experimental

Plant material

The above ground parts of A. collina were collected from Rodopa mountain in July 1995. The plant material was identified by Dr. R. Taskova, Institute of Botany, Bulgarian Academy of Sciences, and a voucher specimen (CO-528) was deposited in the Herbarium of the same Institute.

Extraction and isolation

The air-dried plant material (60 g) was extracted with chloroform (2 ¥ 500 ml) to give, after evaporation of the solvent under reduced pressure, a brownish gum (2.0 g). It was then defatted by dissolving in 50% aq. MeOH (20 ml). After filtration, the filtrate was first extracted with hexane (3 ¥ 25 ml), then concentrated by evaporation the methanol, and finally extracted with chloroform (3 ¥ 10 ml) to give, after removing the solvent the crude lactone fraction (0.7 g). It was separated into 9 fractions by column chromatography (CC) on silica gel (100 g) using chloroform-acetone mixtures as eluents. Selected fractions (IR control) were subjected further to repeated CC and/or prep TLC to yield 1 (2 mg), 2 (16 mg), 3 (11 mg), 4 (3 mg), 5 (6 mg), 6 (2 mg), 7 (10 mg) and 8 (4 mg).

8α-tigloyloxy-11β,13-dihydroparthenolide (2): oil, EIMS (70 eV), m/z (rel. int.): 348 [M]+ (1), C20H28O5; 248 [M-100]+ (31); 220 [248-28]+ (23); 83 (100), 55 (64). 1H NMR: in Table I.

8α-acetylshonachalin A (4): oil; EIMS (70 eV), m/z (rel. int.): 308 [M]+ (1) C17H24O5; 248 [M-60]+ (12), 230 (70), 215 (20), 202 (32), 174 (57), 149 (61), 121 (62), 95 (78), 69 (77), 55 (83). 1H NMR: in Table I.

8α-acetylartapshin (5): oil; EIMS (70 eV), m/z (rel. int.): 308 [M]+ (1) C17H24O5; 248 [M-60]+ (10), 230 [M-60-18]+ (100), 215 (22), 202 (32), 174 (57), 149 (61), 121 (62), 95 (78), 69 (77), 55 (83). 1H NMR: in Table I.
Results and Discussion

The chloroform extract of the aerial parts of *A. collina* was worked up as described in the Experimental, to give the following lactones in order of their elution: 11βH,13-dihydroparthenolide (1) (Ruangrungsi and Rivepiboon, 1988), 2, balchanolide (3) (Suchy et al., 1963), 4, 5, 1β-hydroperoxy-8α-hydroxygermacra-4,10(14)-dien-6β,7α,11βH-12,6-olide (6) (Marco, 1989), 8α-hydroxy-11βH,13-dihydrobalchanin (7) (Fernandez et al., 1987) and artapshin (8) (Fernandez et al., 1987).

Compound 2, isolated as an oil, was a germacranolide, the structure of which followed from the 1H NMR spectrum (Table I). The latter indicated an 8α-tigloyloxy derivative of 1. The presence of the tigloyl ester moiety was further confirmed by the MS spectrum which displayed, alongside a molecular ion (m/z 348) with very low intensity a fragment at m/z 248 due to loss of the aliphatic acid (C5H8O2). Moreover, the 1H NMR data were very similar to those described for the lactones 9 (Jakupovic et al., 1992) and 10 (Talapatra et al., 1970). However, the chemical shift of the H-8 signal in 2 (δ 4.96) which appeared in the same region as that in 10 (δ 4.85) indicated that the hydroxyl group in 9 is replaced by a tigloyl group. The configuration at C-5/ C-8 and C-11 followed from the observed coupling constants, and from their good coincidence with those of the germacranolides 9 and 10. Accordingly, the new lactone 2 was identified as 8α-tigloyloxy-11βH,13-dihydroparthenolide.

The MS and 1H NMR data of the lactone 4 clearly showed that we were again dealing with 11βH,13-dihydrogermacranolide bearing a hydroxyl group and an acetate ester side chain. The location of these substituents at C-1 and C-8, respectively, and the stereochemistry followed from the 1H NMR spectra (Table I) and COSY experiment. Furthermore, the 1H NMR data of 4 were, with the exception of the chemical shift of the H-8 signal, in good accordance with those described for shonachalin A (11) (Serkerov and

Table I. 1H NMR spectral data of lactones 2, 4 and 5 in CDCl₃ (250 MHz).

<table>
<thead>
<tr>
<th>H</th>
<th>2</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.22 brdd (14.1,13.0)</td>
<td>3.85 brs</td>
<td>3.52 dd (4.0, 11.7)</td>
</tr>
<tr>
<td>5</td>
<td>2.64 d (8.9)</td>
<td>5.19 d (9.8)</td>
<td>1.90–2.00*</td>
</tr>
<tr>
<td>6</td>
<td>4.02 t (8.9)</td>
<td>4.45 t (9.8)</td>
<td>4.13 t (10.9)</td>
</tr>
<tr>
<td>7</td>
<td>2.30–2.45*</td>
<td>2.25–2.45*</td>
<td>1.85 –2.00*</td>
</tr>
<tr>
<td>8</td>
<td>4.96 brdd (6.8, 12.6)</td>
<td>5.07 brt (9.8)</td>
<td>5.12 ddd(10.7, 10.7, 4.5)</td>
</tr>
<tr>
<td>11</td>
<td>2.60 dq (6.9, 11.0)</td>
<td>2.48 dq (6.7, 11.5)</td>
<td>2.54 dq (6.6, 11.5)</td>
</tr>
<tr>
<td>13</td>
<td>1.45 d (6.9)</td>
<td>1.35 d (6.7)</td>
<td>1.30 d (6.6)</td>
</tr>
<tr>
<td>14</td>
<td>1.83 brs</td>
<td>5.27 brs (2H)</td>
<td>0.88 s</td>
</tr>
<tr>
<td>15</td>
<td>1.30 s</td>
<td>1.69 s</td>
<td>4.85 brs; 5.00 brs</td>
</tr>
<tr>
<td>OR</td>
<td>1.82 brd (6.9)</td>
<td>2.10 s</td>
<td>2.07 s</td>
</tr>
<tr>
<td></td>
<td>1.86 brs</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.89 qq (6.9, 1.2)</td>
<td></td>
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</tr>
</tbody>
</table>

* Overlapped signal.
Aleskerova, 1985). Hence, the new germacrano-
lide 4 was identified as 8-acetylshonachalin A.

The structure of the lactone 5 could be readily
deduced from its $^1$H NMR spectrum. It was very
close to that of artapshin (8) (Fernandez et al.,
1987) but the observed downfield shift of the H-8
signal to $\delta$ 5.12 and the additional methyl singlet
at $\delta$ 2.07 suggested the presence of an acetate in-
stead of the hydroxyl group at C-8. This conclusion
was further confirmed by the MS fragments at
Compound 5 is thus the 8-acetate of the known
lactone artapshin (8).

As it was mentioned above, the species of the
A. millefolium group are recognized as having sig-
nificant medicinal properties and for pharmaceuti-
cal reasons much attention was focused on the
content of proazulenes which are precursors of
chamazulene. Based on chemical and cytological
investigations of different A. millefolium taxa col-
lected throughout Central Europe, the Vienna
school demonstrated that guaianolides (both pro-
azulene and nonazulenogenic) characterized all
diploid species, but only A. collina and A. cereta-
mica of the tetraploid species (Kubelka et al.,
1999). In contrast, A. collina of Bulgarian origin
was proved to produce germacranoles and
eudesmanolides only. Moreover, not even one of
them has been found in Achillea species so far.
Taking into account the observed difference in the
sesquiterpene pattern on one hand, and on the
other – the generally recognized polymorphism,
introgression and high ecological plasticity of the
taxa belonging to the A. millefolium group, one
could presume that the Bulgarian species A. col-
lina represent a new chemotype.

Acknowledgements

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Foundation for Scientific Research of Bulgaria
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