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EUDESMANOLIDES FROM *ACHILLEA PRATENSIS**

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Key Word Index—*Achillea pratensis*; Compositae, eudesmanolides; sesquiterpene lactones.

Abstract—From flower heads of *Achillea pratensis* five eudesmanolides were isolated by repeated column chromatography and HPLC. The compounds were identified as tauremisin, arglanin, 4-epi-arglanin, 4α-hydroperoxy-4α-dehydroxy-arglanin and santamarin.

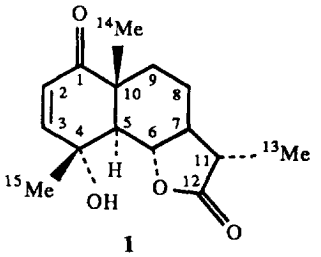
INTRODUCTION

Achillea pratensis Saukel & Länger has been described recently as a new species within the *Achillea millefolium* group [1]. This tetraploid taxon is widely spread in nutritious meadows throughout Middle Europe, e.g. in Northern Italy, Austria and Germany. Local habitants use *A. pratensis* for the preparation of herbal teas with antiphlogistic and spasmolytic effects in the traditional therapy of intestinal diseases [2]. In contrast to the requirements of ÖAB, *A. pratensis* does not give a blue oil after steam distillation. The lack of proazulenes, which are responsible for the antiphlogistic activity [3] on the one hand and the use of the drug in folk medicine on the other required an investigation of its chemistry.

RESULTS AND DISCUSSION

Air-dried flower heads of *A. pratensis* were extracted exhaustively with dichloromethane. After removal of the solvent the residue was extracted with 50% methanol. Further purification was performed by column chromatography over silica gel and reversed-phase HPLC, and afforded five eudesmanolides (**1**–**5**). EI-MS, ^{1}H- and ^{13}C NMR and 2DNMR (^{1}H–^{1}H COSY, ^{13}C–^{1}H COSY) confirmed the structure of **1** as tauremisin (= vulgarin), already described for some *Artemisia* species [4–7]. The stereochemistry at C-4 was proofed by NOE difference spectroscopy, which gave clear effects between Me-14, Me-15 and H-6β.

Compound **2** showed chromatographical behaviour as **1**, but differed with respect to molecular mass and formula ($C_{15}H_{18}O_4$). The obvious lack of two protons in comparison with **1** was explained by the presence of an α-methylene-γ-lactone represented by the pair of protons at δ6.18 and 5.52, respectively. Compound **2** was identified



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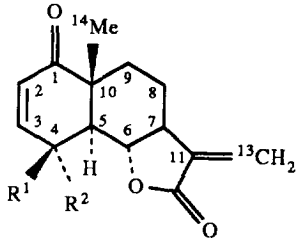
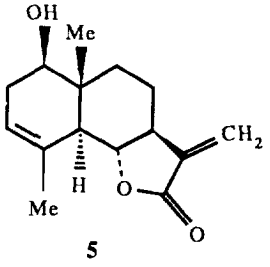


	R^1	R^2
2	Me	OH
3	OH	Me
4	Me	OOH



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*This article is part of the thesis of S. Glasl, University of Vienna, in preparation. Some of the results were reported at the 41st Annual Congress on Medicinal Plant Research, Düsseldorf, 31st August–4th September 1993: (Glasl, S., Kastner, U., Baumann, A., Jurenitsch, J. and Kubelka, W. 1993) *Planta Med.* **59**, Suppl. Issue, A 596.

as arglanin, described for a Mexican *Artemisia* species [8–10].

Compound **3** showed the same mass spectral behaviour as **2** and a remarkably similar ^{1}H NMR spectrum, which differed only in the chemical shifts of the protons near to C-4. Whereas the signal of H-5 was shifted to higher field, the signals corresponding to H-6β and Me-15 were at lower field (Table 1). The ^{13}C NMR spectrum permitted identification of **3** as the C-4 epimer of arglanin.

Compound **4** (M^+ at *m/z* 278, $C_{15}H_{18}O_5$) showed a positive reaction for peroxides [11] and had a signal at low field (δ7.81) typical of the proton of a hydroperoxide. The coupling constants as well as most of the chemical shifts of the other signals (except for H-2, H-3 and H-5, which were all shifted to lower field, Table 1) were almost identical. Therefore, **4** was assigned as 4α-hydroperoxy-4α-dehydroxyarglanin. The stereochemistry for **2** and **4** at C-4 was confirmed by NOE experiments.

By means of mass spectrometry and ^{1}H, ^{13}C and 2D NMR experiments, **5** was identified as santamarin (=balchanin), which was in good accordance with reported data [12, 13].

The eudesmanolides **1–5** as the main sesquiterpenoids, are obviously characteristic for *A. pratensis*. The chemistry of most other taxa of the *A. millefolium* group is dominated by guaianolides [14, 15] which are precursors of azulenes yielding a dark blue essential oil. From the pharmaceutical point of view it should be stressed that **1–5** are almost completely extracted into the herbal tea prepared from *A. pratensis* [16]. These results make desirable further pharmacological studies of the antiphlogistic activity of the various eudesmanolides, so as to define the position of *A. pratensis* among medicinal plants.

EXPERIMENTAL

Plant material. Achillea pratensis was collected in the area of St. Johann/Tirol (Austria) in summer 1991. Vouchers are deposited in Herbarium of the Institute of Pharmacognosy, University of Vienna.

Isolation of compounds. Air-dried flower heads (1 kg) of *A. pratensis* were extracted exhaustively with CH_2Cl_2. After removal of the solvent the residue was extracted with MeOH–H_2O (1:1). Further purification was performed by CC and HPLC as described previously [17]. The procedure yielded the following amounts of chromatographically pure compounds: 3.8 mg **1**, 28.0 mg **2**, 5.3 mg **3**, 5.3 mg **4** and 42.8 mg **5**. Due to some difficulties during purification the isolated amounts do not represent the concentration in the drug material in the plant. With respect to the loss during isolation the total amount comes to *ca* 0.15%, determined by means of HPLC.

Structure elucidation. MS: ion source: 220°, probe: 70°; CI MS, reactant gas NH_3. Compound **1**: *m/z*: 265 [M

Table 1. NMR data of **2–5**

H	2	3	4	5	C	2	3	4	5
1	—	—	—	3.68	1	201.4	201.4	201.3	75.3
2α	5.92	5.91	6.05	2.40	2	125.7	125.4	127.8	32.8
2β	—	—	—	1.97					
3	6.63	6.52	6.75	5.35	3	151.6	150.1	150.4	121.3
4	—	—	—	—	4	70.1	68.1	81.4	133.4
5	2.57	2.15	2.95	2.35	5	55.0	51.5	47.4	51.1†
6	4.15	4.32	4.11	3.95	6	79.7	79.4	79.0	81.5
7	2.60	2.59	2.60	2.50	7	49.7	49.5	49.7	51.0†
8α	2.19	2.15‡	2.18	2.09	8	21.1	21.3	21.0	21.2
8β	1.65*	1.63‡	1.60*	1.65					
9α	1.65*	1.64‡	1.72*	1.31	9	34.0	32.3	33.9	34.2
9β	2.08	2.01	2.09	2.05					
10	—	—	—	—	10	46.2	45.9	46.2	40.9
11	—	—	—	—	11	137.5	138.2	138.0	138.9
12	—	—	—	—	12	169.5	169.5	170.4	171.0
13	6.18	6.16	6.15	6.08	13	118.7	118.2	118.1	116.8
13′	5.52	5.49	5.48	5.42					
14	1.21	1.33	1.22	0.88	14	19.7	20.6	20.4	11.0
15	1.58	1.64	1.52	1.84	15	23.8	31.7	18.3	23.3
OH	2.81	2.62	—	1.55					
OOH	—	—	7.81	—					

*Overlapping signals, determined by ^{1}H–^{1}HCOSY.

†Signals can be exchanged.

‡Approximative values.

Coupling constants in Hz: **2–4**: 2,3 = 10.4; 5,6 = 11.6; 6,7 = 11.2; 7,13 = 7,13′ = 3.3; 7,8α = 3.3; 7,8β = 11.2; 8α,8β = 12.6; 8α,9α = 2.9; 8α,9β = 2.9; 8β,9α = 11.0; 8β,9β = 2.9; 9α,9β = 13.6; 11,13 = 6.9; **5**: 1,2α = 4.9; 1,2β = 9.8; 1,OH = 5; 2α,3 = 2.8; 2β,3 = 2.8; 2α,2β = 17.6; 5,6 = 6,7 = 10.9; 7,13 = 7,13′ = 3.0; 7,8α = 6.9; 7,8β = 12.1; 8α,8β = 13.7; 8α,9α = 4.0; 8α,9β = 11.0; 8β,9β = 4.4; 8β,9α = 11.0; 9α,9β = 11.0; 3,15 = 1.5.

$+ 1]^+$, 282 $[M + NH_4]^+$, **4** *m/z*: 280 $[M + 2]^+$, 296 $[M + NH_4]^+$, 247 $[M + 2 - 33]^+$, 245 $[M - 33]^+$. EI-MS, 20 and 70 eV, MS at 70 eV, **1** *m/z* (rel. int.): 262 (9.6), 247 (75.0), 229 (25.0), 211 (7.8), 201 (17.2), 183 (13.8), 147 (20.7), 119 (28.5), 98 (59.5), 56 (64.6), 45 (100.0). Compound **4**: *m/z* (rel. int.) (M^+: 278, not detectable), 262 (5.6), 247 (28.2), 229 (7.0). Compound **5**: *m/z* (rel. int.) 248 (25.0), 230 (8.6), 175 (8.7), 152 (47.1), 119 (31.7), 107 (99.0), 91 (83.7), 81 (80.8), 66 (62.5), 53 (100.0).

^1H, ^{13}C and 2D NMR measurements were performed in $CDCl_3$ on a Bruker AM 400, with TMS as int. standard, data are given in Table 1.

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