PERGAMON

Phytochemistry 50 (1999) 629–631

PHYTOCHEMISTRY

Two proazulenes from *Achillea ceretanica* Sennen[1,2]

S. Glasl[a,*], A. Presser[b], I. Werner[a], Ch. Wawrosch[a], U. Kastner[a], J. Jurenitsch[a], E. Haslinger[b], W. Kubelka[a]

[a]*Institut für Pharmakognosie, Universität Wien, Pharmaziezentrum, Althanstr. 14, A-1090 Wien, Austria*
[b]*Institut für Pharmazeutische Chemie, Universität Graz, Schubertstraße 1, A-8010 Graz, Austria*

Revised 6 July 1998

Abstract

From flower heads of a tetraploid clone of *Achillea ceretanica* two further guaianolides were isolated by repeated column chromatography and HPLC. The constitution and the stereochemistry of these new, unstable compounds were determined by MS, one- and two-dimensional NMR-techniques (^{1}H-, ^{13}C-NMR, COSY-, HSQC-, TOCSY-, selective TOCSY- and NOE-experiments) as well as by comparison to the data of the literature. The two substances were identified as 2α,8α-dihydroxy-1α,5α,6β,7α,11βH-guaia-3,10(14)-dien-12,6-olide and its 8α-acetate. © 1998 Elsevier Science Ltd. All rights reserved.

Keywords: Achillea ceretanica; Compositae; Guaianolides; Sesquiterpene lactones; Proazulenes

1. Introduction

Achillea ceretanica (tetraploid, 2n = 36) is established in SW Europe, belongs to the *Achillea millefolium* group and represents a proazulene containing species (Glasl, Kastner, Werner, Wawrosch, Schubert-Zsilavecz et al., 1997). One single plant had been collected and propagated by tissue culture in order to provide sufficient plant material for phytochemical investigations (Wawrosch, Kopp, Stöckl, Glasl & Kubelka, 1997). In addition to the previously reported sesquiterpenoids (Glasl et al., 1997) a further guaianolide (**1**) was isolated and structurally elucidated by MS, one- and two-dimensional NMR measurements.

The stereochemistry of this extremely unstable compound was determined by modern DPFGSE-NOE (double pulse field gradient-NOE (Stott, Stonehouse, Keeler, Hwang & Shaka, 1995)) experiments. The second compound (**2**) had been shown to be the acetyl-derivative of **1** by ^{1}H-, ^{13}C-NMR, HH- and CH-COSY experiments recently (Glasl et al., 1997), its relative stereochemistry could now be assigned by comparison to the data of the literature.

2. Results and discussion

The dichloromethane extract of the air-dried flower heads of *Achillea ceretanica* was purified and fractionated as described previously (Glasl et al., 1997). The guaianolides **1** and **2** were isolated from the polar fractions by HPLC on RP 8 material using methanol-water as mobile phase and by purification over silica gel cartridges. Both compounds are proazulenes, characterized by extreme lability under the influence of light, oxygen and room temperature. After TLC-analysis on silica gel and detection with modified acetic acid-phosporic acid-reagent (Stahl, 1967) they yield blue spots, showing clearly a different polarity (**1**: $R_f = 0.06$; **2**: $R_f = 0.38$).

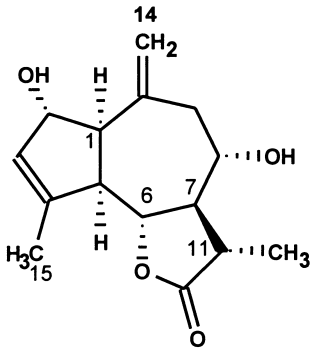
Perfect accordance was found, comparing the ^{1}H- and the ^{13}C-data of **2** (Glasl et al., 1997) with those of a guaianolide which was described recently for the

[1] Dedicated to Univ. Prof. Dr. G. Heinisch, Institut für Pharmazeutische Chemie, Universität Innsbruck, on the occasion of his 60th birthday.

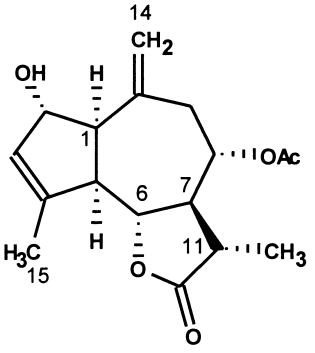
[2] This article is part of the thesis of I. Werner, University of Vienna, in preparation.

* Corresponding author.

0031-9422/98 $ - see front matter
PII: S0031-9422(98)00595-0



1



2

aerial parts of *Artemisia arborescens* collected in Tunisia. Therefore **2** ($C_{17}H_{22}O_5$; $m/z = 306$) is 8α-acetoxy-2α-hydroxy-1α,5α,6β,7α,11βH-guaia-3,10(14)-dien-12,6-olide.

The EI and CI mass spectra of **1** correlated well, indicating a molecular mass of $m/z = 264$ ($C_{15}H_{20}O_4$). The lack of fragment $m/z = 43$ as well as the reduction of 42 molecular units hinted a deacetylated analogon to compound **2**. This was confirmed by the ^{13}C- and ^{1}H-spectra lacking the carboxylic carbon ($\delta = 170.1$ ppm) and the signals of CH_3-Ac ($\delta = 21.1$ ppm, $\delta = 2.09$ ppm). The assignment of ^{1}H- and ^{13}C-resonances was achieved by combining the information obtained by COSY-, selective TOCSY- and HSQC-experiments.

The slight chemical shifts for ^{1}H ($\Delta\delta < 0.26$ ppm, exceptional H-7 and H-8) and ^{13}C ($\Delta\delta < 2.9$ ppm, exceptional C-9) of both compounds **1** and **2** indicate related structures. The differences are due to the addition of 10% CD_3OD to the solution of compound **1** in $CDCl_3$ to improve its solubility. Especially the proton resonances are very similar, only H-8 (near the acetylation side) is shifted to higher frequency ($\Delta\delta = 1.42$ ppm) as well as H-7 ($\Delta\delta = 0.37$ ppm). In addition, nearly the same coupling constants were observed. A similar compound though with different orientations of the protons H-1 (β) and H-2 (α) had been described by Serkerov and Aleskerova (1984) for *Artemisia fragrans*, giving only fragmentary NMR-data and no stereochemistry at C-11. Therefore selective NOE experiments with **1** were necessary in order to determine the stereochemistry: irradiation at the resonance of H-1 (α) gives NOEs at the signals of H-14 (Z), H-5 (α), H-9 (α) and H-7 (α); from H-6 (β) NOEs at H-2 (β), H-8 (β), CH_3-15 and a small signal at H-11 were induced, indicating β-position for H-11 and α-position for CH_3-13. Therefore the stereochemistry given in the figures was confirmed.

Whereas **1** represents a new structure, **2** has already been described for *Artemisia arborescens*. Both compounds are new for the genus *Achillea*.

3. Experimental

3.1. Plant material

Tetraploid (2n = 36), *in vitro* propagated *Achillea ceretanica* Sennen (Wawrosch et al., 1997) originating from one single individuum, was gathered in the second year of cultivation in the garden of the Institute in summer 1995. Vouchers are deposited in the Herbarium of the Institute of Pharmacognosy, University of Vienna.

3.2. Isolation of compounds

Air dried flower heads (200 g) were treated as described previously (Glasl et al., 1997), yielding 5.3 mg **1** and 5.8 mg **2**. Due to the lability of the compounds these amounts do not represent the real concentrations in the plants.

3.3. Thin layer chromatography

Silica gel 60 Merck plates (0.25 mm) were used with dichloromethane-acetone (9 + 1, v/v) as mobile phase and a dichloromethane extract of chamomile as reference (matricin: $R_f = 0.36$). **1** ($R_f = 0.06$) and **2** ($R_f = 0.38$) gave blue spots after detection with modified acetic acid-phosphoric acid-reagent (Stahl, 1967) (water 20 g, acetic acid 100% 50 g, phosphoric acid 85% 5 g, dimethylaminobenzaldehyde 250 mg) and heating at 140°C.

3.4. Structure elucidation

MS: Shimadzu QP-1000 EX MSPAC 200 direct inlet, EI-mode: ion source: 220°C, 70 eV; vacuum: 4×0^{-6} torr, scan: 40–500/2 s; heating rate of sample vial: 40°C/min; CI-mode: ion source: 180°C, 200 eV; reactant gas: ammonia 2.6, pre-pressure 1 bar, vacuum 5×10^{-6} torr, scan 40–600/2 s; heating of sample vial: 40°C/min. NMR spectra: Varian Unity Inova 400, 600

(297 K) 5 mm tubes, solvent resonance ($CDCl_3$) as internal standard. Before NOE experiments were performed, dissolved oxygen was removed by bubbling Ar through the solution.

*3.4.1. 2α,8α-Dihydroxy-1α,5α,6β,7α,11βH-guaia-3,10(14)-dien-12,6-olide (**1**)*

CIMS *m/z* (rel. int.): 264 $[M]^+$ (66.1), 265 $[M+1]^+$ (12.1), 282 $[M+NH_4]^+$ (81.7), 247 $[M-H_2O+1]^+$ (100.0), 229 $[M-2H_2O+1]^+$ (6.8). EIMS *m/z* (rel. int.): 264 $[M]^+$ (1.0), 246 $[M-H_2O]^+$ (3.5), 218 $[M-H_2O-CO]^+$ (1.7), 164 (37.8), 145 (12.1), 118 (24.8), 91 (100.0). ^{1}H NMR (400 MHz, $CDCl_3$ + 10% CD_3OD, 24°C): 5.47 (1H, *br s*; H-3), 4.79 (1H, *s*; H-14$_E$), 4.73 (1H, *s*; H-14$_Z$), 4.53 (1H, *br s*; H-2β), 3.76 (1H, *t*, *J* = 10.3 Hz; H-6), 3.46 (1H, *td*, *J* = 9.4 and 5.1 Hz; H-8β), 2.85 (1H, *t*, *J* = 8.5 Hz; H-5), 2.69 (1H, *dd*, *J* = 8.0 and 3.6 Hz; H-1), 2.50 (1H, *dd*, *J* = 12.5 and 4.9 Hz; H-9β), 2.37 (1H, *dq*, *J* = 10.9 and 7.1 Hz; H-11), 2.02 (1H, *dd*, *J* = 12.5 and 8.5 Hz; H-9α), 1.88 (1H, *q*, *J* = 9.7 Hz; H-7), 1.68 (3H, *br s*; Me-C$_{15}$), 1.21 (3H, *d*, *J* = 6.9 Hz; Me-C$_{13}$). ^{13}C NMR (100 MHz, $CDCl_3$, 24°C): 179.8 (C-12), 147.1, 142.5 (C-4, C-10), 126.6 (C-3), 115.0 (C-14), 80.6 (C-6), 78.8 (C-2), 74.8 (C-8), 55.5 (C-1), 55.0 (C-5), 55.0 (C-7), 46.8 (C-9), 41.0 (C-11), 16.6 (Me-C$_{15}$), 15.7 (Me-C$_{13}$).

*3.4.2. 8α-Acetoxy-2α-hydroxy-1α,5α,6β,7α,11βH-guaia-3,10(14)-dien-12,6-olide (**2**)*

m/z (rel. int.): EI: 306 $[M]^+$ (not detectable), 288 $[M-H_2O]^+$ (1.9), 246 $[M-HOAc]^+$ (2.0), 228 $[M-HOAc-H_2O]$ (6.7), 200 $[M-HOAc-H_2O-CO]^+$ (3.8), 185 (6.2), 164 (29.8), 145 (10.1), 143 (9.6), 118 (16.3), 105 (13.9), 91 (67.8), 69 (18.7), 43 $[Ac]^+$ (100.0). ^{1}H NMR (400 MHz, $CDCl_3$, 24°C): 5.65 (1H, *br s*; H-3), 5.01 (1H, *s*; H-14′), 4.99 (1H, *s*; H-14), 4.88 (1H, *ddd*, *J* = 10.0 and 7.2 Hz; H-8β), 4.75 (1H, *br s*; H-2β), 3.95 (1H, *t*, *J* = 10.0 Hz; H-6), 2.99 (1H, *t*, *J* = 8.0 Hz; H-5), 2.89 (1H, *dd*, *J* = 8.0 and 4.0 Hz; H-1), 2.69 (1H, *dd*, *J* = 13.2 Hz; H-9β), 2.44 (1H, *dq*, *J* = 11.7 and 7.2 Hz; H-11), 2.25 (1H, *ddd*, *J* = 11.7, 10 and 7.2 Hz; H-7), 2.22 (1H, *dd*, *J* = 13.2 and 5.5 Hz; H-9α), 2.09 (3H, *s*; Me-Ac), 1.87 (3H, *br s*, *J* = 1.1 Hz; Me-C$_{15}$), 1.27 (3H, *d*, *J* = 7.2 Hz; Me-C$_{13}$). ^{13}C NMR (100 MHz, $CDCl_3$, 24°C): 177.7 (C-12), 170.1 (Me-CO), 145.9, 140.9 (C-4, C-10), 129.5 (C-3), 117.3 (C-14), 80.7 (C-6), 79.5 (C-2), 75.9 (C-8), 57.5 (C-1), 55.7 (C-5), 53.0 (C-7), 41.5 (C-9), 40.8 (C-11), 21.1 (Me-Ac), 17.2 (Me-C$_{15}$), 15.6 (Me-C$_{13}$).

Acknowledgements

The MS apparatus was financed by support of the Austrian 'Bundesministerium für Wissenschaft und Verkehr'.

References

Glasl, S., Kastner, U., Werner, I., Wawrosch, Ch., Schubert-Zsilavecz, M., Jurenitsch, J., & Kubelka, W. (1997). *Pharm. Pharmacol. Lett., 7,* 119–120.

Serkerov, S. V., & Aleskerova, A. N. (1984). *Khim. Prir. Soedin., 5,* 595–598.

Stahl, E. (1967). *Dünnschicht-Chromatographie, Reagens Nr. 65* (p. 825). Berlin, Heidelberg, New York: Springer Verlag.

Stott, K., Stonehouse, J., Keeler, J., Hwang, T. L., & Shaka, A. J. (1995). *J. Am. Chem. Soc., 117,* 4199–4200.

Wawrosch, Ch., Kopp, B., Stöckl, J., Glasl, S., & Kubelka, W. (1997). *Pharm. Pharmacol. Lett., 7,* 116–118.