Sesquiterpenes from *Achillea pannonica* Scheele[§]

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From dichloromethane extracts of flowerheads of *Achillea pannonica* Scheele three sesquiterpenes were isolated and identified: 11,13-dehydrodesacetylmatricarin, (6E)-5-tigloxy-9-hydroxynerolidol and α-longipin-2-en-1-one. The structures were determined by MS, IR and NMR spectroscopic analyses. (6E)-5-Tigloxy-9-hydroxynerolidol is reported here for the first time. Additionally spathulenol, a compound of the essential oil was identified using GC-MS and GC-FTIR.

Key words: Achillea pannonica, Sesquiterpenes

Introduction

Yarrow (*Achillea millefolium* L. s.l.) is widely used in folk medicine due to its broad spectrum of pharmacological activities (Wichtl, 1997). The *Achillea millefolium* group includes a number of species differing in ploidy, morphology and chemistry. One of them, growing especially in the eastern part of Central Europe and Southeast Europe, is the octoploid *Achillea pannonica.* In addition to a recent paper dealing with the flavonoid composition of this species (Kasaj *et al.*, 2001), we report the study of sesquiterpenes which are known to be important for the anti-inflammatory activity of the plant (Kastner *et al.,* 1993; Sosa *et al.,* 2001). Furthermore, this work contributes to chemotaxonomic conclusions about the *Achillea millefolium* group (Kubelka *et al.,* 1999).

Experimental

General

One- and two-dimensional NMR-spectra were recorded on a Varian Unity Inova 400 NMR-Spectrometer at 297 K. Tubes: 5 mm diameter (Kontes). Dual probe head with shielded *z*-gradients or broadband probe (400 MHz). Chemical shifts are given in ppm. TMS was used as internal standard. The HMBC-experiments were optimised for a long-range coupling constant of 8 Hz. Before NOE experiments were performed, dissolved oxygen was removed by bubbling Ar through the solution.

MS-spectra were recorded on a Shimadzu GCMS-QP5050A instrument with direct inlet and two possible ionisation modes (EI-MS, CI-MS). EI-MS: ion source: 250 °C, 70 eV; vacuum: 5.32×10^{-4} Pa; scan: 40–500/2 s; heating rate of sample vial: 80 °C/min; CI-MS: ion source: 180 °C, 200 eV; reactant gas: NH_3 3.0; vacuum: 6.65×10^{-3} Pa; scan: 40–500/2 s; heating rate of sample vial: 80 °C/min.

IR Spectra were recorded on a Perkin Elmer 2000 GC IR System on silicon plates (13 mm × 1 mm, Korth Kristalle Gmbh, Altenholz, Germany) from a solution of dichloromethane. Resolution 4 cm^{-1}, J-stop resolution: 7.77 cm^{-1}, apodization: strong, gain: 1, OPD velocity: 2 cm/s, interferogram: bi-directional double sided, number of scans: 1, scan range: 5200–370 cm^{-1}, interval: 1.0 cm^{-1}.

UV-spectra were recorded on line in methanol-water by diode array detection during an HPLC run.

Optical rotation was determined with a Perkin Elmer Polarimeter 341 and photomultiplier 1P28A at 20 °C.

Analytical HPLC was performed on a Perkin Elmer Series 200 Liquid Chromatograph, with 600 LINK Controller and LC-235 diode array detector. Column: Hewlett Packard LiChrospher®100

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Rp-8 5 μm, 250 × 4.0 mm, guard column: Hewlett Packard LiChrospher®100 Rp-8 5 μm, 4 × 4 mm. Solvents: MeOH (A) and H_2O (B). Gradient elution: from 20% A to 80% A (v/v) in 90 min (linear; rate = 0.67%/min). Flow rate: 1.0 ml/min. Detection at 220 and 255 nm at room temperature.

Preparative HPLC was carried out on two Perkin Elmer series 10 pumps with an Aston LC controller and a LC135 UV detector. Solvents: MeOH and H_2O in varying mixtures.

Silica Gel 60 for CC was obtained from Merck.

TLC Silica gel plates (Merck, Germany) 0.25 mm, System: dichloromethane-acetone (9 + 1, v/v). Reference: Dichloromethane extract of camomile (matricin R*f* = 0.5). Detection: acetic acid-phosphoric acid reagent (AP-reagent) (Stahl, 1967), after heating (140 °C); anisaldehyde sulfuric acid reagent (AA-reagent) (Dequeker, 1964), after heating (140 °C).

GC-FID and GC-FTIR were performed on a Perkin Elmer Autosystem. Column: HP-5 crosslinked 5% Ph Me silicone column, 50 m × 0.32 mm × 0.52 μm. Precolumn: HP Retention Gap (uncoated, deactivated) 5 m × 0.25 mm. Carrier gas: N_2 5.0, 2 ml/min. Split ratio: 1:10. Temperature program: 60 °C to 270 °C, rate: 3 °C/min (system1), 140 °C to 270 °C, rate: 2.5 °C/min (system2). Detection: FID Detector (H_2 5.0, synth. air 5.0). IR: MCT-Detector, cooled with liquid N_2.

GC-MS was carried out on a Shimadzu GC-17A with a GCMS-QP5050A in EI mode. Column: Macherey-Nagel SE-54-CB Fused Silica Capillary Column, 50 m × 0.25 mm × 0.44 μm Precolumn: HP Retention Gap (uncoated, deactivated) 5 m × 0.25 mm. Carrier gas: He 5.0, 1.5 ml/min.

Plant material

The aerial parts of *Achillea pannonica* were collected in Falkenstein, Buschberg and Oberweiden, Austria, in 1995, 1997 and 1998. The material was identified by J. Saukel, Institute of Pharmacognosy, University of Vienna and voucher specimens were deposited in the herbarium of the institute.

Extraction and isolation

Extraction was carried out soon after collection and drying (room temperature) of the plant material because of the known lability of some sesquiterpenes; the material from all locations was combined according to TLC and GC. Dried flowerheads (927 g/1995, 593.4 g/1997, 907.5 g/1998) were extracted three times with dichloromethane (1:10 w/v) by ultrasonication for 10 min. After concentration of this extract under vacuum to dryness the oily dark yellow residue (33.1 g/1995, 19.9 g/1997, 50.4 g/1998) was redissolved in dichloromethane and purified by extraction with the same volume methanol-water (1:1 v/v). Evaporation of the dichloromethane at 40 °C maximum yielded an oily sticky residue which was filtered and extracted again. After extraction of the unified methanol-water (1:1 v/v) fractions with dichloromethane a yellow oily residue resulted (4.7 g/1995 = extr1, 10.3 g/1997 = extr2, 11.3 g/1998 = extr3). Separation of these purified extracts was performed by flash chromatography using silica gel as stationary phase and dichloromethane, dichloromethane-acetone and dichloromethane-methanol with increasing polarity as mobile phases yielding 23 fractions from extr1 (P I-XXIII), 10 fractions from extr2 (V I-X) and 17 fractions from extr3 (D I–XVII). Compound **1** (2.2 mg) was isolated from fraction V IV, compound **2** (2.8 mg) from fraction D V and compound **3** (2.0 mg) from fraction D II after repeated HPLC (RP-8) and CC (silica gel). Spathulenol was identified by means of GC-MS and GC-FTIR from fraction P VII.

11,13-dehydrodesacetylmatricarin (**1**). TLC R*f*: 0.3, fluorescence quenching, no reaction with AP- or AA-reagent. R*t*-HPLC: 25 min. UV: λ_{max} = 260 nm (MeOH-H_2O). ^{1}H NMR (400 MHz, δ, acetone-d_6): 2.29 (s br, 3H, H-14), 2.38 (s, 3H, H-15), 2.44 (dd, *J* = 13.6, 2.3 Hz, 1H, H-9β), 2.88 (t, *J* = 13.6 Hz, 1H, H-9α), 3.17 (m, 1H, H-7), 3.71 (m, 1H, H-5), 3.72 (m, 1H, H-6), 3.92 (t br, *J* = 2.3 Hz, 1H, H-8), 6.08 (m, 1H, H-13a), 6.13 (s br, 1H, H-3), 6.24 (m, 1H, H-13b) ppm. ^{13}C NMR (100 MHz, δ, acetone-d_6): 19.5 (C-14), 21.0 (C-15), 49.2 (C-9), 51.8 (C-5), 58.2 (C-7), 68.2 (C-8), 82.4 (C-6), 121.6 (C-13), 134.2 (C-1), 135.9 (C-3), 139.2 (C-11), 146.1 (C-10), 170.5 (C-4), 170.6 (C-12), 195.5 (C-2) ppm. EI-MS ($C_{15}H_{16}O_4$): *m/z* (% rel. int.) 260 $[M]^+$ (100), 245 $[M\text{-}CH_3]^+$ (6.10), 242 $[M\text{-}OH]^+$ (8.16), 227 $[M\text{-}CH_3\text{-}OH]^+$ 23.10, 224 (6.99), 214 $[M\text{-}OH\text{-}C{=}O]^+$ (10.3), 199 (24.15), 196 (15.45), 171 (19.34), 147 (20.13), 91 (69.54). CI-MS (ammonia): *m/z* (% rel. int.) 278 $[M+NH_4]^+$ (100), 269 (57.96), 261 $[M+H]^+$ (70.81), 251 (25.30), 243

$[M\text{-}OH]^+$ (35.95). IR: ν_{max} cm^{-1} 3424 (m, OH), 2925 (m, CH), 2855 (w, CH), 1772 (s, C = O, γ-lactone), 1686 (s, C = O, ketone), 1638 (s, C = C conjugated with C = O), 1618 (s, C = C conjugated with C = O), 1139 (m, C-O γ-lactone).

(6E)-5-tigloxy-9-hydroxynerolidol (**2**). TLC R*f*: 0.5, AP-reagent: blue-green, AA-reagent: dark blue. R*t*-HPLC: 70 min. UV: λ_{max} = 202 nm (MeOH-H_2O). $[\alpha]_D^{20}$ = + 32.14 ($CHCl_3$, 0.2%). ^{1}H NMR (400 MHz, δ, acetone-d_6): 1.24 (s, 3H, H-15), 1.61 (d, *J* = 0.9 Hz, 3H, H-13), 1.64 (d, *J* = 0.9 Hz, 3H, H-12), 1.76 (s, 3H, H-4′), 1.78 (s, 3H, H-5′), 1.79 (s, 3H, H-14), 1.79 (m, 1H, H-4a), 2.01 (m, 1H, H-4b), 2.06 (m, 1H, H-8a), 2.18 (m, 1H, H-8b), 3.34 (d, *J* = 3.7 Hz, 1H, OH-9), 3.63 (s, 1H, OH-3), 4.43 (m, 1H, H-9), 4.93 (dd, *J* = 20.7, 1.7 Hz, 1H, H-1a), 5.10 (m, 1H, H-10), 5.20 (dd, *J* = 17.2, 1.7 Hz, 1H, H-1b), 5.21 (s br, 1H, H-6), 5.78 (m, 1H, H-5), 5.95 (m, 1H, H-2), 6.79 (q br, *J* = 7.0 Hz, 1H, H-3′) ppm. ^{13}C NMR (100 MHz, δ, acetone-d_6): 12.0 (C-5′), 14.2 (C-4′), 17.0 (C-14), 18.1 (C-13), 25.5 (C-12), 27.8 (C-15), 47.7 (C-4), 48.9 (C-8), 66.7 (C-9), 69.1 (C-5), 72.0 (C-3), 110.9 (C-1), 128.4 (C-6), 129.8 (C-2′), 129.9 (C-10), 133.0 (C-11), 136.4 (C-7), 136.9 (C-3′), 146.9 (C-2), 167.1 (C-1′) ppm. EI-MS ($C_{20}H_{32}O_4$): *m/z* (% rel. int.) 336 $[M]^+$ (0.22), 335 (0.99), 235 $[M\text{-}1\text{-}tigl]^+$ (0.93), 201 (2.22), 185 (4.64), 171 (6.98), 155 (9.85), 135 (8.08), 119 (8.38), 109 (7.15), 101 (26.38), 93 (10.75), 85 (67.42), 83 $[tigl]^+$ (71.29), 71 (100). CI-MS (ammonia): *m/z* (% rel. int.) 354 $[M+NH_4]^+$ (98.9), 337 $[M+H]^+$ (1.53), 336 $[M]^+$ (2.08), 319 $[M+H\text{-}OH]^+$ (6.47), 254 $[M+NH_4\text{-}tigl]^+$ (16.15), 237 $[M+H\text{-}tigl]^+$ (6.71), 219 $[M+H\text{-}tigl\text{-}OH]^+$ (48.54), 201 $[M+H\text{-}tigl\text{-}OH\text{-}OH]^+$ (2.5), 170 (23.18), 135 (78.95), 102 (100), 83 $[tigl]^+$ (6.2). IR ν_{max} cm^{-1}: 3409 (m, OH), 2929 (s, CH), 2856 (m, CH), 1706 (s, C=O tigl), 1653 (m, C=C), 1609 (w, C=C), 1264 (s, C-O tigl).

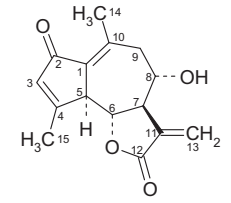
α-*longipin-2-en-1-one* (**3**). TLC R*f*: 0.6, fluorescence quenching, no reaction with AP- or AA-reagent. R*t*-HPLC: 72 min. UV λ_{max} = 260 nm (MeOH-H_2O). ^{1}H NMR (400 MHz, δ, acetone-d_6): 0.78 (s, 3H, H-12), 0.84 (s, 3H, H-14), 0.90 (s, 3H, H-13), 1.45 (m, 2H, H-7), 1.68 (m, 2H, H-8), 1.73 (m, 2H, H-9), 1.94 (d, *J* = 1.5 Hz, 3H, H-15), 2.00 (s, 1H, H-5), 2.59 (m, 1H, H-11), 5.57 (s br, 1H, H-2) ppm. ^{13}C NMR (100 MHz, δ, acetone-d_6): 21.8 (C-8), 23.0 (C-15), 24.7 (C-14), 27.4 (C-13), 27.8 (C-12), 39.1 (C-7), 42.1 (C-9), 50.4 (C-4), 58.4 (C-11), 67.1 (C-5), 122.9 (C-2) ppm. EI-MS ($C_{15}H_{22}O_1$): *m/z* (% rel. int.) 218 $[M]^+$ (32.45), 203 $[M\text{-}CH_3]^+$ (23.66), 175 (30.6), 161 (38.89), 148 (100), 135 (64.16), 121 (56.62), 109 (50.38), 91 (61.38). CI-MS (iso-butane): *m/z* (% rel. int.) 219 $[M+H]^+$ (100). IR: ν_{max} cm^{-1} 2926 (s, CH), 2854 (m, CH), 1675 (C=O).

spathulenol (**4**). TLC: R*f*: 0.75, AP-reagent: blue, AA-reagent: blue-violet. R*t*-GC: 39 min (system1), 12.2 min (system2). GC-MS (EI): *m/z* 220 $[M]^+$, 205 $[M\text{-}CH_3]^+$, 202 $[M\text{-}OH]^+$, 187, 177, 159, 145, 131, 119, 105, 91, 84, 79. GC-FTIR: ν_{max} cm^{-1} 3639 (w, OH), 3089 (w, = CH_2), 2954 (s, CH), 2875 (m, CH), 1638, 1457, 1382, 1098.

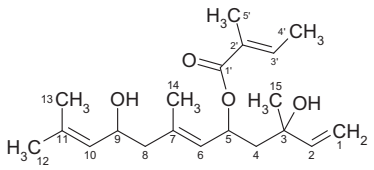
Results and Discussion

TLC screening of single plants of *Achillea pannonica* collected in Falkenstein, Buschberg, Oberweiden, all Lower Austria, showed some characteristic bands occurring in many of the samples but no distinct pattern. To be sure that these differences were not due to heterogeneous material the essential oil as a further chemotaxonomical marker was checked as well. As main compounds α-pinene and 1,8-cineole besides remarkable amounts of β-pinene, sabinene and β-caryophyllene could be identified. The composition of the essential oil corresponded well to samples investigated by Rauchensteiner *et al.* (2002) and showed no remarkable heterogeneity. Therefore the plant material of all locations was combined before extraction. Extraction of the plant material collected in three different years (1995, 1997, 1998) was carried out soon after collection and drying because of the known lability of some sesquiterpenes leading to three extracts (extr1–3).

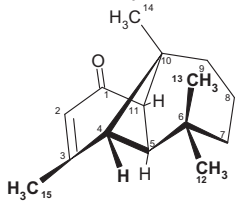
From these dichloromethane extracts of *Achillea pannonica* three sesquiterpenes were isolated by CC on silicagel by gradient elution with dichloromethane and dichloromethane-acetone mixtures. Further purification by HPLC on reversed phase material and CC on silicagel yielded 11,13-dehydrodesacetylmatricarin (**1**) and (6E)-5-tigloxy-9-hydroxynerolidol (**2**) from extr3 and α-longipin-2-en-1-one (**3**) from extr2. 11,13-dehydrodesacetylmatricarin (**1**), which has been reported for *Achillea setacea* (Zitterl-Eglseer *et al.*, 1991) and α-longipin-2-en-1-one (**3**), already known from *A. millefolium* "DIS A" (Kastner *et al.*, 1996), could



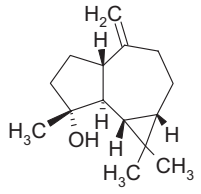
1 11,13-dehydro-desacetylmatricarin



2 (6E)-5-tigloxy-9-hydroxynerolidol



3 α-longipin-2-en-1-one



4 spathulenol

Fig. 1. Structural formulas of compounds **1–4**.

be identified by comparison of their MS-, IR- and NMR-data with the literature. In contrast to matricarin derivatives, which are quite common in the genus *Achillea*, up to now longipinane derivatives have only been reported for *A. millefolium* "DIS A".

CI-MS of **2** showed peaks at *m/z* 354 ($M+NH_4^+$) and 337 ($M+H^+$) suggesting the molecular weight to be 336 amu. Combination of the mass spectra with the information of the NMR experiments indicated the molecular formula $C_{20}H_{32}O_4$ (5 double bond equivalents). A loss of 100 amu as well as a peak at *m/z* 83 in the EI-MS implied an unsaturated C-5 carboxylic acid. This was confirmed by ^{1}H NMR which showed the typical signals of a tiglinate reported in the literature (Joseph-Nathan *et al.*, 1984). In addition to the tiglic moiety, the NMR spectra revealed the presence of three double bonds (^{13}C NMR signals at 110.9, 128.4, 129.9, 133.0, 136.4, 146.9 ppm) and two hydroxyl groups (^{1}H NMR signals at 3.34, 3.63 ppm). Considering the molecular formula ($C_{20}H_{32}O_4$) indicating 5 double bond equivalents, compound **2** could not be a cyclic sesquiterpene. The NMR signals were typical for a nerolidol derivative (De Pascual Teresa *et al.*, 1986) assuming an acid moiety substituted in position 5. This compound is reported for the first time, similar structures (nerolidol derivatives) have been described for the genus *Achillea* only in *Achillea odorata* (Barrero *et al.*, 1990).

Additionally a compound that gave a remarkable spot on TLC (AP blue) was identified by means of GC-MS and GC-FTIR as spathulenol (**4**), a component of the essential oil.

In contrast to literature a germacrane derivative, isolated from *Achillea pannonica* collected in Slovakia (Sosa *et al.*, 2001) could not be detected in this material. Compared to di- and tetraploid taxa of the *Achillea millefolium* group the octoploid *Achillea pannonica* is characterised – similar to hexaploid taxa – by a bigger number of skeletons and greater intraspecific variations concerning the sesquiterpenes.

Barrero A. F., Alvarez-Manzaneda R. E. J., and Alvarez-Manzaneda R. R. (1990), Bisabolene derivatives and other constituents from *Achillea odorata.* Phytochemistry **29**, 3213–3216.

Dequeker R. (1964), Over een niet gewone Handelspolygala. Pharm. Tijdschr. Belg. **41**, 39–47.

De Pascual Teresa J., Bellido I. S., González M. S., and Vicente S. (1986), Tetracyclic triterpenes and nerolidol derivatives from *Santolina oblongifolia.* Phytochemistry **25**, 185–190.

Joseph-Nathan P., Wesener J. R., and Günther H. (1984), A two-dimensional NMR study of angelic and tiglic acid. Org. Magn. Reson. **22**, 190–191.

Kasaj D., Krenn L., Prinz S., Hüfner A., Haslinger E., Yu S. S., and Kopp B. (2001), Flavon- and flavonolglycosides from *Achillea pannonica* Scheele. Z. Naturforsch. **56 c**, 521–525.

Kastner U., Sosa S., Tubaro A., Breuer J., Rücker G., Della Loggia R., and Jurenitsch J. (1993), Anti-edematous activity of sesquiterpene lactones from different taxa of the *Achillea millefolium* group. Planta Med. **59**, Suppl. Iss. A 699.

Kastner U., Jurenitsch J., Glasl S., Follrich B., Gavanelli A., Schröder H., Schubert-Zsilavecz M., Schmidt W., Haslinger E., and Kubelka W. (1996), Longipinen- und Achillifolin-Derivate aus *Achillea millefolium*-Typ "DIS A". Pharmazie **51**, 503–505.

Kubelka W., Kastner U., Glasl S., Saukel J., and Jurenitsch J. (1999), Chemotaxonomic relevance of sesquiterpenes within the *Achillea millefolium* group. Biochem. Syst. Ecol. **27**, 437–444.

Rauchensteiner F., Nejati S., Werner I., Glasl S., Saukel J., Jurenitsch J., and Kubelka W. (2002), Determination of taxa of the *Achillea millefolium* group and *Achillea crithmifolia* by morphological and phytochemical methods I. Characterisation of Central European taxa. Sci. Pharm. **70**, 199–230.

Sosa S., Tubaro A., Kastner U., Glasl S., Jurenitsch J., and Della Loggia R. (2001), Topical anti-inflammatory activity of a new germacrane derivative from *Achillea pannonica.* Planta Med. **67**, 654–658.

Stahl E. (1967), Dünnschicht-Chromatographie. Reagens Nr. 65. Springer Publ., New York.

Wichtl M. (1997), *Teedrogen und Phytopharmaka.* Wissenschaftl. Verlagsges. mbH, Stuttgart, pp. 395–399.

Zitterl-Eglseer K., Jurenitsch J., Korhammer S., Haslinger E., Sosa S., Della Loggia R., Kubelka W., and Franz Ch. (1991), Entzündungshemmende Sesquiterpenlactone von *Achillea setacea*. Planta Med. **57**, 444–446.