Sesquiterpenes and Flavonoid Aglycones from a Hungarian Taxon of the *Achillea millefolium* Group

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The investigation of a dichloromethane extract of flower heads of a Hungarian taxon of the *Achillea millefolium* group led to the isolation of three flavonoid aglycones, one triterpene, one germacranolide and five guaianolides. Their structures were elucidated by UV-VIS, EI- and CI-MS, ^{1}H NMR and ^{13}C NMR spectroscopic methods as well as by 2D-NMR studies and by selective 1D-NOE experiments. Besides apigenin, luteolin and centaureidin, β-sitosterol, 3β-hydroxy-11α,13-dihydro-costunolide, desacetylmatricarin, leucodin, achillin, 8α-angeloxy-leucodin and 8α-angeloxy-achillin were isolated. Both latter substances are reported here for the first time. Their NMR data were compared with those of the other guaianolides. The stereochemistry of 3β-hydroxy-11α,13-dihydro-costunolide was discussed and compared with data of the literature.

Introduction

Yarrow represents a widespread medicinal plant which is used against inflammations and spasms during gastrointestinal disorders. The application of infusions showed positive effects on wound healing and hemorrhages (Wichtl, 1997). Besides the flavonoids, which may contribute to the pharmacological activity of the plant (Della Loggia *et al.*, 1992), anti-edematous effects have been shown for several sesquiterpenes (Kastner *et al.*, 1993). This led to intensive morphological (Saukel and Länger, 1992a, 1992b, 1992c) and phytochemical (Kubelka *et al.*, 1999) investigations resulting in a new definition of the polyploid *Achillea millefolium* group. The fact that up to now mainly Central European species were examined and that a main part of the commercial drug available in Austria comes from Eastern Europe led to further collections at several sites in Hungary, Serbia and Slovakia. A first TLC screen of the samples showed high homogeneity for only one Hungarian habitat and a pattern with clear differences compared to the Central European species. Here we investigated the main compounds of the flowerheads of this tetraploid taxon and we report the isolation and characterization of three flavonoid aglycones, one triterpene and six sesquiterpenes. In addition two guaianolides are described here for the first time.

Experimental

General

NMR-spectra were recorded on a Varian Unity Inova 400 NMR spectrometer at 297 K. Sample tubes: 5 mm diameter (Kontes Glass Company, The Gerresheimer Group, Düsseldorf). Dual probe head with shielded *z*-gradients or broadband probe (400 MHz). Internal standard: TMS. Solvents: chloroform-*d*, acetone-d_6, methanol-d_4. HMBC experiments were optimized for a long-range coupling constant of 8 Hz. Before NOE experiments were performed, dissolved oxygen was removed by bubbling Ar through the solution.

EI- and CI-MS data were recorded on a Shimadzu QP-1000 EX MSPAC 200 with direct inlet and two possible ionization modi. EI-mode: ion source: 250 °C, 70 eV; vacuum: 5.33×10^{-4} Pa;

scan: 40–500/2 s; heating rate of sample vial: 80 °C/min. CI-mode: ion source: 180 °C, 200 eV; reactant gas: ammonia 2.6, pre-pressure: 1×10^5 Pa; vacuum: 6.67×10^{-3} Pa; scan: 40–500/2 s; heating rate of sample vial: 80 °C/min.

For IR spectra a solution of the respective compound in dichloromethane (sesquiterpenes) or in methanol (flavonoids) was dropped on a silicium plate (13 × 1 mm, polished optically, Korth Kristalle GmbH, Altenholz) leaving a slight film. Spectra were recorded with a Perkin Elmer System 2000GC IR (software Spectrum for Windows 1.30); 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; phase correction: self 64; number of scans: 1; scan range: 5200–370 cm^{-1}; interval: 1.0 cm^{-1}.

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

UV spectra were recorded on line in methanol-water by DAD detection during the HPLC runs.

Semipreparative and analytical HPLC was performed on a Merck Hitachi liquid chromatograph consisting of a Rheodyne injection unit, a L-7100 pump, a L-7450 diode array detector (monitoring wavelength 220 nm) and a D-7000 interface. All computations were performed using the Merck D-7000 HSM data system. Stationary phase: Hewlett-Packard LiChrospher®100-RP8 5 μm column (250 × 4.0 mm) guarded by a Hewlett-Packard LiChrospher®100-RP8 5 μm guard column (4 × 4 mm). The mobile phase consisted of varying methanol-water mixtures (v/v), flow rate: 1.0 ml min^{-1}. System 1: 30% methanol (isocratic). System 2: 50% methanol (isocratic). System 3: 60% methanol (isocratic). System 4: start at 20% methanol to 80% methanol in 90 min (linear gradient; rate = 0.66%/min). Detection at 220 nm and 255 nm, room temperature.

Silica gel, Sephadex®-LH-20, and Polyamide S6 used for CC were obtained from Merck (Germany), Pharmacia Biotech (Uppsala, Sweden), Riedel-De Haen AG (Seelze-Hannover, Germany).

TLC Silica gel plates (Merck, Germany), 0.25 mm. System A: CH_2Cl_2-acetone (9:1 v/v). System B: $CHCl_3$-methanol (99:1 v/v). System C: cyclohexane-EtOAc (1:1 v/v). System D: benzene-acetone (9:1 v/v). System E: benzene-acetone (95:5 v/v). System F: CH_2Cl_2. System G: cyclohexane-EtOAc (4:1 v/v). System H: toluol-ethylformiate-formic acid (5:4:1 v/v/v). After development at room temperature, chromatograms were examined under $UV_{255\,nm}$ and $UV_{366\,nm}$. Additional detection was performed by spraying with anisaldehyde sulfuric acid reagent (Dequeker, 1964) or sulfuric acid reagent (Wagner *et al.*, 1983) and heating subsequently.

Reference sesquiterpene and flavonoids

Apigenin (**7**), luteolin (**8**) and β-sitosterol (**10**) were obtained from C. Roth GmbH & Co, Karlsruhe. Desacetylmatricarin (**3**) was isolated from *Achillea ceretanica* (Glasl *et al.*, 1997).

Plant material

The aerial parts were collected in Szekszard, South Hungary, in July 1998. The material was examined by J. Saukel, Institute of Pharmacognosy, University of Vienna, and classified as belonging to the *Achillea millefolium* group. The number of chromosomes was determined as tetraploid by W. Wlach, Institute of Pharmacognosy, University of Vienna, using flow cytometry. A voucher specimen has been deposited in the herbarium of the institute.

Extraction and isolation

Dried, pulverized flower heads (50 g) were extracted for 24 h at room temperature under stirring with 1000 ml $CHCl_3$. After concentration of this extract under vacuum to dryness and redissolving in a small amount of i-PrOH, CC on Sephadex®-LH-20 (50 g) was performed. i-PrOH was used as eluent which yielded seven fractions (I-VII).

3 mg of compound **10** were obtained by purification of fraction II on silica gel (20 g) which was eluted with benzene-acetone (9:1 v/v).

Fraction IV was purified repeatedly by CC on silica gel using benzene-acetone (9:1 v/v), cyclohexane-EtOAc (1:1 v/v) and $CHCl_3$ as eluents to obtain 25 mg of compound **1** and 1.6 mg of compound **4**. Recrystallization from benzene-acetone (9:1 v/v) yielded 2 mg of compound **6** and 4 mg of compound **3**. Compounds **2** and **5** required additional separation by HPLC (stationary phase:

LiChrospher®100-RP8 5 μm; mobile phase: 50% methanol, isocratic) yielding 0.6 mg of compound **2** and 0.8 mg of compound **5**.

Fraction V was further purified by CC on polyamide (10 g) using $CHCl_3$-MeOH-methylethylketone (20:2:1 v/v/v) as eluent yielding 5 mg of compound **9** and 3 mg of compound **7**.

Recrystallisation of fraction VII from MeOH afforded 3 mg of compound **8**. The amounts of the substances do not correspond to the real concentrations in the plant.

Leucodin (= desacetoxymatricarin, leucomisin, **1**). TLC R*f*: 0.78 (system A); detection: quenching under $UV_{255\,nm}$; no colour with spraying reagents. R*t*-HPLC: 66.8 min (system 1). UV λmax (30% MeOH) nm: 260. $[\alpha]_D^{20}$ +61 (*c*, 0.067 $CHCl_3$). IR νmax cm^{-1}: 3021 (s, C-H), 1777 (s, C = O lactone), 1683 (s, C = O ketone), 1636 (m, C = C), 1619 (w, C = C), 1181(m, C-O lactone). Molecular formula: $C_{15}H_{18}O_3$. EI-MS *m/z* (% rel. int.): 246 $[M]^+$ (100), 231 $[M\text{-}CH_3]^+$ (13), 218 [M-C = O]$^+$ (7), 217 (29), 203 $[M\text{-}CH_3\text{-}C = O]^+$ (6), 190 (10), 173 (39), 145 (27), 91 (74). CI-MS (ammonia) *m/z* (% rel. int.): 264 $[M+NH_4]^+$ (80), 247 $[M+H]^+$ (100). ^1H NMR (400 MHz, methanol-d_4): see Table I. ^{13}C NMR (100 MHz, δ, methanol-d_4): 12.7 (C-13), 20.2 (C-15), 22.1 (C-14), 27.1 (C-8), 38.7 (C-9), 42.3 (C-11), 53.9 (C-5), 57.4 (C-7), 86.2 (C-6), 133.5 (C-1), 136.1 (C-3), 155.5 (C-10), 174.0 (C-4), 180.4 (C-12), 198.7 (C-2) ppm.

8α-Angeloxy-leucodin (**2**). TLC R*f*: 0.85 (system A); detection: quenching under $UV_{255\,nm}$; no colour with spraying reagents. R*t*-HPLC: 37.5 min (system 2). UV λmax (50% MeOH) nm: 260. $[\alpha]_D^{20}$ +29 (*c*, 0.069 $CHCl_3$). IR νmax cm^{-1}: 2934 (w, C-H), 1783 (s, C = O lactone), 1711 (m, C = O ang), 1690 (s, C = O ketone), 1643 (m, C = C), 1619 (m, C = C), 1265 (w), 1232 (m), 1158 (m, C-O ang). Molecular formula: $C_{20}H_{24}O_5$. EI-MS *m/z* (% rel. int.): 344 $[M]^+$ (8), 261 [M-ang]$^+$ (3), 244 [M-ang-OH]$^+$ (25), 229 $[M\text{-ang-OH-}CH_3]^+$ (11), 216 (4), 201 $[M\text{-ang-OH-}CH_3\text{-CO}]^+$ (7), 159 (12), 83 [M-ang]$^+$ (41). ^1H NMR (400 MHz, acetone-d_6): see Table I. ^{13}C NMR (100 MHz, δ, acetone-d_6): 10.0 (C-13), 10.8 (C-4′), 14.5 (C-15), 15.3 (C-5′), 15.5 (C-14), 35.6 (C-11), 39.7 (C-9), 46.6 (C-5), 54.0 (C-7), 65.7 (C-8), 76.6 (C-6), 123.0 (C-2′), 129.3 (C-1), 130.6 (C-3), 134.6 (C-3′), 139.8 (C-10), 161.8 (C-1′), 165.7 (C-4), 172.1 (C-12), 190.1 (C-2) ppm.

Desacetylmatricarin (= austricin, austrisin, **3**). TLC R*f*: 0.24 (system A); detection: quenching under $UV_{255\,nm}$; no colour with spraying reagents. R*t*-HPLC: 11.0 min (system 2). UV λmax (50% MeOH) nm: 275. $[\alpha]_D^{20}$ +11 (*c*, 0.294 $CHCl_3$). IR νmax cm^{-1}: 3422 (m, O-H), 2934 (m, C-H), 1770 (s, C = O lactone), 1682 (s, C = O ketone), 1636 (m, C = C), 1616 (w, C = C), 1165 (m, C-O). Molecular formula: $C_{15}H_{18}O_4$. EI-MS *m/z* (% rel. int.): 262 $[M]^+$ (100), 247 $[M\text{-}CH_3]^+$ (3), 244 [M-OH]$^+$ (5), 229 $[M\text{-}CH_3\text{-OH}]^+$ (8), 216 (14), 201 $[M\text{-}CH_3\text{-OH-CO}]^+$ (18), 189 (29), 173 (28), 171 (27), 159 (25). CI-MS (ammonia) *m/z* (% rel. int.): 263 $[M+H]^+$ (100). ^1H NMR (400 MHz, chloroform-*d*): see Table I. ^{13}C NMR (100 MHz, δ, chloroform-*d*): 15.5 (C-13), 19.9 (C-15), 21.7 (C-14), 41.3 (C-11), 49.1 (C-9), 51.6 (C-5), 61.5 (C-7), 69.7 (C-8), 81.0 (C-6), 133.1 (C-1), 135.7 (C-3), 145.2 (C-10), 169.9 (C-4), 177.4 (C-12), 195.3 (C-2) ppm.

Achillin (**4**). TLC R*f*: 0.78 (system A); detection: quenching under $UV_{255\,nm}$; no colour with spraying reagents. R*t*-HPLC: 65.0 min (system 1). UV λmax (30% MeOH) nm: 260. $[\alpha]_D^{20}$ +103 (*c*, 0.136 $CHCl_3$). IR νmax cm^{-1}: 2939 (m, C-H), 1777 (s, C = O lactone), 1682 (s, C = O ketone), 1637 (m, C = C), 1618 (w, C = C), 1267(m), 1208 (w, C-O lactone). Molecular formula and mass spectrometric data are identical to compound **1**. ^1H NMR (400 MHz, methanol-d_4): see Table I. ^{13}C NMR (100 MHz, δ, methanol-d_4): 10.4 (C-13), 20.2 (C-15), 22.1 (C-14), 24.8 (C-8), 38.7 (C-9), 41.0 (C-11), 53.1 (C-7), 53.9 (C-5), 85.7 (C-6), 133.5 (C-1), 136.1 (C-3), 155.5 (C-10), 174.0 (C-4), 180.4 (C-12), 198.7 (C-2) ppm.

8α-Angeloxy-achillin (**5**). TLC R*f*: 0.85 (system A); detection: quenching under $UV_{255\,nm}$; no colour with spraying reagents. R*t*-HPLC: 36.5 min (system 2). UV λmax (50% MeOH) nm: 260. $[\alpha]_D^{20}$ +105 (*c*, 0.041 $CHCl_3$). IR νmax cm^{-1}: 2981 (w, C-H), 1785 (s, C = O lactone), 1714 (m, C = O ang), 1691 (s, C = O ketone), 1643 (w, C = C), 1619 (w, C = C), 1265 (s), 1232 (m), 1158 (m, C-O ang). Molecular formula and mass spectrometric data are identical to compound **2**. ^1H NMR (400 MHz, acetone-d_6): see Table I. ^{13}C NMR (100 MHz, δ, acetone-d_6): 9.6 (C-13), 15.8 (C-4′), 19.5 (C-15), 20.5 (C-5′), 20.9 (C-14), 38.5 (C-11), 44.2 (C-9), 52.0 (C-5), 55.4 (C-7), 67.5 (C-8), 81.5 (C-6), 128.3 (C-2′), 134.2 (C-1), 135.8 (C-3), 139.3 (C-3′), 145.0 (C-10), 167.2 (C-1′), 170.9 (C-4), 177.6 (C-12), 192.0 (C-2) ppm.

3β-Hydroxy-11α,13-dihydro-costunolide (11α, 13-dihydro-hanphyllin, 11α,13-dihydro-3-epi-tamaulipin B, **6**). TLC R*f*: 0.47 (system A); detection: green with anisaldehyde sulfuric acid reagent. R*t*-HPLC: 33.0 min (system 4). UV λmax (system 4) nm: 205. $[\alpha]_D^{20}$ +134 (*c*, 0.1 $CHCl_3$). IR νmax cm^{-1}: 3422 (m, OH), 2942 (s, C-H), 1764 (s, C = O lactone), 1458 (m), 1215 (s, C-O lactone). Molecular formula: $C_{15}H_{22}O_3$. EI-MS *m/z* (% rel. int.): 250 $[M]^+$ (100), 233 $[M\text{-}OH]^+$ (83), 205 $[M\text{-}OH\text{-}CO]^+$ (72), 177 (43), 159 (44). CI-MS (ammonia) *m/z* (% rel. int.): 268 $[M+NH_4]^+$ (100), 251 $[M+H]^+$ (7). ^{1}H NMR (400 MHz, δ, methanol-d_4): 1.26 (d, *J* = 7.7 Hz, 13-CH_3), 1.49 (s, 14-CH_3), 1.72 (q, *J* = ~13 Hz, 8-H_β), 1.75 (s, 15-CH_3), 1.85 (dd, *J* = 14.5, 5.8 Hz, 8-H_α), 2.08 (t, *J* = 12.5 Hz, 9-H_α), 2.24 (q, *J* = 9.3 Hz, 7-H), 2.24–2.34 (m, 2-H_β), 2.36–2.42 (m, 2-H_α), 2.37–2.44 (m, 9-H_β), 2.70 (quint., *J* = 7.8 Hz, 11-H), 4.20 (dd, *J* = 10.2, 5.7 Hz, 3-H), 4.79 (d, *J* = 9.7 Hz, 5-H), 4.95 (d, *J* = ~12.5 Hz, 1-H), 5.01 (t, *J* = 9.7 Hz, 6-H). ^{13}C NMR (100 MHz, δ, methanol-d_4): 11.2 (C-13), 12.0 (C-15), 16.6 (C-14), 26.2 (C-8), 36.0 (C-2), 42.2 (C-9), 42.6 (C-11), 50.5 (C-7), 79.2 (C-3), 82.7 (C-6), 126.1 (C-5), 126.4 (C-1), 139.2 (C-10), 143.1 (C-4), 183.2 (C-12) ppm.

Apigenin (**7**). TLC R*f*: 0.52 (system I). R*t*-HPLC: 8.0 min (system 3). UV λmax (60% MeOH) nm: 270, 330. IR νmax cm^{-1}: 3299 (s, O-H), 2959 (s, C-H), 1730 (s, C = O aromatic), 1653, 1607, 1583 (m, C = C aromatic), 1272 (s). Molecular formula: $C_{15}H_{10}O_5$. EI-MS *m/z* (% rel. int.): 270 $[M]^+$ (74), 242 $[M\text{-}CO]^+$ (11), 153 [A-ring fragment]$^+$ (18), 121 (14), 97 (21). CI-MS (ammonia) *m/z* (% rel. int.): 271 $[M+H]^+$ (100).

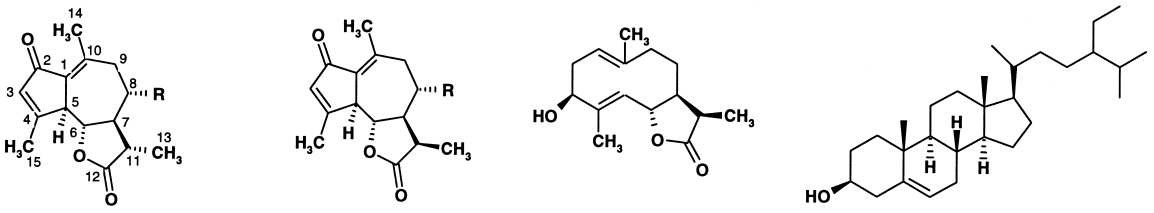
Luteolin (**8**). TLC R*f*: 0.47 (system I). R*t*-HPLC: 5.6 min (system 3). UV λmax (60% MeOH) nm: 256, 267sh, 348. IR νmax cm^{-1}: 3327 (s, O-H), 2928 (s, C-H), 1730 (s, C = O aromatic), 1656, 1606, 1580 (m, C = C aromatic), 1272 (s). Molecular formula: $C_{15}H_{10}O_6$. EI-MS *m/z* (% rel. int.): 286 $[M]^+$ (100), 258 $[M\text{-}CO]^+$ (19), 153 [A-ring fragment]$^+$ (31), 124 (17), 98 (42).

Centaureidin (**9**). TLC R*f*: 0.13 (system D); detection: yellow at daylight, intensification with sulfuric acid reagent; quenching under $UV_{255\ nm}$. R*t*-HPLC: 61.0 min (system 4). UV λmax (system 4) nm: 257, 271sh, 351. IR νmax cm^{-1}: 3391 (s, O-H), 2940 (s, C-H), 1654, 1611, 1586 (s, C = C aromatic), 1471 (s), 1371 (s), 1272 (s). Molecular formula: $C_{18}H_{16}O_8$. EI-MS *m/z* (% rel. int.): 360 $[M]^+$ (100), 345 $[M\text{-}CH_3]^+$ (27), 342 $[M\text{-}OH]^+$ (12), 330 $[M\text{-}2CH_3]^+$ (4), 327 $[M\text{-}CH_3\text{-}OH]^+$ (8), 317 $[M\text{-}CH_3\text{-}CO]^+$ (21), 299 $[M\text{-}CH_3\text{-}CO\text{-}OH]^+$ (12). ^{1}H NMR (400 MHz, δ, methanol-d_4): 3.83 (s, 3-OCH_3), 3.92 (s, 6-OCH_3), 3.99 (s, 4′-OCH_3), 6.53 (s, 8-H), 7.12 (d, *J* = 8.6 Hz, 5′-H), 7.65 (d, *J* = 2.2 Hz, 2′-H), 7.69 (dd, *J* = 8.6, 2.2 Hz, 6′-H). ^{13}C NMR (100 MHz, δ, methanol-d_4): 56.9 (OCH_3-4′), 61.1 (OCH_3-3), 61.4 (OCH_3-6), 95.8 (C-8), 106.6 (C-10), 112.9 (C-5′), 116.6 (C-2′), 122.6 (C-6′), 124.9 (C-1′), 133.6 (C-6), 140.1 (C-3), 148.3 (C-3′), 152.3 (C-4′,C-5), 154.7 (C-9), 158.4 (C-2), 160.7 (C-7), 180.8 (C-4) ppm; (100 MHz, δ, DMSO-d_6): 55.6 (OCH_3-4′), 59.7 (OCH_3-3), 59.9 (OCH_3-6), 93.9 (C-8), 104.5 (C-10), 111.9 (C-5′), 114.9 (C-2′), 120.3 (C-6′), 122.3 (C-1′), 131.2 (C-6), 137.6 (C-3), 146.3 (C-3′), 150.2 (C-4′), 151.6 (C-9), 152.3 (C-5), 155.2 (C-2), 157.7 (C-7), 178.1 (C-4) ppm.

β-Sitosterol (**10**). TLC R*f*: 0.63 (system A); 0.25 (system B); 0.37 (system E); 0.24 (system F); 0.40 (system G); detection: blue with anisaldehyde sulfuric acid reagent; violet with sulfuric acid reagent.

Results and Discussion

Flowerheads of a tetraploid taxon of *A. millefolium* s.l. were extracted by dichloromethane and yielded β-sitosterol (**10**), three flavonoid aglycones (**7–9**), five guaianolides (**1–5**) and one germacrene (**6**) (Fig. 1). Repeated CC on Sephadex®-LH-20 (i-PrOH) and silica gel (benzene-acetone) yielded β-Sitosterol (**10**) which was identified by R*f*-TLC in different systems and by colouring with anisaldehyde sulfuric acid reagent and sulfuric acid reagent using a reference substance. Isolation of **7** and **9** required CC on Sephadex®-LH-20 (i-PrOH) and Polyamide ($CHCl_3$-MeOH-methylethylketone), **8** crystallized directly from MeOH after CC on Sephadex®-LH-20. Comparison of R*f*-TLC, R*t*-HPLC, UV, IR spectroscopic and MS data with those of authentic substances revealed the structures of apigenin (**7**), luteolin (**8**) and centaureidin (**9**). **7** and **8** are widespread in contrast to centaureidin (**9**) which is rather exceptional for the *A. millefolium* group (Valant-Vetschera and Wollenweber, 1988; Wierzchowska-Renke *et al.*, 1997). Its structure was identified by NMR spectroscopic analysis and by comparison with data of the literature (Barberá *et al.*, 1986; Parodi and Fischer, 1988; Narantuya *et al.*, 1999).

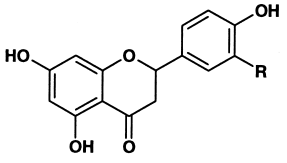


1 (R=H) leucodin
2 (R=OAng) 8α-angeloxy-leucodin
3 (R=OH) desacetylmatricarin

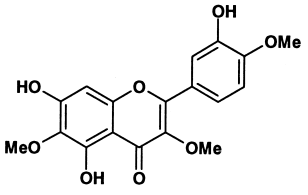
4 (R=H) achillin
5 (R=OAng) 8α-angeloxy-achillin

6 3β-hydroxy-11α,13-dihydro-costunolide

10 β-sitosterol



7 (R=H) apigenin
8 (R=OH) luteolin



9 centaureidin

Fig. 1. Structural formulas of compounds **1–10.**

The sesquiterpenes were obtained after repeated CC on silica gel using eluents with different polarities (see experimental). **3** and **6** crystallized easily as pure compounds whereas **1** and **4** as well as their corresponding 8α-angeloxy derivatives **2** and **5** required further separation by HPLC on RP-8 (methanol-water). Compound **3** was assigned as 8-desacetylmatricarin due to the correlation of R*f*-TLC, R*t*-HPLC, UV, IR spectroscopic and MS data with those of a reference substance.

The structures of compounds **1**, **2**, **4** and **5** were elucidated by NMR, the comparison to data of the literature revealed leucodin for compound **1** and achillin for compound **4** (Martíñez and Muñoz-Zamora, 1988). The respective 8α-angeloxy-derivatives (**2** and **5**) have not been described up to now. Substances with tiglic acid in this position exist but are not known for the genus *Achillea.* The compounds **1/2** and **4/5** represent pairs of respective isomers with different stereochemistry in position 13. **1** (leucodin) and **2** (8α-angeloxy-leucodin) are characterized by α orientation of CH_3-13 whereas in **4** (achillin) and **5** (8α-angeloxy-achillin) the methyl group of the lactone ring is β orientated. This does not influence the carbon shifts but remarkably affected the chemical shifts of the protons in positions 6 and 7. H-6 and H-7 of the achillin derivatives showed a shift of Δ = 0.2 ppm and 0.5 ppm to higher frequencies, respectively. If there was no oxygen in 8α, the proton in position 11 of achillin (**4**) was also shifted to higher frequencies by 0.3 ppm. This effect was less developed with an angeloxy rest in position 8: CH_3-13 of the respective compound 8α-angeloxy-achillin (**5**) showed a shift to lower frequencies by 0.2 ppm. Compared to desacetylmatricarin (**3**), substitution of the OH group in position 8 with angelic acid caused reduction of the shielding and shifted the geminal proton for more than 1 ppm to higher frequencies.

The IR spectra of the acid substituted derivatives were characterized by one additional strong band at 1711 cm^{-1} originating from the carboxylic C = O of angelic acid. The differences between the respective stereomers were only slight: the achillin analogues showed a stronger band at 1267 cm^{-1} than the corresponding leucodins.

Table I. ^{1}H NMR data of compounds **1–5** (400 MHz, d in ppm, *J* in Hz).

Position	**1** methanol-d_4	**4** methanol-d_4	**2** acetone-d_6	**5** acetone-d_6	**3** chloroform-*d*
3	6.07 s	6.07 s	6.15 s	6.15 s	6.18 s
5	3.53 d (10.3)	3.53 d (10.3)	3.70 d (10.1)	3.69 d (10.1)	3.39 d (10.0)
6	3.60 t (10.3)	3.80 t (10.3)	3.93 t (9.8)	4.13 t (10.4)	3.66 t (10.0)
7	1.95–2.06 m	2.45–2.54 m	2.59–2.68 m	3.03–3.10 m	2.08–2.17 m
8α	1.89–1.96 m	1.77–1.84 m	–	–	–
8β	1.31 q (~13)	1.37 q (~13)	5.01 t (10.5)	4.89 td (10.9, 2.0)	3.75 t (10.3)
9α	2.41–2.50 m	2.41–2.50 m	2.94 dd (13.5, 11.0)	2.82 t (13.1)	2.81 t (13.8)
9β	2.23–2.30 m	2.23–2.30 m	2.39–2.44 m	2.47 dd (13.1, 2.0)	2.25–2.42 m
11	2.26–2.35 m	2.63 quint. (7.6)	2.66–2.72 m	2.78 quint. (7.5)	2.53–2.59 m
13	1.14 d (6.9)	1.04 d (7.7)	1.27 d (6.6)	1.13 d (7.6)	1.47 d (6.9)
14	2.33 s	2.33 s	2.41 s	2.41 s	2.44 s
15	2.22 s	2.22 s	2.28 s	2.27 s	2.31 s
3′	–	–	6.21 q (7.0)	6.20 q (7.2)	–
4′	–	–	2.01 d br (7.2)	2.00 d br (7.2)	–
5′	–	–	1.92 s br	1.90 s br	–

Multiplicities and coupling constants of overlapping signals were estimated from selective 1D TOCSY and 1D NOESY experiments.

Selective NOE experiments were necessary to confirm the stereochemistry of the compounds **2** and **5**: in compound **2**, NOEs were induced from H-6 (*β*) to H-8 (*β*) and H-11 (*β*); irradiation at the resonance of H-8 (*β*) gave a nuclear *Overhauser* effect at the signals of H-6 (*β*), H-9 (*β*) and H-11 (*β*) (weak), leading to the relative configuration shown. The beta orientation of CH_3-13 in compound **5** was confirmed by positive NOEs from H-6 (*β*) to H-8 (*β*) and CH_3-13 (*β*) as well as from H-8 (*β*) to H-6 (*β*), H-9 (*β*) and CH_3-13 (*β*).

The structure of compound **6** is shown in Fig. 1. Its relative stereochemistry was assigned by selective 1D TOCSY and NOE experiments and by the accompanying coupling constants. A similar compound named artabin was described for the first time from *Artemisia* by Akhmedov *et al.* (1970a, b). These papers deal with the stereochemistry of the lactone ring but do not give information about the stereochemistry of the hydroxyl group in position 3. However, later papers mentioning artabin simply refer to Akhmedov *et al.* (1970a) and indicate the structural formula with 3*β*-OH, without presenting further data to proof this fact (e. g. optical rotation, NMR-data). Akhmedov *et al.* (1970b) indicated the optical rotation of artabin to be $[\alpha]_D^{20}$ +220 (*c*, 1.5 $CHCl_3$). In contrast, compound **6** which is described here for the first time for the *A. millefolium* group showed an optical rotation of $[\alpha]_D^{20}$ +134 (*c*, 0.1 $CHCl_3$). The relative stereochemistry of compound **6** was determined by selective 1D-NOE experiments: irradiation at the resonance of H-5 (α) gave NOEs at the signals of H-3 (*α*) and H-7 (*α*); from H-7 (*α*) NOEs at H-5 (*α*) and H-11 (*α*) were induced, indicating the *β*-position of OH-3 and CH_3-13. In addition, positive NOEs from H-6 (*β*) to CH_3-13 (*β*) and CH_3-15 (*β*) were observed.

The sesquiterpene composition of the presently investigated taxon is new and shows clear differences to other tetraploid species as *A. collina* (proazulene type) or *A. pratensis* (eudesmanolide type). Due to their chromatographic and spectral properties compounds **1–6** are easily detectable in a sample (Glasl *et al.*, 2002).

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