Sesquiterpenes and Phenolic Compounds from *Achillea clypeolata*

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The investigation of a dichloromethane extract of flower heads of *Achillea clypeolata* collected in Bulgaria led to the isolation of one guaiane (4,10,11-trihydroxy-guaiane, **1**), four eudesmanes (4(15)-eudesmene-1β,11-diol, **2**, clypeotriol, **3**, 3-*epi*-clypeotriol, **4**, cryptomeridiol, **5**), one diterpene (sugeroside, **6**) and two phenolic compounds (centaureidin, **7** and scopoletin, **8**). Their structures were elucidated by UV/vis, EI- and CI-MS as well as by one- and two-dimensional NMR experiments. 4,10,11-Trihydroxy-guaiane (**1**) and 3-*epi*-clypeotriol (**4**) are reported here for the first time.

Key words: Achillea clypeolata, Sesquiterpenes, Phenolic Compounds

Introduction

The drug Herba Millefolii often consists of material collected from wild resources in South Eastern Europe. Beside the common *Achillea* species which are also widespread in Central Europe further species like *A. clypeolata* are occuring in this region. In contrast to the most common Central European species which are quite well investigated, up to now only little phytochemical information about *Achillea clypeolata* is available.

Achillea clypeolata is a diploid species with yellow inflorescences belonging to the *A. filipendulina* group. Nevertheless, this species is able to hybridize with species of the *A. millefolium* group [1].

As the sesquiterpenes are important for pharmacological effects and chemotaxonomy the main focus was put on this class of compounds.

Experimental Section

General

TLC was performed on RP plates (Merck, Germany), 0.25 mm. System A: RP2, Methanol 70 % (v/v). System B: RP8 methanol 80 % (v/v). After development at r. t., chromatograms were examined under $UV_{255\,nm}$ and $UV_{366\,nm}$. Additional detection was performed by spraying with anisaldehyde sulfuric acid reagent [2] and subsequent heating.

HPLC was performed on a Perkin Elmer Series 200 system consisting of an Autosampler, a pump, a diode array detector (monitoring wavelength 205 nm) and a LINK interface. As additional detector a Sedex 75 (Sedere, France) light scattering device was used. All computations were performed using the Perkin Elmer Turbochrom software. 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 methanol-water in a linear gradient system starting at 50 % (v/v) with a rate of 1 %/min up to 100 % methanol, flow rate: 1.0 mL min^{-1}. Light scattering detector: temperature: 40 °C, sensitivity: 8, pressure: 3.5 bar.

The NMR spectra were measured on a Varian Unity Inova 400 (^{1}H at 400 MHz, ^{13}C at 100 MHz) and a Varian Unity Inova 600 (^{1}H at 600 MHz, ^{13}C at 150 MHz) instrument at 24 °C. The TMS resonance was used as internal standard. ^{1}H- and ^{13}C-resonances were assigned using 1D proton and carbon experiments as well as 2D COSY, HSQC, HSQC-TOCSY, and HMBC techniques. The latter were optimized for a 7 Hz heteronuclear coupling constant. Spin systems were identified in COSY, HSQC, and HSQC-TOCSY spectra. Subsequently, these spin systems and the quaternary carbons were connected by correlation found in the HMBC experiment. The relative stereochemistry was assigned by selective NOE- and 2D NOESY-experiments. ^{1}H- and ^{13}C-resonances are numbered as given in the formulae. Assignments marked with an asterisk are interchangeable.

EI- and CI-MS data were recorded on a Shimadzu QP-1000 EX MSPAC 200 with direct inlet and two possible

ionisation modi. EI-mode: ion source: 250 °C, 70 eV; vacuum: $5 \cdot 10^{-4}$ 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: $1 \cdot 10^5$ Pa; vacuum: $6.67 \cdot 10^{-3}$ Pa; scan: 40 – 500/2 s; heating rate of sample vial: 80 °C/min.

LC-MS was performed on a PE Sciex API 150EX with an APCI ion source in the positive mode. Solvent: Methanol-water (80 + 20); 5 mmol/L ammonium acetate was added to the water. Scan Mode: Profile, Scan Type: Positive Q1 Scan, Q1 Resolution: Unit Resolution, Nebulizer Gas: 5, Curtain Gas: 12, Ion Spray Voltage: 5000.0, Temperature: 300.0, Declustering Potential: 10.0, Focusing Potential: 129.5, Entrance Potential: −5.1, Ion Energy: 1.0, Deflector: −400.0, CEM: 2700.0, Software: PE Sciex Analyst 1.1.

HR-MS was performed on a PE-SCIEX Qstar QTOF mass spectrometer using the APCI ion source in the positive mode (conditions see above), exact mass calibration with parthenolid m/z = 266.1756 (100 %), $[M+NH_4]^+$.

Reference substances

Centaureidin was isolated from a Hungarian *Achillea* [3], scopoletin from *Cynara cardunculus* [4].

Plant material

The plant material originated from seeds collected in Bulgaria 1998 (Golo Burdo). The plants were cultivated at the botanical garden of the Department of Pharmacognosy in Vienna. The plant material for the chemical investigations was harvested in 2001. Voucher specimens have been deposited in the herbarium of the Department.

Extraction and isolation

Dried, pulverized flower heads (660 g) were extracted at r. t. with 4 × 5000 mL $CHCl_3$for 14 d. The evaporation residue (30 g) was redissolved in 250 mL of $CHCl_3$ and extracted with MeOH-H_2O (1 + 1, v/v, 8 × 250 mL). After filtration this fraction (*ca.* 2100 mL) was divided into three parts and reextracted by shaking with $CHCl_3$ (each 8 × 100 mL). Both fractions ($CHCl_3$ and MeOH-H_2O, 1 + 1, v/v) were evaporated to dryness to yield a crude extract ($CHCl_3$ 4 g and MeOH-H_2O 2 g).

The $CHCl_3$ extract (4 g reextracted from 50 % MeOH) was separated on a silica gel column with benzene-acetone mixtures of increasing polarity. A total of 226 fractions (50 mL each) were collected and analysed by TLC.

Fractions 4 – 5 were further separated on a silica gel column with cyclohexane-ethyl acetate (1 + 1, v/v) using an automatic fraction collector. A total of 80 fractions were collected (2 mL each) to yield compound **7** (5 mg, fractions 27 – 39) and compound **8** (2 mg, fractions 51 – 62).

Fractions 6 – 7 were further separated on a silica column (Megabond SI) using CH_2Cl_2-acetone (9 + 1, v/v) followed by a separation on RP8 material with 60 % MeOH to give compound **2** (2.5 mg).

Fractions 14 – 25 were further separated on silica gel with cyclohexane-ethyl acetate (1 + 1, v/v) using an automatic fraction collector. A total of 88 fractions were collected (2 mL each) to yield compound **5** (7 mg, fractions 34 – 50).

Fractions 26 – 34 were separated on a RP8 column with 60 % MeOH yielding compound **4** (3 mg).

Fractions 56 – 61 were separated on a silica gel column (Megabond SI) with benzene-acetone (7 + 3, v/v) and on an RP8 column with 60 % MeOH giving compound **3** (4.3 mg).

Fractions 130 – 146 were separated on a silica gel column with benzene-acetone (1 + 1, v/v) using an automatic fraction collector. A total of 135 fractions were collected (2 mL each) and combined according to TLC (silica gel, benzene-acetone (3 + 7, v/v); RP8, 80 % MeOH) to 5 fractions. Further separation of fractions 4 – 5 on an RP8 column with 60 % MeOH yielded compound **1** (2.5 mg).

Compound **6** (7.8 mg) crystallized from the fractions 187 – 188. After recrystallization from acetone it was directly used for spectroscopic characterisation and enzymatic hydrolysis.

Enzymatic hydrolysis of compound **6**: 3.5 mg were dissolved in 3 mL of MeOH-H_2O (1 + 2, v/v) and treated with β-glycosidase for 24 h at 35 °C. The crystallizing aglycone was analysed by TLC (benzene-acetone (7 + 3, v/v)) using iodine vapour or AS-reagent (anisaldehyde-sulfuric acid) for spot visualization. After recrystallization from MeOH the aglycone (**6a**, 1.5 mg) was used to determine the optical rotation power.

*Guaia-4,10,11-triol (**1**).* TLC: R_f = 0.68 (system A), 0.70 (system B); detection: dark blue with anisaldehyde sulfuric reagent. R_t-HPLC = 10.4 min. $[\alpha]_D^{20}$ = −37.5 (c = 0.1 $CHCl_3$). Molecular formula: $C_{15}H_{28}O_3$. – HR-MS: m/z (%) = 274.2365 (85) (calcd. 274.2382 for $C_{15}H_{32}O_3N$, $[M+NH_4]^+$). – APCI-MS: m/z (%) = 274 $[M+NH_4]^+$ (86), 257 $[M+H]^+$ (17), 256 $[M+NH_4–H_2O]^+$ (59), 239 $[M+H–H_2O]^+$ (16), 238 $[M+NH_4–2H_2O]^+$ (4), 235 (26), 221 $[M+H–2H_2O]^+$ (76), 203 $[M+H–3H_2O]^+$ (100). – EI-MS: m/z (%) = 220 $[M–2H_2O]$ (13), 205 $[M–2H_2O–CH_3]$ (21), 202 (11), 187 (12), 177 (16), 165 (19), 163 (20), 162 (76), 159 (18), 149 (45), 147 (42), 134 (18), 122 (37), 121 (30), 119 (21), 107 (30), 99 (25), 95 (27), 93 (34), 82 (21), 81 (40), 79 (33), 71 (37), 59 (49), 43 (100). – ^{1}H NMR (600 MHz, [D$_5$]pyridine): δ = 1.28 (q br, J = 11.9 Hz, H-6α), 1.37 (s, 3H, CH_3-12), 1.40 (s, 3H, CH_3-13), 1.45 (s, 3H, CH_3-14), 1.46 – 1.53 (m, H-8α), 1.51 (s, 3H, CH_3-15), 1.75 (t br, J = 10.3 Hz, 2α), 1.79 – 1.84 (m, H-9β), 1.90 (td, J = 10.9, 3.5 Hz, H-3α), 1.99 – 2.04 (m, H-3β), 2.06 – 2.11 (m, H-2β), 2.17 – 2.22 (m, H-7), 2.18 – 2.23 (m, H-9α), 2.31 – 2.37 (m, H-8β), 2.40 (d, J = 12.0 Hz,

H-6β), 2.50 (t br, J = 9.9 Hz, H-5), 3.17 – 3.22 (m, H-1), 5.27 (s, OH-11), 5.28 (s, OH-10), 5.46 (s, OH-4). – ^{13}C NMR (150 MHz, [D$_5$]pyridine): δ = 25.3 (C-15), 26.2 (C-2, C-12), 26.4 (C-8), 27.2 (C-6), 28.2 (C-13), 30.0 (C-14), 38.2 (C-9), 39.8 (C-3), 50.2 (C-7), 52.9 (C-1), 54.9 (C-5), 72.7 (C-11), 73.7 (C-10), 81.2 (C-4).

4(15)-Eudesmene-1β,11-diol (**2**). TLC: R_f = 0.55 (system A), 0.54 (system B); detection: dark blue with anisaldehyde sulfuric reagent. R_t-HPLC = 18.0 min. $[\alpha]_D^{20}$ = +40 (c = 0.25 $CHCl_3$). Molecular formula: $C_{15}H_{26}O_2$. – APCI-MS: m/z (%) = 239 (13), 221 (51), 203 (100). – ^{1}H NMR (400 MHz, [D$_6$]acetone): δ = 0.67 (s, 3H, CH$_3$-14), 1.12 (td, J = 13.0, 3.6 Hz, H-9$_{ax}$), 1.15* (s, 3H, CH$_3$-12), 1.16* (s, 3H, CH$_3$-13), 1.18 – 1.28 (m, H-6$_{ax}$), 1.21 – 1.31 (m, H-8$_{ax}$), 1.27 – 1.37 (m, H-7), 1.48 – 1.59 (m, H-2$_{ax}$), 1.69 (d, J = ~ 11 Hz, H-8$_{eq}$), 1.72 (d, J = ~ 11 Hz, H-5), 1.72 (d, J = ~ 12 Hz, H-6$_{eq}$), 1.72 – 1.78 (m, H-2$_{eq}$), 2.03 (d, J = 13.0 Hz, H-9$_{eq}$), 2.11 (td, J = 13.3, 5.1 Hz, H-3$_{ax}$), 2.27 (ddd, J = 13.3, 5.1, 2.2 Hz, H-3$_{eq}$), 3.09 (s, OH-11), 3.33 – 3.40 (m, H-1), 3.48 (d, J = 5.0 Hz, OH-1), 4.51 (s br, H-15$_{(Z)}$), 4.72 (s br, H-15$_{(E)}$). – ^{13}C NMR (100 MHz, [D$_6$]acetone): δ = 10.7 (C-14), 23.1 (C-8), 25.4 (C-6), 27.3* (C-12), 27.8* (C-13), 32.6 (C-2), 35.2 (C-3), 38.2 (C-9), 41.1 (C-10), 48.6 (C-5), 50.0 (C-7), 71.8 (C-11), 79.0 (C-1), 106.5 (C-15), 151.0 (C-4).

Clypeotriol (**3**). TLC: R_f = 0.58 (system A), 0.60 (system B); detection: grey with anisaldehyde sulfuric reagent. R_t-HPLC = 15.0 min. $[\alpha]_D^{20}$ = −7.7 (c = 0.22 $CHCl_3$). Molecular formula: $C_{15}H_{26}O_3$. – APCI-MS: m/z (%) = 272 $[M+NH_4]^+$ (32), 255 $[M+H]^+$ (8), 237 $[M+H-H_2O]^+$ (82), 219 $[M+H-2H_2O]^+$ (100), 201 $[M+H-3H_2O]^+$ (83). – ^{1}H NMR (400 MHz, [D$_6$]acetone): δ = 0.68 (s, 3H, CH$_3$-14), 1.15 (d, J = 9.0 Hz, H-1$_{eq}$), 1.21* (s, 3H, CH$_3$-12), 1.22* (s, 3H, CH$_3$-13), 1.25 (dd, J = 8.3, 2.7 Hz, H-9$_{eq}$), 1.43 – 1.57 (m, H-6$_{ax}$ + H-6$_{eq}$), 1.52 (d, J = ~ 9 Hz, H-8$_{eq}$), 1.66 – 1.74 (m, H-9$_{ax}$), 1.67 – 1.77 (m, H-8$_{ax}$), 1.71 – 1.80 (m, H-2$_{ax}$ + H-2$_{eq}$), 1.75 – 1.86 (m, H-1$_{ax}$), 2.87 (d, J = ~ 12 Hz, H-5), 4.21 (s, H-3), 4.49 (t, J = 1.9 Hz, H-15$_{(E)}$), 4.83 (s br, H-15$_{(Z)}$). – ^{13}C NMR (100 MHz, [D$_6$]acetone): δ = 15.1 (C-14), 25.0 (C-12 + C-13), 27.2 (C-8), 29.8 (C-6), 31.2 (C-2), 36.1 (C-10), 36.3 (C-1), 37.1 (C-9), 38.9 (C-5), 73.5 (C-3), 75.2 (C-7), 76.2 (C-11), 107.3 (C-15), 154.6 (C-4).

3-Epi-clypeotriol (**4**). TLC: R_f = 0.60 (system A), 0.61 (system B); detection: blueviolet with anisaldehyde sulfuric reagent. R_t-HPLC = 15.8 min. $[\alpha]_D^{20}$ = −9.3 (c = 0.43 $CHCl_3$). Molecular formula: $C_{15}H_{26}O_3$. – HR-MS: *m/z* (%) = 272.2213 (46) (calcd. 272.2225 for $C_{15}H_{30}O_3N$, $[M+NH_4]^+$). – APCI-MS: m/z (%) = 272 $[M+NH_4]^+$ (45), 255 $[M+H]^+$ (4), 237 $[M+H-H_2O]^+$ (18), 219 $[M+H-2H_2O]^+$ (64), 201 $[M+H-3H_2O]^+$ (16). – ^{1}H NMR (400 MHz, [D$_6$]acetone): δ = 0.70 (s, 3H, CH$_3$-14), 1.17 (d, J = ~ 12 Hz, H-1$_{eq}$), 1.22 (s, 6H, CH$_3$-12 + CH$_3$-13), 1.25 (d, J = ~ 10 Hz, H-9$_{eq}$), 1.47 – 1.57 (m, H-6$_{ax}$ + H-6$_{eq}$), 1.51 (d, J = ~ 10.5 Hz, H-8$_{eq}$), 1.56 (t, J = ~ 12 Hz, H-1$_{ax}$), 1.66 (t, J = ~ 10 Hz, H-9$_{ax}$), 1.68 – 1.78 (m, H-8$_{ax}$), 1.72 – 1.81 (m, H-2$_{ax}$), 1.95 (d, J = ~ 14 Hz, H-2$_{eq}$), 2.67 (d br, J = 11.2 Hz, H-5), 4.32 – 4.34 (m, H-3), 4.71 (t, J = 1.8 Hz, H-15$_{(E)}$), 4.97 (s br, H-15$_{(Z)}$). – ^{13}C NMR (100 MHz, [D$_6$]acetone): δ = 15.1 (C-14), 25.0 (C-12 + C-13), 27.0 (C-2), 27.1 (C-8), 29.6 (C-6), 35.9 (C-10), 36.8 (C-1), 37.0 (C-9), 39.7 (C-5), 75.1 (C-7), 76.1 (C-11), 86.4 (C-3), 111.2 (C-15), 150.1 (C-4).

Cryptomeridiol (**5**). TLC: R_f = 0.45 (system A), 0.41 (system B); detection: blueviolet with anisaldehyde sulfuric reagent. R_t-HPLC = 26.4 min. $[\alpha]_D^{20}$ = −25.6 (c = 0.09 $CHCl_3$). Molecular formula: $C_{15}H_{28}O_2$. – APCI-MS: m/z (%) = 258 $[M+NH_4]^+$ (69), 241 $[M+H]^+$ (4), 240 $[M+NH_4-H_2O]^+$ (20), 223 $[M+H-H_2O]^+$ (10), 222 $[M+NH_4-2H_2O]^+$ (16), 205 $[M+H-2H_2O]^+$ (100). – EI-MS: m/z (%) = 222 $[M-H_2O]$ (6), 204 $[M-2H_2O]$ (28), 189 $[M-2H_2O-CH_3]$ (34), 164 (30), 161 (29), 149 (73), 135 (18), 133 (18), 123 (19), 122 (17), 121 (20), 109 (31), 108 (25), 107 (26), 105 (19), 95 (24), 93 (27), 91 (26), 81 (34), 79 (28), 77 (17), 71 (23), 67 (27), 59 (100), 55 (28), 43 (77). – ^{1}H NMR (400 MHz, [D$_6$]acetone): δ = 0.84 (s, 3H, CH$_3$-14), 0.96 – 1.05 (m, H-6$_{ax}$), 1.02 (s, 3H, CH$_3$-15), 1.03 (t, J = ~ 13 Hz, H-1$_{ax}$), 1.09 (t, J = ~ 12 Hz, H-9$_{ax}$), 1.11* (s, 6 H, CH$_3$-12, CH$_3$-13), 1.18 (dd, J = 12.4, 2.6 Hz, H-5), 1.25 – 1.36 (m, H-8$_{ax}$), 1.26 (t, J = ~ 11 Hz, H-7), 1.32 (d, J = ~ 13 Hz, H-1$_{eq}$), 1.35 (t, J = 12.4 Hz, H-3$_{ax}$), 1.38 (d, J = ~ 12 Hz, H-9$_{eq}$), 1.43 – 1.57 (m, H-2), 1.55 – 1.63 (m, H-8$_{eq}$), 1.68 (d, J = 12.4 Hz, H-3$_{eq}$), 1.97 – 2.05 (m, H-6$_{eq}$). – ^{13}C NMR (100 MHz, [D$_6$]acetone): δ = 19.1 (C-14), 20.9 (C-2), 22.3 (C-6), 23.0 (C-15), 23.2 (C-8), 27.6* (C-12), 27.9* (C-13), 35.1 (C-10), 42.1 (C-1), 44.3 (C-3), 45.8 (C-9), 51.0 (C-7), 55.5 (C-5), 71.4 (C-4), 72.0 (C-11).

Sugeroside (**6**). TLC: R_f = 0.65 (system A), 0.63 (system B); detection: grey with anisaldehyde sulfuric reagent. R_t-HPLC = 19.0 min. $[\alpha]_D^{20}$ = −45 (c = 0.1 MeOH). Molecular formula: $C_{26}H_{42}O_8$. – APCI-MS: m/z (%) = 500 $[M+NH_4]^+$ (79), 483 $[M+H]^+$ (3), 465 $[M+H-H_2O]^+$ (53), 447 $[M+H-2H_2O]^+$ (12), 303 $[M+H-H_2O$–hexose$]^+$ (100), 285 $[M+H-2H_2O$–hexose$]^+$ (79). – ^{1}H NMR (400 MHz, [D$_6$]acetone): δ = 1.03 (s, 3H, CH$_3$-19), 1.07 (s, 3H, CH$_3$-18), 1.10 (s, 3H, CH$_3$-20) 1.15 (d, J = 7.8 Hz; H-9), 1.41 – 1.47 (m, H-1$_\alpha$), 1.42 (d, J = ~ 14 Hz, H-15$_a$), 1.49 – 1.59 (H-6$_\alpha$ + H-6$_\beta$), 1.51 (d, J = ~ 8 Hz, H-5), 1.51 – 1.59 (m, H-7$_a$), 1.54 – 1.63 (m, H-12$_a$), 1.55 (d, J = ~ 14 Hz, H-15$_b$), 1.57 – 1.71 (m, H-11$_\alpha$ + H-11$_\beta$), 1.62 – 1.68 (m, H-7$_b$), 1.66 (d, J = ~ 11 Hz, H-14$_a$), 1.66 – 1.72 (m, H-12$_b$), 1.91 (d, J = 11.6 Hz, H-14$_b$), 1.98 – 2.05 (m, H-1$_\beta$), 2.09 – 2.12 (m, H-13), 2.46 – 2.51 (m, H-2$_\alpha$ + H-2$_\beta$), 3.22 (t, J = 8.5 Hz, H-2′), 3.25 – 3.30 (m, H-5′), 3.26 – 3.32 (m, H-4′), 3.37 (t, J = ~ 8 Hz, H-3′), 3.51 (d, J = 10.3 Hz, H-17$_a$), 3.64 – 3.69 (m, H-6′$_a$), 3.87 (d, J = 11.6 Hz, H-6′$_b$), 4.22 (d, J = 10.3 Hz, H-17$_b$), 4.28 (d, J = 7.8 Hz, H-1′). – ^{13}C NMR (100 MHz, [D$_6$]acetone): δ = 18.6 (C-20), 20.1 (C-11), 21.6

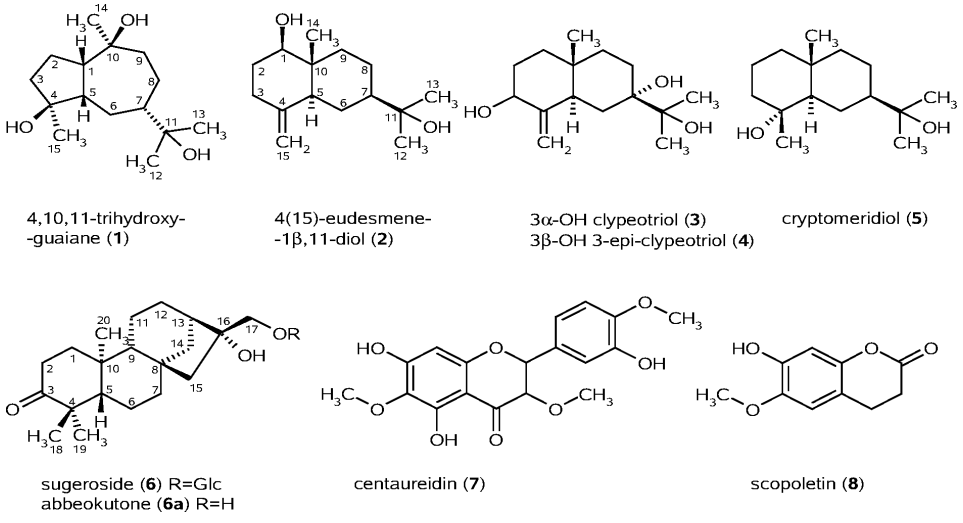

Fig. 1. Structural formulae of compounds **1** – **8**.

(C-19), 23.0 (C-6), 27.3 (C-12), 28.0 (C-18), 35.3 (C-2), 38.0 (C-14), 40.0 (C-10), 40.7 (C-1), 42.3 (C-7), 45.8 (C-8), 47.0 (C-13), 48.6 (C-4), 53.5 (C-15), 55.7 (C-5), 57.1 (C-9), 63.1 (C-6′), 72.0 (C-4′), 75.3 (C-17), 75.6 (C-2′), 78.2 (C-3′), 78.4 (C-5′), 82.3 (C-16), 105.6 (C-1′), 221.3 (C-3).

Abbeokutone (***6a***, obtained by encymatic hydrolysis of **6**). $[\alpha]_D^{20} = -60$ ($c = 0.15$ $CHCl_3$).

Results and Discussion

From a chloroform extract of flowerheads of *Achillea clypeolata* five sesquiterpenes (**1** – **5**), one diterpene (**6**) and two phenolic compounds (**7**, **8**) were isolated by repeated CC on silica gel and RP8 material. This purification step yielded clypeotriol (**3**), which had been described before as found in *Achillea clypeolata* [5]. Its 3-epimer (**4**) and compound **1** are new natural compounds, the compounds **2** and **5** – **8** were isolated for the first time from this *Achillea* species (Fig. 1).

The NMR spectra of **1** showed signals of fifteen carbons, none of them olefinic, and 25 protons. Three carbons showed shifts typical for a substitution with a hydroxyl group, two of them were additionally substituted with a methyl group, the third was part of an isopropyl moiety. In combination with the MS data (indicating the molecular formula $C_{15}H_{28}O_3$), the structure was established as guaia-3,10,11-triol. The entire relative stereochemistry was assigned by 2D NOESY experiments.

The comparison of the NMR data and the optical rotation of compound **2** with data from the literature [6, 7] revealed its structure as 4(15)-eudesmene-1β,11-diol, a sesquiterpene which has not been found in *Achillea* before.

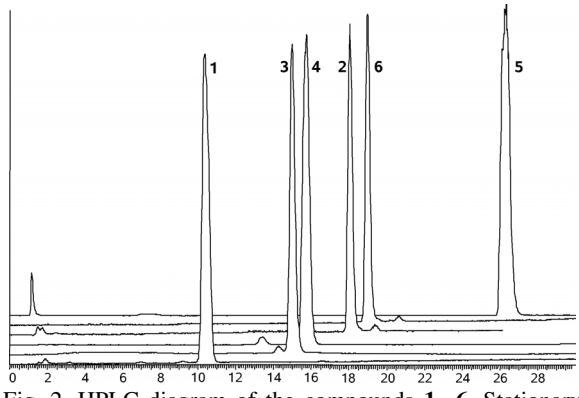

Fig. 2. HPLC diagram of the compounds **1** – **6**. Stationary phase: Hewlett-Packard LiChrospher®100-RP8 5 μm column (250 × 4.0 mm) guarded by a Hewlett-Packard LiChrospher®100-RP8 5 μm guard column (4 × 4 mm); mobile phase: methanol-water linear gradient system, start at 50 % (v/v) up to 100 % methanol, rate: 1 %/min, flow rate: 1.0 mL min^{-1}; detection: light scattering detector.

Compound **3** was identified by its NMR data as clypeotriol, a sesquiterpene already known from *Achillea clypeolata* [5]. It was nearly identical to compound **4**, but differences in the NMR shifts of C-3 and the respective NOE effects suggested an inversion of the hydroxyl group at C-3. Compound **4** was therefore determined as 3-*epi*-clypeotriol, which has not yet been described in the literature.

The ^{1}H and ^{13}C resonances and the optical rotation of compound **5** were in good accordance with cryptomeridiol [7 – 9], a sesquiterpene which is quite

common but has not been reported for *Achillea* until now.

Compound **6** could be established as sugeroside previously isolated from *Artemisia sacrorum* [10]. Its stereochemistry was confirmed by one- and two-dimensional NMR spectroscopy and by the optical properties of the compound itself [11] as well as of its aglycone [12] which was obtained by enzymatic hydrolysis. The α-orientation of the hydroxyl group in position 16 was confirmed by NMR, because the carbon shifts of C-13 and C-16 show remarkable differences in the 16α-OH and 16β-OH isomers [13].

Comparison of UV, IR spectroscopic and MS data as well as co-chromatography on TLC with authentic substances revealed compound **7** as centaureidin [3] and **8** as scopoletin [4].

In addition to the structure elucidation of these compounds a method for analytical investigations *via* HPLC with focus on the terpenes was developed. As the isolated terpenes possess no chromophor they can not be detected by UV as usual. Therefore ELSD (evaporative light scattering detection) was applied as an alternative detection method. This technique allows the detection of any non-volatile analyte independent of its absorption characteristics. After nebulization and evaporation of the solvent the resulting microparticles lead to light scattering and can thus be detected. The separation was carried out on an RP8 column with methanol-water as mobile phase in a linear gradient system starting at 50 % (v/v) with a rate of 1 %/min up to 100 % methanol. The elution order of the substances in this system is shown in Fig. 2.

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