Flavonoids from *Achillea nobilis* L.

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The detailed investigation of a methanolic extract of aerial parts of *Achillea nobilis* resulted in the isolation of 10 flavonoids. A new C-glycosylflavone, luteolin-6-C-apiofuranosyl-(1‴ →2″)-glucoside, was isolated besides orientin, isoorientin, vitexin, isoschaftoside, luteolin-7-O-β-glucuronide, luteolin-4′-O-β-glucoside and quercetin-3-O-methyl ether and two rare flavonolglycosides, quercetin-3-O-α-arabinosyl-(1‴→6″)-glucoside and quercetin-3-O-methylether-7-O-β-glucoside. The structures were established either by comparison with authentic substances or by UV, ^{1}H NMR and ^{13}C NMR spectroscopic methods including 2D-NMR techniques and ESI-MS.

Key words: Achillea nobilis, Flavonoids, Luteolin-6-C-apiofuranosyl-(1‴ → 2″)-glucoside

Introduction

The use of herbal teas from different species of the *Achillea millefolium* group against gastrointestinal disorders is quite common because of the antiphlogistic, spasmolytic and antimicrobial activities (Wichtl, 2002). Although the taxon *Achillea nobilis* L. does not belong to the *millefolium* group, the differentiation might be difficult due to morphological similarities (Kastner *et al.*, 1995). But *A. nobilis* can be distinguished from most of the species of the *Achillea millefolium* group by its flavonoid pattern (Valant-Vetschera, 1987, Valant, 1978). To identify possible adulterations by *A. nobilis* in the quality control of Millefolii Herba and herbal medicinal products thereof, an analytical method for the identification and quantification of the flavonoids from *A. nobilis* by capillary electrophoresis was developed (Marchart, 2001). For the validation of this method besides the known compounds, orientin, isoorientin and vitexin (Valant, 1978) also minor accompanying flavonoids were necessary. In this paper we report the isolation and structure elucidation of seven additional flavonoids from a methanolic extract.

Material and Methods

General

NMR-spectra were recorded on Bruker AX 300 NMR-spectrometer. 5 mm sample tubes, 1H-NMR: SF = 300.13 MHz; 13C-NMR: SF = 50.3 MHz, internal standard: TMS; solvent: CD_3OD or DMSO/CD_3OD 1:1 (v/v).

ESI-MS were recorded on a PE Sciex API 150 EX single quadrupole instrument, configurated for negative ionisation, the orifice plate voltage set at – 20 and – 80 V. Full scan spectra were acquired over the range 200 –700 mz. Scan time: 2 s.

GC-MS identification and determination of the absolute configuration of monosaccharide units were performed on a Shimadzu 5050A quadrupol mass spectrometer according to (De Bettignies-Dutz *et al.*, 1991).

Capillary electrophoresis (CE) was performed on SpectraPHORESIS 1000 according to (Marchart, 2001).

Analytical HPLC was performed on a Perkin-Elmer Series 200 Liquid Chromatograph, with 600 LINK Controller, LC-235 diode array detector and series 200 autosampler according to (Kasaj *et al.*, 2001).

Preparative HPLC was carried out on two ISCO 2350 HPLC pumps with a Linear UVIS-205 absor-

bance detector on a Nucleosil 100–5C 18, 20 × 250 mm column. Gradient elution was performed with methanol (A) and water (B). The gradient profile was 5 min. 25% A, within 5 min up to 40% A and further 20 min isocratic at 40% A at a flow rate of 12 ml min^{-1}. Detection at 340 nm.

For droplet counter current chromatography separations a DCC-A apparatus (300 tubes; *i.d.* 2.0 mm, Tokyo Rikakikai, Tokyo, Japan) was used with chloroform-methanol-isopropanol-water (9 + 12 + 1 + 8 v/v/v/v) in the descending mode.

Centrifugal partition chromatography (CPC) was performed on a PharmaTech 1000 (PharmaTech, Baltimore, Maryland, USA) instrument using EtOAc-water-MeOH (100 + 100 + 10 v/v/v) with the lower phase as stationary and a flow of 2 ml min^{-1} of the mobile phase at 1000–1100 rpm.

UV spectra were recorded on Beckmann DU 640 Spectrophotometer using MeOH as blank. The preparation of shift-reagent solutions and analyses of the flavonoids after derivatisation were carried out by standard procedures (Mabry *et al.*, 1970).

Polyamide, Sephadex®-LH-20 and Amberlite XAD-2 used for CC were obtained from ICN Pharmaceuticals (Eschwege, Germany), Pharmacia Biotech (Uppsala, Sweden) and Supelco (Bellefonte, Pennsylvania, USA), respectively.

TLC Silica gel plates (Merck, Germany), 0.25 mm; System A: EtOAc-HOAc-HCO_2H-H_2O (100:11:11:26). System B: EtOAc-butanone-HCO_2H–H_2O (50:30:10:10). Detection: 1% MeOH solution of diphenyl-boric acid-ethanolamine complex (= Naturstoffreagens A) and additionally with 5% EtOH solution of PEG 400. After drying the plates were controlled under UV_{366}.

Reference flavonoids

Orientin (**1**), isoorientin (**2**) and vitexin (**3**) were obtained from K. Roth, Karlsruhe, Germany. Isoschaftosid and luteolin-7-O-β-glucuronide were isolated from *Passiflora incarnata* (Rahman, 1997) and *Achillea collina* (Kasaj *et al.*, 2001a), respectively.

Plant material

A. nobilis was cultivated in the botanical garden of the Institute of Pharmacognosy, University of Vienna. The aerial parts were collected in 1991 and 1995. Voucher specimen are deposited in the herbarium of the institute.

Extraction and isolation

Dried, pulverised aerial parts (1670 g) of *A. nobilis* were sonicated twice with CH_2Cl_2 for the removal of apolar substances. The purified drug was dried and extracted exhaustively with 40% MeOH under reflux. The aq. methanolic extract (220 g) was separated in twelve portions by CC on polyamide (90 × 3 cm) using H_2O-MeOH mixtures as solvent. The resulting fractions were combined according to their composition to five fractions (fr. A–E).

Fr. A (8.6 g) was purified by CC on Sephadex® LH-20 (75 × 5 cm, eluent 10% to 100% MeOH, 24 fr.). The resulting fr. A4–A8 (3 g) were separated by CC on Amberlite XAD-2 (80 × 2.5 cm) with H_2O-MeOH mixtures yielding 13 fractions. Droplet counter current chromatography (conditions see above) of fr. 10 (400 mg) and a further purification step by CC on Sephadex® resulted in 18 mg compound **4**.

In fr. B (9.5 g) **1** and **2** as the main flavonoids and **3** and **10** as minor compounds were detected. Fractionation of 200 mg of fr. B by centrifugal partition chromatography (conditions see above) yielded 4.5 mg of **10**.

The separation of fr. C (3.9 g) was performed by CC on Sephadex® LH-20 (75 × 5 cm, eluent 10% to 100% MeOH, 21 fr.). CC of fr. C16–C17 (400 mg) on polyamide (60 × 2.5 cm, eluent 50% to 100% EtOH, 9 fr.) and of the resulting fr. 4 (95 mg) again on Sephadex® LH-20 (60 × 1 cm, eluent 10% to 100% MeOH) yielded 60 mg **6**.

After CC of fr. D (9.7 g) on Sephadex® LH-20 (70 × 5 cm, eluent 10% to 100% MeOH, 28 fr.) from the resulting fr. D8–D15 (550 mg) 6 mg **5** were isolated by repeated CC on polyamide and on Sephadex® LH-20. From fr. D20–D21 (1.4 g) by repeated CC on Sephadex® LH-20 and Amberlite XAD-2 as well as preparative HPLC 8 mg **7** and 4 mg **10** were yielded, respectively. 3 mg **8** resulted from fr. D26 (75 mg) after a purification step on Sephadex® LH-20 and preparative TLC on silica with chloroform-methanol-water (80 + 10 + 1 v/v/v) as mobile phase.

Orientin (**1**). TLC R*f*: 0.57 (system A); 0.66 (system B).

Isoorientin (**2**). TLC R*f*: 0.43 (system A); 0.58 (system B).

Vitexin (**3**). TLC R*f*: 0.66 (system A); 0.74 (system B).

Isoschaftoside (**4**). TLC R*f*: 0.23 (system A); 0.19 (system B); *Rt*-HPLC: 5.09 min. CE migration time: 9.39 min. UV λmax MeOH nm: 274, 300 sh, 334; + NaOAc: 282, 302sh, 386; + NaOAc + H_3BO_3: 276, 285 sh, 325, 342 sh; + $AlCl_3$: 280, 305, 348, 383 sh; + $AlCl_3$ + HCl: 279, 304, 343, 383 sh; + NaOH: 283, 335, 402. Negative ESI-MS ($C_{26}H_{28}O_{14}$) *m/z*: 563 $[M-H]^-$.

Luteolin-7-O-β-glucuronide (**5**). TLC R*f*: 0.54 (system A); 0.59 (system B). *Rt*-HPLC: 9.27 min. CE migration time: 10.80 min. UV λmax MeOH nm: 256, 267 sh, 348; + NaOAc: 262, 291 sh, 403; + NaOAc + H_3BO_3: 262, 291 sh, 370; + $AlCl_3$: 273, 296 sh, 424; + $AlCl_3$ + HCl: 272, 295 sh, 355, 389; + NaOH: 245 sh, 269, 301 sh, 394. Negative ESI-MS ($C_{21}H_{18}O_{12}$) *m/z*: 461 $[M-H]^-$, 285 $[M-H-176]^-$ = $[agycone-H]^-$.

Luteolin-4′-O-β-glucopyranoside (**6**). TLC R*f*: 0.64 (system A); 0.65 (system B); 0.53 (system C). *Rt*-HPLC: 13.67 min. CE migration time: 9.66 min. UV λmax MeOH nm: 269, 336; + NaOAc: 273, 369; + NaOAc + H_3BO_3: 270, 339; + $AlCl_3$: 259 sh, 278, 295 sh, 351, 387 sh; + $AlCl_3$ + HCl: 256 sh, 280, 293 sh, 345, 387 sh; + NaOH: 269, 302 sh, 379. Negative ESI-MS ($C_{21}H_{20}O_{11}$) *m/z*: 447 $[M-H]^-$; 285 $[M-H-162]^-$ = $[aglycone-H]^-$. ^1H NMR (300 MHz, MeOH): δ ppm 3.40 (1H, t, H-4″), 3.50 (2H, obs, H-3″ and H-5″), 3.55 (1H, t, H-2″) 3.72 (1H, dd, H-6a″), 3.91 (1H, dd, H-6b″), 4.95 (1H, d, *J* = 7.5 Hz, H-1″), 6.22 (1H, d, *J* = 2.1 Hz, H-6), 6.43 (1H, d, *J* = 2.1 Hz, H-8), 6.62 (1H, s, H-3), 7.32 (1H, d, *J* = 8.4 Hz, H-5′), 7.45 (1H, d, *J* = 2.4 Hz, H-2′), 7.46 (1H, dd, $J_{5',6}$ = 8.4 Hz, $J_{2',5'}$ = 2.4 Hz, H-6′). ^{13}C NMR: δ ppm 62.4 (C-6″), 71.3 (C-4″), 74.8 (C-2″), 77.5 (C-3″), 78.5 (C-5″), 95.2 (C-8), 100.3 (C-6), 103.2 (C-1″), 105.2 (C-3), 105.4 (C-10), 114.9 (C-2′), 117.9 (C-5′), 119.8 (C-6′), 127.2 (C-1′), 148.6 (C-3′), 149.9 (C-4′), 159.4 (C-9), 163.2 (C-5), 165.4 (C-7), 166.2 (C-2), 183.7 (C-4).

Luteolin-6-C-apiofuranosyl-(1‴→2″)-glucopyranoside (**7**) UV λmax MeOH nm: 268, 352; + NaOAc: 272, 385; + NaOAc + H_3BO_3: 267, 386; + $AlCl_3$: 276, 425; + $AlCl_3$ + HCl: 274, 355, 386; + NaOH: 269, 334, 402. Negative ESI-MS ($C_{26}H_{28}O_{15}$) *m/z*: 579 $[M-H]^-$, 447 $[M-H-132]^-$, 285 $[M-H-132-162]^-$ = $[aglycone-H]^-$. ^1H NMR (300 MHz, MeOH): δ ppm 2.70 (1H, m, H-4a‴), 3.22 (1H, obs, H-5a‴), 3.25 (1H, obs, H-4b‴), 3.35 (1H, obs, H-5″), 3.40 (1H, obs, H-5b‴), 3.46 (1H, t, H-4″), 3.58 (1H, t, H-3″), 3.71 (1H, dd, H-6a″), 3.78 (1H, t, H-2″), 3.79 (1H, t, H-2‴), 3.86 (1H, dd, H-6b″), 4.85 (1H, d, *J* = 7 Hz, H-1″), 5.31 (1H, s, H-1‴), 6.48 (1H, s, H-8), 6.55 (1H, s, H-3), 6.89 (1H, d, *J* = 8.5 Hz, H-5′), 7.38 (1H, dd, $J_{5',6}$ = 8.5 Hz, $J_{2',5'}$ = 2.0 Hz, H-6′), 7.39 (1H, d, *J* = 2.0 Hz, H-2′). ^{13}C NMR: δ ppm 62.9 (C-6″), 66.1 (C-5‴), 71.8 (C-4″), 73.1 (C-1″), 75.0 (C-4‴), 76.7 (C-2″), 78.0 (C-2‴), 80.6 (C-3‴), 80.9 (C-3″), 82.6 (C-5″), 95.3 (C-8), 103.9 (C-3), 105.0 (C-10), 110.0 (C-6), 111.0 (C-1‴), 114.1 (C-2′), 116.8 (C-5′), 120.3 (C-6′), 123.5 (C-1′), 147.1 (C-3′), 151.2 (C-4′), 158.7 (C-5), 162.0 (C-9), 165.4 (C-7), 166.3 (C-2), 183.9 (C-4).

Quercetin-3-O-methyl ether (**8**) UV λmax MeOH nm: 256, 364; + NaOAc: 272, 402; + NaOAc + H_3BO_3: 267, 384; + $AlCl_3$: 276, 438; + $AlCl_3$ + HCl: 267, 364, 402; + NaOH: 269, 330, 401. Negative ESI-MS ($C_{16}H_{12}O_7$) *m/z*: 315 $[M-H]^-$. ^1H NMR (300 MHz, MeOH): δ ppm 3.77 (3H, s, -OCH_3), 6.18 (1H, d, *J* = 2 Hz, H-6), 6.37 (1H, d, *J* = 2 Hz, H-8), 6.90 (1H, d, *J* = 8.5 Hz, H-5′), 7.52 (1H, dd, $J_{5',6}$ = 8.5 Hz, $J_{2',5'}$ = 2.2 Hz, H-6′), 7.61 (1H, d, *J* = 2.2 Hz, H-2′). ^{13}C NMR: δ ppm 94.9 (C-8), 100.1 (C-6), 105.6 (C-10), 116.4 (C-2′), 116.4 (C-5′), 122.3 (C-1′), 122.9 (C-6′), 139.5 (C-3), 146.5 (C-3′), 150.0 (C-4′), 157.9 (C-2), 158.5 (C-9), 163.1 (C-5), 166.9 (C-7), 179.9 (C-4).

Quercetin-3-O-methylether-7-O-β-glucopyranoside (**9**) UV λmax MeOH nm: 264, 352; + NaOAc: 270, 378; + NaOAc + H_3BO_3: 264, 386; + $AlCl_3$: 275, 439; + $AlCl_3$ + HCl: 269, 362, 400; + NaOH: 269, 402. Negative ESI-MS ($C_{22}H_{22}O_{12}$) *m/z*: 477 $[M-H]^-$, 315 $[M-H-162]^-$ = $[aglycone-H]^-$. ^1H NMR (30 MHz, MeOH): δ ppm 3.45 (1H, t, H-4″), 3.49 (1H, t, H-2″), 3.53 (1H, t, H-3″), 3.62 (1H, m, H-5″), 3.72 (1H, dd, H-6a′′), 3.92 (1H, dd, H-6b″), 5.12 (1H, d, *J* = 7.5 Hz, H-1″), 6.47 (1H, d, *J* = 2.1 Hz, H-6), 6.80 (1H, d, *J* = 2.1 Hz, H-8), 6.94 (1H, d, *J* = 10.0 Hz, H-5′), 7.60 (1H, dd, $J_{5',6}$ = 10.0 Hz, $J_{2',5'}$ = 2.0 Hz, H-6′), 7.68 (1H, d, *J* = 2.0 Hz, H-2′). ^{13}C NMR: δ ppm 60.2 (-OCH_3), 62.3 (C-6″), 71.0 (C-4″), 74.4 (C-2″), 77.6 (C-3″), 78.0 (C-5″), 95.5 (C-8), 100.3 (C-6), 101.4 (C-1″), 107.3 (C-10), 116.1 (C-2′), 116.2 (C-5′), 122.2 (C-1′), 122.3 (C-6′), 139.5 (C-3), 146.3 (C-3′), 150.1

(C-4′), 157.5 (C-9), 157.7 (C-2), 162.4 (C-5), 164.3 (C-7), 179.7 (C-4).

Quercetin-3-O-[α-arabinopyranosyl-(1‴→6″)-β-glucopyranoside] (**10**). UV λmax MeOH nm: 262, 370; + NaOAc: 269, 390; + NaOAc + H_3BO_3: 265, 390; + $AlCl_3$: 269, 435; + $AlCl_3$ + HCl: 265, 360 sh, 397; + NaOH: 269, 392. Negative ESI-MS ($C_{26}H_{28}O_{16}$) *m/z*: 595 $[M-H]^-$, 463 $[M-H-132]^-$, 301 $[M-H-132-162]^-$ = $[aglycone-H]^-$. ^1H NMR (300 MHz, MeOH): δ ppm 3.14 (1H, dd, H-5a‴), 3.19 (1H, dd, H-3‴), 3.30 (1H, obs, H-4″), 3.36 (1H, t, H-2‴), 3.40–3.42 (2H, m, H-3″and H-5″), 3.50 (1H, t, H-2″), 3.60 (1H, dd, H-6a″), 3.63 (1H, m, H-4‴), 3.67 (1H, dd, H-5b‴), 3.89 (1H, dd, H-6b″), 4.03 (1H, d, J = 7 Hz, H-1‴), 5.19 (1H, d, J = 7.5 Hz, H-1″), 6.20 (1H, d, J = 2.1 Hz, H-6), 6.40 (1H, d, J = 2.1 Hz, H-8), 6.87 (1H, d, J = 8.2 Hz, H-5′), 7.67 (1H, dd, $J_{5',6}$ = 8.2 Hz, $J_{2',5'}$ = 2.0 Hz, H-6′), 7.70 (1H, d, J = 2.0 Hz, H-2′). ^{13}C NMR: δ ppm 66.6 (C-5‴), 69.2 (C-6″), 69.4 (C-4‴), 71.5 (C-4″), 72.4 (C-2‴), 74.0 (C-3‴), 75.7 (C-2″), 78.1 (C-3″), 78.2 (C-5″), 94.8 (C-8), 99.9 (C-6), 104.1 (C-1″), 104.8 (C-1‴), 105.8 (C-10), 116.1 (C-5′), 117.4 (C-2′), 123.1 (C-1′), 123.5 (C-6′), 135.7 (C-3), 146.0 (C-3′), 149.9 (C-4′), 158.5 (C-2), 158.7 (C-9), 163.1 (C-5), 166.1 (C-7),), 179.5 (C-4).

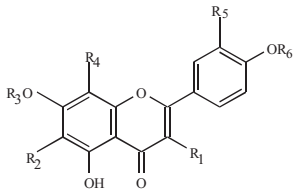
Results and Discussion

From a 40% methanolic extract of aerial parts of *A. nobilis* ten flavonoids were isolated by repeated CC on polyamide, Sephadex® LH-20 and Amberlite XAD-2 by gradient elution with H_2O–MeOH and H_2O-EtOH mixtures of decreasing polarity. Further purification was performed by preparative TLC on silica, HPLC on C 18, DCCC with chloroform-methanol-isopropanol-water (9 + 12 + 1 + 8 v/v/v/v) in the descending mode and CPC using EtOAc-water-MeOH (100 + 100 + 10) with the lower phase as stationary. The separations yielded orientin (**1**), isoorientin (**2**) and vitexin (**3**), which had been described before in *A. nobilis* (Valant, 1978; Valant-Vetschera, 1981). In addition isoschaftoside (**4**), luteolin-7-O-glucuronopyranoside (**5**), luteolin-4′-O-β-glucopyranoside (**6**), luteolin-6-C-apiofuranosyl-(1‴→2″)-β-glucopyranoside (**7**) quercetin-3-O-methyl ether (**8**), quercetin-3-O-methylether-7-O-β-glucopyranoside (**9**) and quercetin-3-O-α-arabinopyranosyl-1‴→6″-β-glucopyranoside (**10**) were isolated for the first time from this *Achillea* species, of which **7** is a new natural compound (Fig. 1).

Comparison of the R*f*-TLC with those from authentic substances revealed the identification of **1–3** as orientin, isoorientin and vitexin, R*t*-HPLC and CE-migration time as well as UV spectroscopic and ESI-MS data confirmed the structures of **4** and **5** as isoschaftoside and luteolin-7-O-β-glucuronide additionally. The structures of **6**, **8** and **9** were established by NMR techniques. The ^1H- and ^{13}C-NMR shifts of **6** were in excellent correspondence to those reported for luteolin-4′-O-β-glucoside (Kellam *et al.*, 1993), the shifts of **8** corresponded to quercetin-3-O-methylether (Agrawal, 1989). The ^1H- and ^{13}C-NMR measurements of **9** resulted in the structure of a monoglucoside of **8**. The data showed good correlation with those of transilin (=quercetin-3-O-methylether-7-O-β-glucoside) (Choi *et al.*, 1996). Structure elucidation of **7** and **10** was performed by UV, ESI-MS, NMR and 2D-NMR-techniques, the sugars and their specific linkages were confirmed after permethylation, acid hydrolysis and trimethylsilylation by GC-MS.

Negative ESI-MS of **7** showed a peak at *m/z* 579 $[M-H]^-$ suggesting the molecular formula ($C_{26}H_{28}O_{15}$). The fragment ion at *m/z* 447 $[M-H-132]^-$ gave the indication of a pentose and their O-glycosidic linkage. The UV spectrum in MeOH gave maxima at 268 (band II) and 352 nm (band I) pointing to a luteolin-type flavone. The bathmochromic shift and unchanged intensity of band I in presence of NaOMe indicated a free 4′-OH and no hydroxylation of C-3, a free 7-OH was deduced from the additional maximum at 334 nm. This was confirmed by the shift of band II after additon of NaOAc. Moreover, the bathmochromic shift for band I, when H_3BO_4 was added to this solution as well as the comparison of the spectra recorded in MeOH/$AlCl_3$ and in MeOH/$AlCl_3$ + HCl, showed the ortho-hydroxylation of ring B (Mabry *et al.*, 1970).

In the ^1H NMR spectrum a 3′,4′-dihydroxylation for ring B (a doublet at 7.39 ppm, $J_{2',5'}$ = 2.0 Hz, for 2′-H, a doublet at 6.89 ppm, J = 8.5 Hz, for 5-H′ and a double doublet at 7.38 ppm, $J_{5',6'}$ = 8.5 Hz, $J_{2',5'}$ = 2.0 Hz, for 6′-H) was proven. At 6.55 ppm the singlet of 3-H and at 6.40 the one of 8-H were detected, the shifts of which in comparison to orientin and isoorientin (Bucar *et al.*, 1998)



Compound	R1	R2	R3	R4	R5	R6
1	H	H	H	glucose	OH	H
2	H	glucose	H	H	OH	H
3	H	H	H	glucose	H	H
4	H	arabinose	H	glucose	H	H
5	H	H	glucuronic acid	H	OH	H
6	H	H	H	H	OH	glucose
7	H	apiosyl-1‴→″-glucose	H	H	OH	H
8	OCH_3	H	H	H	OH	H
9	OCH_3	H	glucose	H	OH	H
10	O-arabinosyl-1‴→6″-glucose	H	H	H	OH	H

Fig. 1. Structures of flavonoids in *Achillea nobilis*.

indicated a luteolin-C-6-glycosyl. Two anomeric protons resonated at 4.85 ppm as doublet (J = 8.5 Hz) and 5.31 ppm as broad singlet. From the latter in accordance with MS data a pentafuranose was deduced (Harborne, 1993). The ^{13}C NMR data clearly showed luteolin as genin and a C-6-glycosylation (Hirobe *et al.*, 1997). Signals of three CH_2-groups among the sugar carbons proved a branched sugar. The subspectrum of the sugars with high digital resolution, the results of HSQC, HMBC and ^1H,^1H-COSY experiments and the absolute values of the coupling constants indicated the presence of an apiofuranosyl and a glucopyranosyl moiety with β-configuration at the anomeric carbon and the attachment of apiose via C-2″ of the glucose. This was aditionally confirmed by the excellent correspondence of the sugar signals with those reported of (Ma *et al.*, 1998). Thus, **7** was unambiguously identified as luteolin-6-C-β-apiofuranosyl-(1‴→2″)-β-glucopyranoside, which to the best of our knowledge is a new natural compound.

Diagnostic UV shifts of compound **10** suggested quercetin as genin with 3-O-glycosidic linkage of the sugar residue. Negative ESI-MS showed a molecule weight of 596, which is 14 less than rutin. The fragment ion at m/z = 301 [M-H-162–132 = aglycone]$^-$ confirmed an O-linked sugar chain and the one at m/z = 463 [M-H-132]$^-$ gave the first indication of a terminal pentose. The structure of 3-O-substituted quercetin was confirmed by the ^1H NMR spectral measurements and comparison with those of rutin (Chaurasia *et al.*, 1987). The anomeric protons of a hexose and a pentose occurred at 5.19 ppm (1″-H) and 4.03 ppm (1‴-H). Due to the coupling of 7 Hz of the anomeric proton the pentose had to be linked as a pyranoside to the hexose (Harborne, 1993). Correlation of the ^1H and ^{13}C NMR data by HSQC, HMBC and ^1H, ^1H-COSY experiments indicated the presence of β-glucose and α-arabinose. Due to the good correlation of the ^{13}C NMR signals of the aglycones with quercetin-3-O-glycosides (Agrawal, 1989; Merfort *et al.*, 1997), and those of the sugar moieties with 6-O-arabinosyl-glucose (Agrawal, 1989; Li *et al.*, 1990) **10** had to be quercetin-3-O-arabinopyranosyl-1‴→6″-glucopyranoside. By GC-MS analysis of the TMS ethers of the methylglycosides after methylation and hydrolysis the sugars and their linkages (De Bettignies *et al.*, 1991) were confirmed additionally.

In accordance with earlier investigations (Valant, 1978) this study showed orientin, isoorientin and vitexin to be the main flavonoids of *A. nobilis*.

The trend to the synthesis of C-glycosyl flavonoids in this *Achillea* species was also underlined by the minor compounds isoschaftoside and luteolin-6-C-apiosyl-(1‴→2″)-glucoside. The marker flavonoids of the *A. millefolium* group apigenin-7-O-glucoside, luteolin-7-O-glucoside and rutin (Kasaj *et al.* 2001, 2001a, 2001b; Smolnig *et al.*, 2000) were not identified in *A. nobilis*.

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