65.3; H, 6.0. $C_{15}H_{16}O_5$ requires: C, 65.2; H, 5.9%); UV λ_{max} log (ε) 260 (3.80), 335 (4.30); IR (nujol) ν, 3580, 1700, 1620, 1267 and 835 cm^{-1}; NMR ($CDCl_3$, δ): 1.33 and 1.45 (*s*, 3H each, gem dimethyl), 2.5 (*s*, 1H, —OH, D_2O exchangeable), 3.2 and 3.36 (*s*, 1H each, Ar—$\underline{CH_2}$—CH<), 4.08 (*s*, 3H, —OCH_3), 4.8 (*t*, 1H, Ar—CH_2—$\underline{CH}$<), 6.14 (*d*, J = 10 Hz, 1H, H-3), 6.92 (*s*, 1H, H-5), 7.7 (*d*, J = 10 Hz, 1H, H-4). Acetylation with Ac_2O and *p*-$CH_3.C_6H_4.SO_3H$ gave the diacetate, mp 162–4°, (Found: C, 63.0; H, 4.9. $C_{18}H_{18}O_7$ requires: C 62.4; H, 5.2%), UV λ_{max} log (ε), 225 (3.65), 245 (3.35), 290 (3.40), 320 (3.76); IR (KBr) ν, 1715, 1625, 1575, 1247 and 950 cm^{-1}. The diacetate (**3**) was kept on a pre-heated block at 330° for 75 sec and the product purified by prep. TLC (C_6H_6–Me_2CO, 24:1). The pyrolysed product was identical with the acetate of E_2 (see later). The mp and spectral values of compound E_1 were found to be identical with the reported values for (−)-2,3-dihydro-9-hydroxy-2(1-hydroxy-1-methylethyl)-7H-furo[3.2 g] [1]-benzopyran-7-one (rutaretin) [4]. Compound E_1 is therefore, rutaretin (**1**).

Compound E_2 crystallized as shining needles (50 mg), mp 198°; $[\alpha]_D^{19}$ − 68.29° (c = 0.41, $CHCl_3$); R_f: 0.33 (C_6H_6–Me_2CO, 9:1); 0.85 ($CHCl_3$–MeOH, 19:1); (Found: M^+ 244; C, 68.5; H, 5.2. $C_{14}H_{12}O_4$ requires: C, 68.9; H, 4.9%); UV λ_{max} log (ε) 265 (3.86), 280 (3.65), 335 (4.30); NaOAc, 265, 280, 335; IR (KBr) ν; 3340, 1675, 1425, 1065 and 900 cm^{-1}; NMR (DMSO-d_6, δ): 1.7 (*s*, 3H, —$\underline{CH_3}$), 3.2 (*m*, 2H, Ar—$\underline{CH_2}$—CH<), 5.0 and 5.17 (*s*, 1H each, =$\underline{CH_2}$), 5.41 (*t*, 1H, Ar—CH_2—$\underline{CH}$<), 6.3 (*d*, J = 10 Hz, 1H, H-3), 7.1 (*s*, 1H, H-5), 8.03 (*s*, J = 10 Hz, 1H, H-4), It gave a positive Gibb's test and a negative ferric reaction. Methylation with CH_2N_2 gave a Me ether, mp 84°; (Found: C, 69.6; H, 6.0. $C_{15}H_{14}O_4$ requires: C, 69.8; H, 5.5%); UV λ_{max} log (ε) 260 (3.48), 330 (3.96); IR (KBr) ν: 1730, 1622 1412, 1085 and 891 cm^{-1}; NMR ($CDCl_3$, δ): 1.8 (*s*, 3H, —OCH_3), 3.28 (*m*, 2H, Ar—$\underline{CH_2}$—CH<), 4.12 (*s*, 3H, —OCH_3), 5.05 and 5.2 (*s*, 1H each, =CH_2), 5.43 (*t*, 1H, Ar—CH_2—$\underline{CH}$<), 6.29 (*d*, J = 10 Hz, 1H, H-3), 6.8 (*s*, 1H, H-5), 7.72 (*s*, J = 10 Hz, 1H, H-4). Compound E_2 on hydrogenation with Pd–C gave the tetrahydro-derivative, a pale yellow semi-solid which failed to crystallize UV λ_{max} 285, 315; IR (KBr) ν, 1725, 1625, 1475, 1078 and 952 cm^{-1}; NMR ($CDCl_3$, δ) 0.82 and 0.96 (2*d*, 3H each, gem dimethyl), 2.98 (*m*, 6H, Ar—$\underline{CH_2}$—CH<, Ar—$\underline{CH_2}$—$\underline{CH_2}$—), 5.1 (*t*, 1H, Ar—CH_2—$\underline{CH}$<), 7.14 (*s*, 1H, H-5). The acetate (**4**) prepared by the Ac_2O–C_5H_5N method crystallized as white needles, mp 110–2°; (Found: C, 67.50; H, 5.2. $C_{16}H_{14}O_5$ requires: C, 67.1; H, 4.9%); UV λ_{max} log (ε), 245 (3.86), 330 (4.37); IR (KBr) ν; 1725, 1620, 1415, 1062 and 897 cm^{-1}. It was found to be identical (mmp, UV, IR) with the pyrolysed product of the diacetate (**3**) of compound E_1. Compound E_2 is, therefore (−)-2,3-dihydro-9-hydroxy-2-isopropenyl-7H-furo [3.2 g] [1]-benzopyran-7-one (**2**).

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C-GLYCOSYLFLAVONES FROM THE GENUS *ACHILLEA*

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INTRODUCTION

Recent studies on the distribution of flavonoids in *Achillea* indicate that most species contain *C*-glycosyl-flavones [1]. Earlier publications on *A. fragrantissima* (Forsk.) Sch.-Bip. [2] report the isolation of vitexin 7-*O*-glucoside but this could not be detected in another specimen examined [1]. Orientin and vitexin have been detected in *Achillea nobilis L.* by comparison with authentic samples [3]. Furthermore, *C*-glycosylation has also been noted in two other genera of the Anthemideae: *Artemisia* [4] and *Otospermum* [5]. The endemic Anatolian genus *Leucocyclus* which is closely related to *Achillea* also contained *C*-glycosyl-flavones [1]. Apart from the mono-*C*-glycosylflavones vitexin, isovitexin, orientin and isoorientin, the 7-methyl ethers of isoorientin and isovitexin respectively and di-*C*-glycosylapigenins are characteristic of the genus *Achillea*. The identification of the latter substances is the subject of the present communication.

RESULTS AND DISCUSSION

Two *C*-glycosylflavones (B, L) were isolated from the leaf extract of *Achillea leptophylla* M. B. (cultivated in the Botanical Garden, A-1018) and their UV spectra determined. The spectral data suggested derivatives of isoorientin and isovitexin, but the addition of NaOAc gave no bathochromic shift of band II. Acid hydrolysis did not produce any sugar residue and led to the 8-isomers which again showed the same reaction with NaOAc. The presence of 7-*O*-methylation was apparent from the chromatographic properties [7]; direct comparison of B and L with authentic swertiajaponin (7-methylisoorientin) and swertisin (7-methylisovitexin) showed complete identity, and the permethyl ethers proved to be identical with permethylisoorientin and permethylisovitexin. Therefore the sugar residue must be glucose in both cases.

From *Achillea setacea* W. & K., collected in E. Austria, Burgenland, two other *C*-glycosylflavones could be isolated by PC of the leaf extract. Both showed the UV spectrum of apigenin and the chromatographic properties of apigenin diglycosides. Permethylation of band 1 led to a major product showing the MS of a PM 6-*C*-arabinosyl-8-*C*-hexosylapigenin (M^+ 704, M − 175 < M − 131 > M − 119 > M − 145) [8] and the R_f of PM 6-*C*-arabinosyl 8-*C*-glucosylapigenin (PM isoschaftoside). Permethylation of band 2 and TLC of the product led to three bands: PM1 PM2 and PM3. The MS of PM1 (M^+ 734, M − permethyl hexosyl 36%, M − permethyl hexosyloxy 100%, j > i) showed a PM 2″-*O*-hexosyl-6-*C*-hexosylapigenin structure [9], but amounts available did not allow identification of the sugar residues. The MS of PM2 indicated a 6-*C*-hexosyl-8-*C*-pentosylapigenin structure (M^+ 704, M − 175 > M − 131) and PM2 co-chromatographed with PM schaftoside (PM 6-*C*-glucosyl-8-*C*-arabinosylapigenin). The MS of PM3 showed the presence of two compounds: a PM di-*C*-hexosylapigenin (M^+ 748) and a PM *C*-pentosyl-*C*-hexosylapigenin (M^+ 704) in the ratio 5:2. These compounds were shown to be PM 6,8-di-*C*-glucosylapigenin (PM vicenin-2) and PM 6-*C*-glucosyl-8-*C*-xylosylapigenin (PM vicenin-3) by co-chromatography with synthetic standards, a conclusion supported by the chromatographic properties of the free compounds.

Thus *Achillea setacea* affords a further example of a species containing di-*C*-glucosyl, *C*-glucosyl-*C*-arabinosyl and *C*-glucosyl-*C*-xylosylapigenins, three glycosylflavones already observed to co-occur in *Flourensia cernua* [10] and *Rynchosia minima* [11]. Indeed this co-occurrence may be easily overlooked owing to the difficulties of separating these compounds in the free state and it would be interesting to ascertain the taxonomic significance of these substances by a systematic MS study of the permethyl ethers obtained from natural 'vicenins'.

EXPERIMENTAL

Dried leaf tissue was thoroughly extracted with 70% EtOH and the aq. residue was 2-D chromatographed on Whatman 3MM paper in BAW (4:1:5)/15% HOAc. To obtain greater amounts of flavonoids a cellulose powder column (Whatman CF 11) eluted with H_2O was used. The fractions were purified by preparative PC in the above mentioned systems. UV spectra were recorded using standard procedures [6], MS on an AEI MS 902 spectrograph (70 eV). Permethylation was carried out using the method previously described [8]. Voucher specimens of the species examined are deposited at the Herbarium of the Institute for Botany, University of Vienna (WU).

UV—Compound B: λ_{max} nm 349, 271, 258, 244 (MeOH); 425, 331 sh, 298 sh, 276 ($AlCl_3$); 381, 364 sh, 297 sh, 277, 261 sh ($AlCl_3$/HCl); 404, 271 (NaOMe); 405, 365 sh, 269 (NaOAc); 430 sh, 374, 267 (NaOAc/H_3BO_3). Wessely-Moser isomer of B; 349, 299 sh, 267 sh, 257 (MeOH); 431, 334 sh, 300 sh, 280 ($AlCl_3$); 390, 357, 298 sh, 279, 269 sh ($AlCl_3$/HCl); 406, 296 sh, 267 (NaOMe); 405, 368 sh, 299 sh, 265 (NaOAc); 378, 298 sh, 264 (NaOAc/H_3BO_3). Compound L: 332, 272 (MeOH); 380 sh, 351, 300, 279, 263 sh ($AlCl_3$); 379 sh, 347, 300, 280, 261 sh ($AlCl_3$/HCl); 391, 304 sh, 272 (NaOMe); 390, 356 sh, 300 sh, 270 (NaOAc); 340, 272 (NaOAc/H_3BO_3). Wessely-Moser isomer of L · 331, 300 sh, 269 (MeOH); 389; 347, 304, 277 ($AlCl_3$). 387, 340, 302, 278 ($AlCl_3$/HCl); 390, 301 sh, 270 (NaOMe); 389, 299, 269 (NaOAc); 407 sh, 343, 269 (NaOAc/H_3BO_3). Vicenins: 332, 272 (MeOH); 387, 352, 304, 280 ($AlCl_3$); 382, 350, 302, 280 ($AlCl_3$/HCl); 400, 331, 281 (NaOMe); 397, 335 sh, 281 (NaOAc); 412, 351 sh, 320, 282, (NaOAc/H_3BO_3).

TLC—R_f × 100, Cellulose, Compound B: 29 (15% HOAc), 46 (BAW 4:1:5), 65 (CAW = $CHCl_3$–HOAc 1:1, H_2O satd). WM isomer of B: 4 (15% HOAc), 24 (BAW), 43 (CAW). Compound L: 48 (15% HOAc), 67 (BAW), 86 (CAW), WM isomer of L: 10 (15% HOAc), 41 (BAW), 66 (CAW). Vicenins, band 1: 0.30; band 2: 0.38 (15% HOAc). Si gel, compound B: 5 ($CHCl_3$–MeOH 5:1), 56 (AME = EtOAc–MeOH–H_2O 63:12:9). Compound L: 38 (AME), 51 (EtOAc–Py–H_2O–MeOH 80:12:10:5). Vicenins, band 1: 0.43; band 2: 0.36–0.42 (EtOAc–Py–H_2O–MeOH 80:20:10:5).

Permethyl ethers ($CHCl_3$–EtOAc–Me_2CO 5:4:1), B: 28; L: 34; vicenins, band 1: 17; band 2: 13 (PM1), 20 (PM2), 29 (PM3).

MS—*m/e* (%), PM vicenins, band 1: M^+ 704 (13), M − 15 (23), M − 31 (100), M − 119 (28), M − 131 (36), M − 145 (19), M − 175 (14). PM vicenins, band 2, PM1: M^+ 734 (6), M − permethyl hexosyl 515 (36), M − permethyl hexosyl 499 /100), i 355 (14), j 341 (56); PM2: M^+ 704 (13), M − 15 (27), M − 31 (100), M − 131 (14), M − 163 (32), M − 175 (43); PM3: M_1^+ 748 (17), M_1 − 15 (29), M_1 − 31 (100), M_2^+ 704 (8), M_2 − 15 (12), M_2 − 31 (38), M_1 − 163 + M_2 − 119 (35), M_1 − 175 + M_2 − 131 (50), M_1 − 189 + M_2 − 145 (12), M_2 − 163 (17), M_2 − 175 (17).

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