FLAVONOIDS OF *ANVILLEA GARCINI*

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ABSTRACT.—Five new and three known methoxylated flavonol glycosides and two 6-methoxyflavone aglycones were isolated from the leaves of *Anvillea garcini* DC (Compositae).

The flavonoids were identified by spectral methods as isorhamnetin 3-glucoside, isorhamnetin 3-rhamnoglucoside, quercetin 3-rhamnoglucoside 3',4'-dimethyl ether, 6-methoxykaempferol 3-galactoside, 6-methoxykaempferol 3-galactoside 7-methyl ether, 6-methoxykaempferol 3-galactoside 7, 4'-dimethyl ether, 6-methoxykaempferol 3-rhamnoglucoside, 6-methoxyquercetin 3-rhamnoglucoside 3'-methyl ether, 6-methoxyapigenin and 6-methoxyluteolin.

Anvillea garcini DC is a perennial shrub of the Compositae widespread in southern Iran. This report of the flavonoid chemistry is the first chemical investigation of this species.

EXPERIMENTAL[1]

PLANT MATERIAL.—Leaves of *Anvillea garcini* were collected in March 1977 near Hajiabad, 1300 km south of Tehran. A specimen of the plant, which was identified by Prof. Dr. A. Siami (Rezaieh-Iran), is deposited in the Herbarium of the Faculty of Agriculture, University of Rezaieh, Iran.

EXTRACTION AND SEPARATION OF THE FLAVONOIDS.—Powdered leaves of the plant (2 kg) were extracted with 95% ethanol in a Soxhlet. The extract was diluted with water (1:1) and then the solution was extracted with chloroform; the latter extract was discarded. The remaining aqueous part was further exhaustively extracted with ethyl acetate. The resulting extract was concentrated to a brown syrup which was rich in flavonoids (15 g).

Five grams of this syrup were separated on a silica gel (7×70 cm) column using a chloroform-methanol gradient elution system, beginning with chloroform: fractions 24–36 (100 ml each; chloroform-methanol, 7:3) contained three main and three minor compounds (yield: 600 mg) which were separated on a polyclar (3×35 cm) column. Each compound obtained from this latter column was further purified over Sephadex LH-20. The major compounds were isorhamnetin 3-glucoside and 3-rhamnoglucoside and quercetin 3-rhamnoglucoside 3',4'-dimethyl ether; the minor compounds were 6-methoxykaempferol 3-galactoside 7,4'-dimethyl ether, 6-methoxykaempferol 3-galactoside 7-methyl ether and 6-methoxykaempferol 3-galactoside. Fractions 38–49 (100 ml each; chloroform-methanol, 7:3, contained 6-methoxykaempferol 3-rhamnoglucoside and 6-methoxyquercetin 3-rhamnoglucoside 3'-methyl ether (yield: 620 mg); they were separated on a polyclar column and cleaned over Sephadex. Fractions 49–79 (100 ml each; chloroform-methanol, 7:3–6:4) yielded 6-methoxyapigenin while 84–110 (100 ml each; chloroform-methanol, 1:1) yielded 6-methoxyluteolin.

IDENTIFICATION OF ISORHAMNETIN 3-GLUCOSIDE.—Isorhamnetin 3-glucoside (1) exhibited uv spectral properties comparable with those of a standard sample; identification was confirmed by pmr (as TMS ether in CCl_4) and ms. Hydrolysis yielded glucose (tlc comparison with a standard sugar) and isorhamnetin (uv and ms comparison).

IDENTIFICATION OF ISORHAMNETIN 3-RHAMNOGLUCOSIDE (NARCISSIN).—Isorhamnetin 3-rhamnoglucoside (2) yielded glucose and rhamnose (tlc comparison with standard sugars) as well as isorhamnetin upon acid hydrolysis. Moreover, the compound exhibited uv spectral

[1]Spectra were recorded with the following instruments: uv, Beckman DB; nmr, Varian HA-100; ms, DuPont 21-491. Adsorbants used for tlc and cc were from E. Merck.

properties similar to those of a standard sample; identification was confirmed by pmr (as TMS ether in CCl_4). Ms, pmr, and uv shifts of the aglycone showed that it was isorhamnetin and that the sugars were attached at C_3 in the glycoside.

IDENTIFICATION OF QUERCETIN 3-RHAMNOGLUCOSIDE 3',4'-DIMETHYL ETHER.—A new glycoside, quercetin 3-rhamnoglucoside 3',4'-dimethyl ether, was obtained as a mixture with isorhamnetin 3-rhamnoglucoside; they were not separated over various columns. However both compounds were detected on cellulose plates under uv light as overlapping purple spots; ammonia vapor and NA reagent spray (Naturstoffreagenz A in methanol) changed the color of isorhamnetin 3-rhamnoglucoside while the new glycoside remained purple with both reagents. Acid hydrolysis of the mixture yielded a 1:1 mixture of glucose and rhamnose (tlc comparison with standard sugars) and two aglycones which were readily separated over a Sephadex LH 20 column. Quercetin 3',4'-dimethyl ether showed a dull yellow color over uv light indicating a C_3-hydroxyl group; therefore, the new compound must have been a 3-O-glycoside. The uv spectra of the aglycone established a 4'-methoxyl group (a lower intensity Band I with NaOMe); $AlCl_3$, $AlCl_3$/HCl and NaOAc shifts indicated free 3,5 and 7-hydroxyl groups.

The pmr spectrum of the aglycone showed two methoxyl resonances at 3.78 and 3.9 ppm indicating 3' and 4'-methoxyl groups. The complete spectral data for quercetin 3',4'-dimethyl ether (3) are as follows: uv λ max (MeOH) 371, (sh), 273 (sh), 257; NaOMe, 420 (lower intensity), 335, 272; $AlCl_3$, 434, 380 (sh), 300 (sh), 265; $AlCl_3$/HCl, 435, 380 (sh), 300 (sh), 264; NaOAc, 384, 305, 268; NaOAc/H_3BO_3, 372, 273 (sh), 257; pmr (as TMS ether in CCl_4) 3.78 (s, OCH_3), 3.90 (s, OCH_3), 6.2 (d, J=2 Hz, C_6-H), 6.55 (d, J=2 Hz, C_8-H), 6.9 (d, J=8 Hz, $C_{5'}$-H), 7.5–7.8 (m, $C_{2'}$-H and $C_{6'}$-H); ms, m/z M^-: 330, B_2: 165, A_1; 152.

IDENTIFICATION OF 6-METHOXYKAEMPFEROL 3-GALACTOSIDE.—Hydrolysis of this new glycoside yielded galactose (tlc comparison with standard sugar) and 6-methoxykaempferol (4, 5) (tlc, uv and ms comparison with a standard sample). The uv spectra of the new glycoside established a free 4'-hydroxyl group (NaOMe). The 25 nm bathochromic shift of Band I with $AlCl_3$/HCl relative to Band I in MeOH indicated the presence of a 5-hydroxyl-6-methoxyl system (6). The presence of Band III at 335 nm in the NaOMe uv spectrum and a small shift in Band II with NaOAc indicated a free 7-hydroxyl group. The color reactions of the glycoside and aglycone as well as uv shifts showed that the sugar is attached at C_3. The uv and ms spectral data are as follows: uv λ max (MeOH) 340, 305 (sh), 272, 265 (sh); NaOMe, 415, 335, 278; $AlCl_3$, 382, 315, 275, 265; $AlCl_3$/HCl, 365, 310 (sh), 278 (sh), 268; NaOAc, 378, 310 (sh), 278; NaOAc/H_3BO_3, 344, 310 (sh), 272, 265 (sh); ms (underivatized), m/z M^+ (agly): 316, M-CH_3: 310, A_1: 182, A_1-CH_3: 167, B_2: 121. There was insufficient material to record a pmr spectrum.

IDENTIFICATION OF 6-METHOXYKAEMPFEROL 3-GALACTOSIDE 7-METHYL ETHER (EUPALITIN 3-GALACTOSIDE).—Eupalitin 3-galactoside (7) showed uv spectral properties similar to those of a standard sample. Hydrolysis yielded eupalitin (tlc and uv comparison) and galactose (tlc comparison); ms (underivatized), M^+ 330, M-CH_3: 315, A_1: 196, A_1-CH_3: 181, B_2: 121.

IDENTIFICATION OF 6-METHOXYKAEMPFEROL 3-GALACTOSIDE 7,4'-DIMETHYL ETHER (MIKANIN 3-GALACTOSIDE).—The new glycoside, 6-methoxykaempferol 3-galactoside 7,4'-dimethyl ether gave a purple color on tlc plates over uv with and without ammonia: furthermore, spraying with NA reagent also did not change this color. These color tests indicated that the B-ring did not contain any free hydroxyl groups. The uv spectrum with NaOMe gave a lower intensity Band I at 365 nm relative to Band I in MeOH. A bathochromic shift of 18 nm of Band I with $AlCl_3$/HCl relative to Band I in MeOH indicated the presence of a 5-hydroxyl-6-methoxyl system. The dull yellow color of the aglycone and the Band I shift in the uv spectrum in MeOH showed that the sugar was attached at C_3. The uv spectral data of the new glycoside are as follows: uv λ max (MeOH) 332, 274, 255 (sh); NaOMe, 365, 305 (sh), 274; $AlCl_3$, 358, 295 (sh), 268; $AlCl_3$/HCl, 350, 277, 266; NaOAc, 367, 310, 276; NaOAc/H_3BO_3, 330, 276, 255 (sh); hydrolysis of the new glycoside yielded galactose (tlc comparison with standard sugar) and mikanin (8, 9, 10) (uv and ms spectral data); ms (underivatized), m/z M^+(agly): 344, M-CH_3: 329, A_1-CH_3: 181, B_2: 135, B_2-CH_3: 120.

IDENTIFICATION OF 6-METHOXYKAEMPFEROL 3-RHAMNOGLUCOSIDE.—The new glycoside upon acid hydrolysis yielded 6-methoxykaempferol (tlc and uv and ms spectra comparison with an authentic sample) and glucose and rhamnose (tlc comparison with standard sugars). The presence of Band III in the NaOMe uv spectrum and a bathochromic shift of Band II in the NaOAc uv spectrum relative to Band II in MeOH indicated a free C_7-hydroxyl group. A 24 nm bathochromic shift of Band I in the $AlCl_3$/HCl uv spectrum relative to Band I in MeOH showed the presence of a 6-methoxyl group. The color changes with reagents of both the glycoside and aglycone as well as the uv spectra of the aglycone indicated that the sugars are at C_3 in the glycoside. The uv and prm spectral data of the new glycoside are as follows: uv λ max (MeOH) 342, 272, 256; NaOMe, 402, 320, 272; $AlCl_3$, 380, 300 (sh), 278, 270; $AlCl_3$/HCl, 368, 280 (sh), 268; NaOAc, 376, 318, 274; NaOAc/H_3BO_3, 342, 273, 258; pmr (as TMS ether in CCl_4) 0.9 (d, rhamnose methyl), 3.25–3.8 (11 H's br. m, glucose and rhamnose), 3.9 (s, OCH_3), 4.85 (1 H, m, rham-H_1) 5.9 (1 H, d, gluc-H_1), 6.8 (d, $C_{3'}$-H and $C_{5'}$-H), 7.5 (d, $C_{2'}$-H and $C_{6'}$-H).

IDENTIFICATION OF QUERCETIN 3-RHAMNOGLUCOSIDE 6,3'-DIMETHYL ETHER (SPINACETIN 3-RHAMNOGLUCOSIDE).—The new glycoside afforded on hydrolysis spinacetin (11, 12) (tlc and uv comparison with an authentic sample) and glucose and rhamnose (tlc comparison with standard sugars). The bathochromic shift of 60 nm (and increase in intensity) of Band I with NaOMe indicated a free 4'-hydroxyl group. In addition, the presence of Band III in the same spectrum as well as an 18 nm bathochromic shift of Band II in the NaOAc spectrum relative to Band II in MeOH indicated a free 7-hydroxyl group. In the $AlCl_3$/HCl uv spectrum a bathochromic shift of 18 nm in Band I showed a 6-methoxyl group. The complete spectral data are as follows: uv λ max 350, 270 (sh), 255; NaOMe, 410, 330, 272; $AlCl_3$, 400 (sh), 370, 310 (sh), 267; $AlCl_3$/HCl, 368, 310 (sh), 275 (sh), 267; NaOAc, 380, 320, 273; NaOAc/H_3BO_3, 352, 270 (sh), 256; ms (underivatized), *m/z* M^+ (agly): 346, M-CH_3: 331, A_1: 182, A_1-CH_3: 167; B_2: 151.

IDENTIFICATION OF 6-METHOXYAPIGENIN (HISPIDULIN=DINATIN).—6-Methoxyapigenin (13, 14) exhibited uv spectral properties similar to those of an authentic sample; tlc and uv spectral comparison proved that the compound was hispidulin.

IDENTIFICATION OF 6-METHOXYLUTEOLIN (NEPETIN=EUPAFOLIN).—6-Methoxyluteolin (15, 16) exhibited uv, pmr, ms, and the tlc properties identical to those of a standard sample.

ACKNOWLEDGMENTS

This study was supported by the National Science Foundation (Grant DEB 76-02703), The Robert Welch Foundation (Grant F-130), and the National Institutes of Health (Grant HD-04488).

Received 23 April 1979.

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