FLAVONOIDS OF *HAPLOPAPPUS SCROBICULATUS* AND *HAPLOPAPPUS SERICEUS*

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ABSTRACT.—Thirteen flavonoids and the coumarin esculetin were isolated from *Haplopappus scrobiculatus*, and five of the flavonoids were also found in *H. sericeus*. Both species yielded quercetin, quercetin 3-β-D-glucoside, isovitexin, vitexin, and vicenin-2 (6,8-di-C-glucosylapigenin). In addition, *H. scrobiculatus* was found to accumulate isorhamnetin, isorhamnetin 3-β-D-glucoside, 6-methoxyluteolin 4'-methyl ether, 6-methoxyluteolin 7-methyl ether, 6-methoxyluteolin, kaempferol 7-methyl ether, and isoschaftoside (6-C-arabinosyl-8-C-glucosylapigenin).

We have previously reported *Haplopappus* flavonoids from *H. canescens* (1) of section *Haplopappus*, *H. rengifoanus* (2) and *H. foliosus* (3) of section *Polyphylla*, and *H. integerrimus* var. *punctatus* (4) of section *Steriphe*. Sections *Haplopappus* and *Steriphe* are closely related and are perhaps the only true *Haplopappus* in South America (5). We report here further studies from a continuing investigation of the systematic relationships between these two sections and between South American *Haplopappus* and its North American relatives. Of note in this study is the accumulation of C-glucosylflavones in both *H. scrobiculatus* (Nees) DC. (section *Haplopappus*) and *H. sericeus* Phil. (section *Steriphe*), compounds which have not previously been found in South American species but which are common in closely related North American taxa (6).

EXPERIMENTAL[1]

PLANT MATERIAL.—Leaves of *H. scrobiculatus* were collected in Chile, 5 km west of Portillo, Prov. Aconcagua, (Clark and Brown 1337, 1338) and on the outskirts of Farellones, Prov. Santiago, (Clark & Brown 1351) in January and February 1979; those of *H. sericeus* (Clark & Brown 1347) were collected on the outskirts of La Parva, Prov. Santiago, in February 1979. Voucher specimens are deposited in the Herbarium at Arizona State University.

EXTRACTION AND ISOLATION OF THE FLAVONOIDS.—All of the general chromatographic techniques employed were described in the previous report on the flavonoids of *H. rengifoanus* (2). Powdered leaves of *H. scrobiculatus* (Nos. 1337, and 1338, 250 g combined; No. 1351, 400 g) and *H. sericeus* (237 g) were separately extracted in a Soxhlet sequentially with petroleum ether (bp 30–60°), chloroform, ethyl acetate, and ethyl alcohol. The flavonoids from *H. scrobiculatus* were found in the ethyl acetate and alcohol extracts, while in *H. sericeus* they were found only in the alcohol extract. Since two-dimensional paper chromatography showed the same flavonoids in the ethyl acetate extracts of each of the collections of *H. scrobiculatus*, they were combined. The combined concentrate (10 g) was chromatographed over a Polyclar column (4 x 50 cm). The column was eluted with Egger's solvent chloroform-methanol-methyl ethyl ketone-acetone (4:2:0.5:0.1); the polarity of the eluate was increased by reducing the percentage of chloroform. All of the compounds from the Polyclar column were cleaned over Sephadex LH-20. The Polyclar column yielded the following compounds: 6-methoxyluteolin 4'-methyl ether (5 mg), 6-methoxyluteolin 7-methyl ether (4 mg), 6-methoxyluteolin (3 mg), and kaempferol 7-methyl ether. The combined alcohol concentrate (10 g) of *H. scrobiculatus* was chromatographed over a Polyclar column (5 x 50 cm). The elution was initiated with

[1]Spectra were recorded with the following instruments: uv Varian Techtron model 635; pmr Varian 90 MHz; ms DuPont 21-491 and AEI 902, direct inlet at 70 eV. Adsorbents for the tlc and cc were from E. Merck, Macharey-Nagel and GAF Corp; Sephadex LH-20 was from Pharmacia.

ethanol, and the polarity was increased by the gradual addition of water up to 100%. The following compounds were obtained: isorhamnetin (6 mg), isorhamnetin 3-β-D-glucoside (5 mg), quercetin (5 mg), esculetin (5 mg), isovitexin (8 mg), vitexin (2 mg), and a mixture (8 mg) of vicenin-2 and a 6,8-di-C-glucosyl compound with a luteolin skeleton (these two compounds were separated on prep tlc plates after permethylation of the mixture). Next, 7 mg of isoschaftoside (6-C-arabinosyl-8-C-glucosylapigenin) with traces of vicenin-2 were obtained. These two compounds were also separated on prep tlc plates after permethylation. Finally, the column yielded quercetin 3-β-D-glucoside (4 mg).

The ethanol extract (yield 7 g of concentrate) of *Haplopappus sericeus* was also chromatographed on a Polyclar column. The column was eluted with ethyl alcohol with gradually increasing percentage of water up to 100%. Quercetin (7 mg), quercetin 3-β-D-glucoside (12 mg), vicenin-2 (4 mg), isovitexin (3 mg) and vitexin (3 mg) were obtained.

QUERCETIN, ISORHAMNETIN, KAEMPFEROL 7-METHYL ETHER, 6-METHOXYLUTEOLIN, 6-METHOXYLUTEOLIN 4'-METHYL ETHER, 6-METHOXYLUTEOLIN 7-METHYL ETHER.—Uv, ms, and tlc comparisons with standard samples established the structures of these compounds.

QUERCETIN 3-β-D-GLUCOSIDE, ISORHAMNETIN 3-β-D-GLUCOSIDE.—Hydrolysis of these compounds with acid (0.1 N TFA) and β-glucosidase yielded the expected aglycones, quercetin and isorhammetin (uv and tlc comparison) and glucose (tlc comparison). Uv spectral data and colors under uv light (366 nm) before and after hydrolysis, tlc comparison with authentic samples, and uv spectral data established the structures of these two glucosides.

ISOVITEXIN, VITEXIN.—Uv, ms and standard sample comparison were employed to establish the structures of these compounds.

VICENIN-2 AND AN UNIDENTIFIED 6,8-DI-C-GLUCOSYLFLAVONE WITH A LUTEOLIN SKELETON.—The uv spectral shifts of this fraction indicated that the major compound (by far) must have an apigenin skeleton. It gave the following spectral data: uv λ max (MeOH): 332, 272; + NaOMe 399 (higher int.), 330, 283; + $AlCl_3$ and $AlCl_3$/HCl 385 (sh), 353, 307, 262 (sh); + NaOAc 368, 296 (sh), 282; + NaOAc/H_3BO_3 415 (sh), 340 (sh), 321, 278. Although the components of this mixture could not be separated by chromatography on Polyclar, permethylation and prep tlc (Si gel) developed with chloroform-ethyl acetate-acetone (5:4:1) gave two major compounds (R_f 0.22 and 0.33). The ms of the latter was that of a permethyl 6,8-di-C-hexosylapigenin (7) and showed the same R_f as PM 6,8-di-C-glucosylapigenin; ms *m/z* (rel int.): M^+748 (9), 733 (29), 717 (100), 645 (11), 585 (29), 573 (48). The lower band showed the same R_f as PM 6,8-di-C-glucosylluteolin, and the ms confirmed the PM 6,8-di-C-hexosylluteolin structure (7); ms *m/z* (rel int.) M^+778 (13), 763 (25), 747 (100), 675 (15), 615 (28), 603 (42). Therefore, this latter natural product must be based on a luteolin or luteolin methyl ether skeleton.

ISOSCHAFTOSIDE.—The uv spectrum was identical to that of agipenin. The light yellow color with diazotized benzidine reagent, as well as the R_f value in 5% acetic acid (0.43), suggested a 6,8-di-C-glycosylapigenin. Tlc of the PM derivative with standard PM isoschaftoside indicated that the natural product was isoschaftoside; the tlc also showed PM vicenin-2 to be present as a minor constituent.

ESCULETIN.—The uv and ir spectra as well as standard sample comparison proved its structure.

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