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Flavonoids from *Baccharis halimifolia, Monarda didyma,* and *Gnaphalium dioicum*

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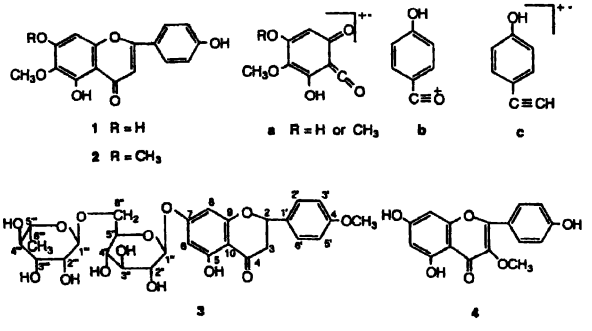
Baccharis halimifolia L. (Compositae) (Sea-myrtle, Silverling) is abundantly found in the coastal areas of Georgia. A decoction of the leaves is reputed in the folklore to possess antirheumatic properties[1]. Earlier investigations on the roots of *B. halimifolia* report the isolation of the triterpene baccharis oxide[2] and some furoclerodane diterpenes[3]. No work appears to have been carried out on the leaves of this plant. In an attempt to isolate pure compounds, using activity-directed fractionation, we have investigated the leaves of *B halimifolia*, and the whole plants *Monarda didyma* L and *Gnaphalim dioicum.* From the 95% EtOH extract of the leaves of *B. halimifolia*, by chromatographic separation, the triterpene oleanolic acid[4] and the flavonoids, hispidulin (dinatin, 5,7,4′-trihydroxy-6-methoxyflavone)[5,6] (**1**) and cirsimaritin (scrophulein, 5,4′-dihydroxy-6,7-dimethoxyflavone)[6,7] (**2**) have been isolated. The structures of these compounds were established by elemental analyses and spectral data, as well as by comparison with authentic samples. The ^{13}C nmr assignments of oleanolic acid have been made by comparison of the values given for methyl oleanolate[8]. The ^{13}C nmr spectral assignments for the two flavonoids have been made by comparison of the values of closely related flavones[9]. Hispidulin **1** has been isolated from *Ambrosia hispida*, *Iva frutiscens*, *Digitalis lanata* and many other plant species[10,11] and cirsimaritin from *Teucrium pelium*, *Cirsium brevistylum*, *Digitalis thapsi* and many other species[10,11].



Monarda didyma L. (Labiatae) is a small minty herb growing on the wooded stream banks of Georgia and has been used in the folk medicine by the Indians for the treatment of colds. From 90% EtOH extract of the whole plant, a crystalline compound was isolated. On the basis of elemental analysis , uv-spectra, physical constants, and comparison with an authentic sample, this flavanone was identified as didymin (isosakuranetin-7-*O*-rutinoside) (**3**). The structure of this flavanoneglycoside was established earlier by degradative and synthetic studies[12,13]. Didymin has been isolated from some citrus species[14]. We have determined the ^{13}C nmr spectrum of **3**, and have assigned the chemical shifts for the carbons by comparison of the values given for rutin and hesperidin[9].

Gnaphalium dioicum (compositae) is found in Eastern Europe. From the 95% EtOH extract, we have isolated a flavonol and on the basis of the elemental analysis, uv, mass spectra, and comparison with an authentic sample, which was identified as isokaemferide (kaemferol-3-*O*-methylether) (**4**). The flavonol **4** has been isolated from *Begonia manicata*, *Centaurea arguta* and many other plant species[10,11].

Experimental

The leaves of *B. halimifolia* L. and *Monarda didyma* were collected around the Milledegeville area (Baldwin county, Georgia) in April 1987; a herbarium specimen was compared with the specimen in the University of Georgia herbarium in the Botany Department. *Gnaphalium dioicum* (Life everlasting) whole plant, was purchased from Starwest Botanicals, Inc., California. Spectra : ir Perkin-Elmer 1420; uv Cary-219; ^1H nmr Perkin-Elmer EM-390 (90 MHz); ^{13}C nmr JEOL FT FX-60; MS Finnegan 4023 GC/MS system (70 eV).

Isolation of hispidulin and cirsimaritin : Dried and powdered leaves of *B. halimifolia* (500 g) were extracted in a Soxhlet apparatus with hexane. Evaporation of the solvent gave 10 g of fatty material. The plant was then extracted with 95% EtOH and evaporation of the extract under reduced pressure furnished 53 g of residue. This residue was partitioned between $CHCl_3$ and water, when an insoluble material (27 g) which was soluble in MeOH was obtained. The $CHCl_3$-soluble part on evaporation gave a residue (18 g). Part of the residue (10 g) was chromatographed on silica gel (PF 254 + 265) by vacuum liquid chromatography (VLC)[15] and 100 ml fractions were collected by elution with hexane containing increasing amounts of EtOAc. The Fr. (6-9) (hexane-30% EtOAc) gave 0.29 g which was further chromatographed and crystallized from EtOH to afford colorless needles, m.p. 299–300°, $[\alpha]_D$ + 75° ($CHCl_3$); *m/z* 456 (M^+). The TLC, m.m.p. and ir spectra were identical

with those of an authentic sample of oleanolic acid; ^{13}C nmr ($CDCl_3$ + CD_3COCD_3) δ 38.3 (C-1), 27.3 (C-2), 78.1 (C-3), 38.9 (C-4), 54.9 (C-5), 17.9 (C-6), 32.4 (C-7), 40.8 (C-8), 47.3 (C-9), 36.6 (C-10), 23.1 (C-11), 122.0 (C-12), 143.5 (C-13), 41.3 (C-14), 27.7 (C-15), 25.3 (C-16), 45.9 (C-17), 40.9 (C-18), 45.6 (C-19), 30.2 (C-20), 33.5 (C-21), 32.5 (C-22), 27.7 (C-23), 15.2 (C-24), 14.8 (C-25), 16.6 (C-26), 26.8 (C-27), 179.6 (C-28), 33.3 (C-29), 22.6 (C-30).

The Fr. (9-12) (hexane-50% EtOAc) (2.5 g) was chromatographed over MN polyamide CC 6 column (15 g) and eluted initially with toluene and then with $CHCl_3$ containing an increasing percent of MeOH. The fraction eluted with $CHCl_3$-70% MeOH gave a mixture (370 mg) which when crystallized fron EtOH afforded hispidulin **1** as pale yellow needles, m.p. 290–91° (lit.[6] 290°) (Found : C, 63.70; H, 3.95. $C_{16}H_{12}O_6$ (MW : 300) calcd. for : C, 64.00; H, 4.00%); *m/z* 300 (M^+; 40%), 285 $[M-CH_3]^+$ (25), 257 $[M-43]^+$ (45), 167 [**a**-15]$^+$ (20), 139 [**a**-43]$^+$ (22), 118 (**c**, 25); ν_{max} (nujol) 3280, 1660, 1640, 1600, 1540, 1460, 1370, 1300, 1235, 1090, 1032, 1010, 990 cm^{-1}; λ_{max} (MeOH) 335, 275; (MeOH + NaOMe) 392, 326, 275; (MeOH + $AlCl_3$) 360, 302, 278; (MeOH + $AlCl_3$ + HCl) 356, 300, 278; (MeOH + NaOAc) 382, 302, 274 nm; ^{13}C nmr (DMSO-d_6) δ 163.9 (C-2), 102.4 (C-3), 182.1 (C-4), 152.4 (C-5), 129.2 (C-6), 157.2 (C-7), 94.3 (C-8), 152.7 (C-9), 103.1 (C-10), 121.2 (C-1′), 128.4 (C-2′, C-6′), 115.9 (C-3′, C-5′), 161.1 (C-4′), 60.0 (OCH_3). The TLC, m.m.p. and ir spectra were identical with those of an authentic sample of hispidulin.

The mother liquor obtained after isolation of hispidulin was purified by centrifugally accelerated thin layer chromatography (Chromatotron)[16] on a silica gel rotor and eluted with hexane-$CHCl_3$. This afforded after crystallization from EtOH, cirsimaritin (**2**) as straw-coloured plates, m.p. 258–59° (Found : C, 64.42; H, 4.59. $C_{17}H_{14}O_6$ (MW 314) calcd. for : C, 64.96; H, 4.46%); *m/z* 314 (M^+, 50%), 313 $[M-1]^+$ (20), 299 $[M-CH_3]^+$ (50), 185 $[M-CO]^+$ (20), 271 $[M-43]^+$ (30), 181 [**a**-15]$^+$ (25), 153 [**a**-43]$^+$ (50), 121 [**b**]; λ_{max} (MeOH) 335, 275; (MeOH + NaOMe) 380, 290, 268; (MeOH + $AlCl_3$) 360, 300, 284, 262; (MeOH + $AlCl_3$ + HCl) 356, 298, 285, 260; (MeOH + NaOAc) 382, 332, 273 nm; ^{13}C nmr (DMSO-d_6) δ 164.1 (C-2), 102.7 (C-3), 182.2 (C-4), 152.6 (C-5), 132.0 (C-6), 158.6 (C-7), 91.5 (C-8), 152.1 (C-9), 105.1 (C-10), 121.1 (C-1′), 128.4 (C-2′, C-6′), 116.0 (C-3′, C-5′), 161.3 (C-4′), 60.0 (C-6 OCH_3), 56.4 (C-7 OCH_3). The TLC, m.m.p. and ir spectra were identical with those of an authentic sample of cirsimaritin.

Isolation of didymin : The powdered whole plant of *M. didyma* (125 g) was extracted in a Soxhlet apparatus with hexane and then 90% EtOH (1.5 l). Evaporation of the EtOH extract (*in vacuo*) to 100 ml and leaving at room temperature for two days gave a solid (3.5 g). Part of this (1.6 g) was crystallized from EtOH (8 ml) and H_2O (70 ml) to afford didymin as colourless crystals (800 mg), m.p. 199–200° (Found : C, 54.94; H, 5.94. $C_{28}H_{34}O_{14}.H_2O$ calcd. for : C, 54.90; H, 5.88%); $[\alpha]_D$-110° (*c* 0.5, MeOH); λ_{max} (MeOH) 210, 225, 284 nm; ^1H nmr (DMSO-d_6) δ2.12 (3H, d, *J* 6 Hz), 4.79 (3H, s), 7.18 (2H, s), 8.00 (2H, d, *J* 9 Hz), 8.50 (2H, d, *J* 9 Hz); ^{13}C nmr (DMSO-d_6) δ 78.4 (C-2), 42.3 (C-3), 197.1 (C-4), 163.1 (C-5), 96.7 (C-6), 165.2 (C-7), 95.6 (C-8), 162.6 (C-9), 103.4 (C-10), 130.4 (C-1′), 128.5 (C-2′), 114.0 (C-3′), 159.6 (C-4′), 159.6 (C-5′), 128.5 (C-6′), 99.6 (C-1″), 73.0 (C-2″), 76.4 (C-3″), 70.8 (C-4″), 75.8 (C-5″), 66.1 (C-6″), 100.6 (C-1‴), 70.4 (C-2‴), 69.7 (C-3‴), 72.3 (C-4‴), 68.4 (C-5‴), 17.9 (C-6‴), 55.2 (OCH_3).

Isolation of isokaemferide : Ground *G. dioicum* (whole plant, 105 g), was extracted with hexane and then with 95% EtOH (800 ml) in a Soxhlet apparatus. Evaporation of the EtOH extract gave a residue (10.3 g) which was chromatographed by VLC twice on a SiO_2 column and eluted with $CHCl_3$: EtOAc (3 : 2). This afforded isokaemferide (50 mg), identical in all respects with an authentic sample.

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