Journal of Natural Products
Vol. 58, No. 8, pp. 1248-1250, August 1995

PHENOLICS OF *PICNOMON ACARNA*

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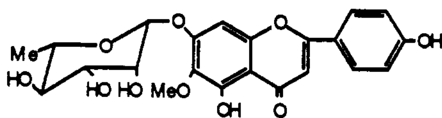
ABSTRACT.—A new flavonoid glycoside, 5,4′-dihydroxy-6-methoxyflavone-7-*O*-α-L-rhamnopyranoside [**1**], together with pectolinarin, linarin, gentisic acid 5-*O*-glucopyranoside, homoplantagin, hyperin, hispidulin, luteolin, quercetin, and 6,7,8-trimethoxycoumarin, was isolated from the aerial parts of *Picnomon acarna*.

Picnomon acarna (L.) Cass. (Asteraceae, tribe Cardueae) is an annual plant that grows in arid areas of the Mediterranean region (1) and has been used as an hemostatic and spasmolytic plant in Greek folk medicine (2). As the result of a phytochemical investigation of *P. acarna*, we report here the isolation and structural elucidation of a novel flavone glycoside, 5,4′-dihydroxy-6-methoxyflavone-7-*O*-α-L-rhamnopyranoside [**1**], along with seven known flavonoids (hispidulin, luteolin, quercetin, pectolinarin, linarin, homoplantagin, hyperin), a phenolic glycoside (gentisic acid 5-*O*-glucoside), and a coumarin (6,7,8-trimethoxycoumarin).

Flavonoid **1** was isolated from nature for the first time by polyamide cc of an *n*-BuOH extract. On acid hydrolysis, it afforded rhamnose and an aglycone. The attachment position of the sugar moiety was determined by comparison of the uv spectra of the aglycone with those of the glycoside, measured in MeOH and after addition of the usual shift reagents (3), and the glycosidation site was found to be at C-7 of the aglycone. Furthermore, uv data gave evidence of an apigenin-like skeleton with a C-6 substituent (3). The eims of the aglycone exhibited a molecular ion peak (100%) at *m/z* 300 in accordance with a flavone containing three hydroxyl groups and one methoxyl group ($C_{16}H_{12}O_6$). Fragments at *m/z* 285 (67%), 282 (18%), 257 (27%), 167 (30%), and 118 (16%) corresponded to $[M-CH_3]^+$, $[M-H_2O]^+$, $[M-CO-CH_3]^+$, $[A_1-CH_3]^+$ and $[B_1]^+$ ions, respectively (4), and tentatively identified the aglycone as 5,7,4′-trihydroxy-6-methoxyflavone (hispidulin).



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A careful study of the ^{1}H-nmr spectrum of **1** showed the presence of an AA′XX′-system in the B-ring, represented by two, two-proton doublets (J=8.4 Hz) at δ 7.90 and 6.88 ppm corresponding to the H-2′,H-6′, and H-3′,H-5′ protons, respectively. Two singlets (1H each) at δ 6.96 and 6.81 arose from the H-8 and H-3 protons. The three-proton singlet at δ 3.76 was assigned to an aromatic methoxyl group, located at C-6 as indicated by the uv spectra and by the eims. In the latter we observed the lower intensity (67%) of the $[M-15]^+$ fragment at *m/z* 285 compared with the $[M]^+$ (100%) at *m/z* 300, as well as the intensity of the $[M-H_2O]^+$ peak (18%>10%) at *m/z* 282 (5). In addition, a broad singlet at δ 5.41 and a 3H doublet (J=6.6 Hz) at δ 0.85 were assigned to the anomeric proton and the methyl group of the α-L-rhamnosyl moiety, respectively (6,7), proving the presence of a 7-*O*-rhamnoside. The α-L-rhamnosyl linkage was confirmed by a successful naringinase-catalyzed hydrolysis coupled with an unsuccessful hydrolysis with β-glucosidase (8). On the basis of the above results, glycoside **1** was identified as 5,4′-dihydroxy-6-methoxyflavone-7-*O*-α-L-

rhamnopyranoside (hispidulin-7-*O*-α-L-rhamnopyranoside), which is a novel natural product.

In spite of the exhaustive isolation procedure, gossypetin-8,3′,4′-trimethyl ether (3,5-dihydroxytrimethoxyflavone), luteolin-7,3′-dimethyl ether, and luteolin-3′-methyl ether, reported previously by Souleles and Laskaris (9), were not found. An examination of the original extracts failed to confirm their presence. Further, a re-examination of the original spectral data showed that they do not correspond to the structures previously reported (9). Thus, the previous report on the occurrence of these three flavonoids was in error.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The ^{1}H-nmr spectrum of **1** was obtained on a JEOL JNM-GX 270 MHz instrument using DMSO-d_6 as solvent while the ^{1}H-nmr spectrum of 6,7,8-trimethoxycoumarin was measured on a Bruker AC-300 instrument running at 300 MHz, using $CDCl_3$ as solvent and TMS as internal standard. The ^{1}H-nmr spectra of all other isolates were measured on a Bruker AC-200 instrument (200 MHz) using DMSO-d_6 as solvent. Eims were obtained on a VG TS-250 instrument (6,7,8-trimethoxycoumarin) and on a VG Trio-2000 (all other isolates), both at 70 eV. Uv spectra were measured on a Hitachi U-2000 spectrophotometer.

Fractions were collected by monitoring with uv light. Tlc was carried out on cellulose plates (Merck 5552) in (a) AcOH (15%), (b) *n*-BuOH-AcOH-H_2O (4:1:5), and (c) $CHCl_3$-AcOH-H_2O (10:9:1) and on silica F_{254} plates (Merck 5554) in $CHCl_3$/MeOH (various percentages) and Me_2CO-H_2O (9:1). Flavonoids were visualized by uv light and by spraying with NA (Naturstoffreagenz-A) in MeOH. Sugars resulting from acid hydrolysis were separated on precoated silica plates that were dipped in 0.3 M NaH_2PO_4 solution, using Me_2CO-H_2O (9:1) as mobile phase (double development). They were visualized by spraying with 4-aminohippuric acid reagent after heating at 110° for 10 min and reheating under the same conditions (10). Sugars were identified by chromatographic comparison against authentic markers. Enzymatic and acid hydrolyses were carried out according to standard procedures (3).

PLANT MATERIAL.—*Picnomon acarna* was collected in May 1985, on Hortiatis Mountain in the Thessaloniki region. The plant was identified by Dr. G. Pavlidis, Department of Botany, Aristotle University of Thessaloniki. A herbarium specimen (G.L.-1) is held at the Laboratory of Pharmacognosy, Aristotle University of Thessaloniki.

EXTRACTION AND ISOLATION.—The air-dried and finely ground (500 g) aerial parts of the plant were extracted in a Soxhlet apparatus successively with petroleum ether, C_6H_6, $CHCl_3$, and MeOH. The last extract (10% of dry wt) was dissolved in boiling H_2O and partitioned successively between Et_2O, EtOAc, and *n*-BuOH. The Et_2O extract was chromatographed on a polyamide (Macherey Nagel SC-6) column, packed with C_6H_6 and eluted with increasing volumes of methyl ethyl ketone and MeOH. The first fractions yielded by the column contained 6,7,8-trimethoxycoumarin (5 mg), followed by hispidulin (30 mg), luteolin (8 mg), and quercetin (1.5 mg). Compounds were further purified over Sephasorb HP Ultrafine (Pharmacia) or Sephadex LH-20 columns, with MeOH as eluent. The *n*-BuOH extract was chromatographed over a polyamide column packed with H_2O. The column was eluted with increasing volumes of MeOH and yielded pectolinarin (25 mg), linarin (24 mg), gentisic acid 5-*O*-glucoside (5.5 mg), and 5,4′-dihydroxy-6-methoxyflavone-7-*O*-α-L-rhamnopyranoside [**1**] (H_2O-MeOH, 40:60). Compounds were purified by successive chromatography over polyamide and Sephadex LH-20 columns, using mixtures of H_2O in MeOH. The final yield of **1** was 10 mg. Similar treatment of the EtOAc extract afforded homoplantagin (9 mg) and hyperin (13 mg).

All compounds were characterized by spectroscopic (^{1}H-nmr, eims, uv) and chemical methods. The 6,7,8-trimethoxycoumarin sample was identified by using the same methods and also on the basis of NOESY measurements. Full details of the isolation and identification of the known compounds are available from the senior author.

*5,4′-Dihydroxy-6-methoxyflavone-7-*O*-α-L-rhamnopyranoside* [**1**].—Obtained as an amorphous powder: uv (MeOH) λ max (log ε) 275 (3.28), 332 (3.37) nm; (MeOH/NaOMe) 274, 305, 356 sh, 379; (MeOH/NaOAc) 274, 393; (MeOH/NaOAc/H_3BO_3) 273, 336; (MeOH/$AlCl_3$) 282, 299, 360; (MeOH/$AlCl_3$/HCl) 284, 229, 354; ^{1}H nmr (DMSO-d_6, 270 MHz) δ 7.90 (2H, d, J=8.4 Hz, H-2′, H-6′), 6.96 (1H, s, H-8)[1], 6.88 (2H, d, J=8.4 Hz, H-3′, H-5′), 6.81 (1H, s, H-3)[1], 5.41 (1H, br s, H-1″ rhamnose), 3.76 (3H, s, OMe-6), 0.85 (3H, d, J=6.6 Hz, H-6″ rhamnose); eims of the aglycone (70 eV) m/z $[M]^+$ 300 (100), 285 (67), 282 (18), 257 (17), 167 (30), 118 (16); tlc (dark fluorescence at 366 nm) R_f system (a)=0.3, R_f system (b)=0.5, R_f system (c)=0.4.

[1]Values may be interchanged.

ACKNOWLEDGMENTS

We thank Prof. Dr. Robert Verpoorte, Division of Pharmacognosy, Center for Biopharmaceutical Sciences, Leiden, The Netherlands, for ^{1}H-nmr and nOe-NOESY measurements, and the Department of Organic Chemistry, Aristotle University of Thessaloniki, and the Mediterranean Agronomic Institute, Chania, Greece, for eims data.

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Received 25 October 1994