Notes

A New Flavonoid Glycoside from *Centaurea horrida*

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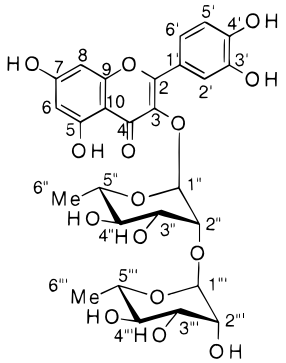
A new natural compound, horridin (**1**), was isolated from the aerial parts of *Centaurea horrida*. Its structure as quercetin 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranoside was determined by spectroscopic methods, including 2D NMR.

Centaurea horrida Bad. (Asteraceae), known in Italy as "fiordaliso spinoso", is a thorny shrub with tortuous, entangled branches. The leaves are completely transformed into pinnate thorns, 20−30 mm long, while the capitula are small (4−5 mm) and pale, with purple apexes. This very peculiar habitus is the result of an adaptive process to the arid marine habitat, and it makes the plant a unique case among the Italian Asteraceae; it is considered a species of great antiquity.[1]

C. horrida is strictly endemic to North Sardinia (Italy), where it lives only in particular separate small areas.[1,2] The plant is not used in Italian folk medicine, although other species of the same genus (i.e., *Centaurea cyanus* and *Centaurea scabiosa*) are used against coughs and as ophthalmic drugs.[3] The studies on this genus deal mainly with sesquiterpene lactones, while flavonoids are less investigated; there have been no previous reports on constituents from *C. horrida*. This paper deals with the isolation and characterization of a new flavonoid glycoside (**1**).



(**1**)

The negative FABMS of horridin (**1**) gave a peak at *m*/*z* 593, $[M - H]^-$, corresponding to the molecular formula $C_{27}H_{30}O_{15}$, supported also by elemental analysis. The ^{13}C NMR spectrum showed 27 resonances, sorted by DEPT experiments into 2 CH_3, 15 CH, and 10 quaternary C. In the ^1H NMR spectrum are two coupled doublets, at δ 6.24 and 6.29, typical of two *meta*-related H-6 and H-8 protons of ring A of a flavonoid unit. The ABM system of ring B (δ 7.31, d, J = 1.9 Hz; 7.28, dd, J = 8.2, 1.9 Hz; 6.89, d, J = 8.2 Hz) permits identification of the aglycon as quercetin. Moreover, a one-proton broad singlet was present at δ 5.32 and an analogous signal, partially overlapped by the water signal, was at δ 4.79. A third one-proton broad singlet was visible at δ 4.21. These three broad singlets were really three doublets with very small J values, as shown by the COSY experiment, which displayed cross-peaks between the resonance at δ 5.32 and that at δ 4.21, and between each of the signals at δ 4.79 and 4.21 and the not-resolved area between 3 and 4 δ. Finally, two partially overlapped three-proton doublets (J = 5.7 Hz) at δ 0.94 and 0.90 were present. This suggested the presence of two deoxysugars having an α-configuration because of the very low J values of their anomeric protons due to *trans*-diequatorial interactions. The H-2 resonance of one sugar was unusually downfield (δ 4.21), probably because of the 1→2 interglycosidic linkage of the sugar moiety. Analysis of the ^{13}C NMR spectrum confirmed the identity of the aglycon as quercetin and the two sugar units as α-L-rhamnose. Due to typical shifts experienced by the inner rhamnose (signals were displaced downfield by 6.0 ppm for C-2″ and upfield of 1.6 and 0.6 ppm for C-1″ and C-3″, respectively, from those of quercetin 3-*O*-rhamnoside[4]), it was possible to confirm the 1→2 interglycosidic linkage. The values are also in good agreement with those from analogous compounds.[5,6] Placement of the disaccharidic moiety at C-3 was determined on the basis of the typical glycosylation shifts that occurred with respect to the aglycon quercetin:[7] downfield shifts of C-2 and C-4 (about 8.5 and 3.0 ppm, respectively) and an upfield shift of C-3 (about 1.5 ppm). Therefore, **1** is quercetin 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranoside, which we have named horridin. This structure is supported by its negative FABMS spectrum where, besides the molecular peak at *m*/*z* 593, $[M - H]^-$, there were peaks due to loss of a rhamnose at *m*/*z* 447, $[M - H - 146]^-$, and of two rhamnose units at *m*/*z* 301, $[M - H - 292]^-$. Finally, acid hydrolysis of **1** liberated rhamnose and quercetin, identified by TLC with authentic samples.

Experimental Section

General Experimental Procedures. Melting points (uncorrected) were determined with a Kofler apparatus; optical rotations were measured on a Perkin-Elmer 241 polarimeter;

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FABMS were recorded (negative mode) with a VG ZAB instrument; ^{1}H and ^{13}C NMR spectra were obtained with a Bruker AC200 spectrometer in CD_3OD, DMSO-d_6, and $CDCl_3$, using TMS as internal standard. All 1D and 2D NMR experiments were performed using the standard Bruker library of microprograms.

The following adsorbents were used for purification: flash chromatography, Merck Kieselgel 60 (230−410 mesh); low-pressure chromatography, Merck Lobar Lichroprep RP_8 and RP_{18} (31 × 2.5 cm); size-exclusion chromatography, Pharmacia Fine Chemicals Sephadex LH-20; analytical TLC, Merck Kieselgel 60 F_{254} precoated plates; chromatograms were visualized under UV light at 254 and 366 nm and/or sprayed with Komarowsky or cerium sulfate or Naturstoffereagenz A-PEG reagents.

Plant Material. The flowered aerial parts of *C. horrida* were collected at Capo Falcone, Stintino, Sassari, Italy, in May 1998. A voucher specimen (no. 3214/97 URB) is deposited in the Herbarium of Urbino Botanical Garden.

Extraction and Isolation. The dried and ground aerial parts (950 g) were extracted successively in a Soxhlet apparatus with *n*-hexane, $CHCl_3$, $CHCl_3$−MeOH (9:1) (4 L × 25 h) and, at room temperature, with MeOH (2.5 L × 7 days × 3). After removal of solvents in vacuo at up to 40 °C, the following residues were obtained: R_H (12.4 g), R_C (7.1 g), R_{CM} (9.1 g), and R_M (18.2 g).

R_M was suspended in MeOH−H_2O (7:3) and extracted, in turn, with EtOAc and *n*-BuOH obtaining, after removal of the solvents, the residues R_{MAc} and R_{MBu}. The former, after size-exclusion chromatography on Sephadex LH-20 with MeOH and Si gel column chromatography eluting with $CHCl_3$−MeOH (7:3) yielded compound **1** (5 mg), horridin, which appears on TLC as an orange spot after treatment with Naturstoffereagenz A-PEG.

Horridin (1): yellowish amorphous solid; $[\alpha]^{20}_D$ −55.6° (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ϵ) 362 (4.12), 265 (sh) (3.95), 261 (4.42) nm; (MeOH + $AlCl_3$) 433 (4.88), 301 (sh) (3.44), 277 (4.94) nm; (MeOH + $AlCl_3$ + HCl) 400 (4.20), 360 (sh) (3.39), 302 (3.51), 274 (4.76) nm; ^{1}H NMR (CD_3OD, 200 MHz) δ 0.94 (3H, d, J = 5.7 Hz, H-6‴), 0.90 (3H, d, J = 5.7 Hz, H-6″), 4.21 (1H, br s, H-2″), 4.79 (1H, br s, H-1‴), 5.32 (1H, br s, H-1″), 6.24 (1H, d, J = 1.9 Hz, H-6), 6.49 (1H, d, J = 1.9 Hz, H-8), 6.89 (1H, d, J = 8.2 Hz, H-5′), 7.28 (1H, dd, J = 8.2, 1.9 Hz, H-6′), 7.31 (1H, d, J = 1.9 Hz, H-2′); ^{13}C NMR (DMSO-d_6), 50 MHz) δ 178.2 (s, C-4), 164.4 (s, C-7), 161.3 (s, C-5), 156.8 (s, C-9), 156.5 (s, C-2), 148.6 (s, C-4′), 145.0 (s, C-3′), 133.8 (s, C-3), 122.2 (s, C-1′), 122.0 (d, C-6′), 116.3 (d, C-5′), 115.7 (d, C-2′), 104.1 (s, C-10), 101.5 (d, C-1⁗), 100.3 (d, C-1″), 99.0 (d, C-6), 94.4 (d, C-8), 76.4 (d, C-2″), 71.9 (d, C-4⁗), 71.8 (d, C-4″), 70.5 (d, C-3‴), 70.4 (d, C-5″), 70.2 (d, C-2‴), 70.0 (d, C-3″), 69.6 (d, C-5⁗), 17.7 (q, C-6″), 17.4 (q, C-6‴); FABMS (negative-ion mode) *m*/*z* 593 $[M - H]^-$ (16), 447 $[M - H - 146]^-$ (7); 301 $[M - H - 292]^-$ (28); *anal.* C 54.82%, H 4.87, calcd for $C_{27}H_{30}O_{15}$, C 54.55%, H 5.09%.

References and Notes

(1) Pignatti, S. *Flora d'Italia;* Edagricole: Bologna, 1982; Vol. 3, p 183.
(2) Desole, L. *Webbia* **1956**, *12*, 251−324.
(3) Poletti, A. *Fiori e Piante Medicinali;* Musumeci Editore: Milano, 1978; Vol. 1, p 152.
(4) Markham, K. R.; Ternai, B.; Stanley, R.; Geiger, H.; Mabry, T. J. *Tetrahedron* **1978**, *34*, 1389.
(5) Li, W. K.; Zhang, R. Y.; Xiai, P. G. *Phytochemistry* **1996**, *43*, 527.
(6) Braca, A.; Bilia, A. R.; Mendez, J., Morelli, I. *Phytochemistry* **1999**, *51*, 1125.
(7) Ternai, B.; Markham, K. R. *Tetrahedron* **1976**, *32*, 565.

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