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Phytochemistry, Vol. 22, No. 10, pp. 2323–2324, 1983.
Printed in Great Britain.

0031–9422/83 $3.00 + 0.00

CHRYSIN 7-GENTIOBIOSIDE FROM THE FLOWERS OF *SPARTIUM JUNCEUM*

SALVATORE DE ROSA and SALVATORE DE STEFANO

Istituto per la Chimica M.I.B. del C.N.R., Via Toiano N.6, 80072 Arco Felice, Napoli, Italy

(*Received* 2 *December* 1982)

Key Word Index—*Spartium junceum*; Leguminosae; flavonoid glycoside; chrysin 7-gentiobioside.

Abstract—Three flavonoids were isolated from the flowers of *Spartium junceum*: the known compounds, chrysin and chrysin 7-glucoside and a new glycoside, chrysin 7-gentiobioside. All three constituents were active in the root growth bioassay.

INTRODUCTION

Spartium junceum L. is known for its diuretic, vasoconstrictor, sedative and other therapeutic properties [1]. There have been several reports [2–4] of B-ring substituted flavonoids and isoflavonoids from this plant. From the flowers of *S. junceum* we now report three unusual flavones (**1**–**3**) with no B-ring substituents.

RESULTS AND DISCUSSION

The flowers of *Spartium junceum* were extracted with acetone and, after evaporation of the solvent, the remaining water was extracted with diethyl ether and then *n*-butanol. Compound **1** was recovered from the ether extract after separation by CC, while **2** and **3** were recovered from the butanol extract.

Compound **1** (mp 288–292°, ethanol) exhibited a molecular ion at *m/z* 254 (100%) in accord with a flavone structure containing two hydroxyl groups. Its UV spectrum suggested the presence of two free hydroxyl groups at C-5 (bathochromic shifts with aluminium chloride, aluminium chloride–hydrochloric acid and sodium methoxide) and at C-7 (bathochromic shifts with sodium acetate). Compound **1** was identified as chrysin (5,7-dihydroxyflavone) from the spectral data and by comparison with an authentic sample.

Compound **2** exhibited UV maxima in methanol at 269 and 306 nm, bathochromic shifts with aluminium chloride, aluminium chloride–hydrochloric acid and sodium methoxide and the absence of a shift with sodium acetate, suggested the presence of a free hydroxyl group at C-5. The ^{1}H NMR spectrum (DMSO-d_6) showed, in addition to the aromatic signals resembling those of **1**, other signals at δ 3.2–4.2, suggesting the presence of a sugar substitution on the hydroxyl group at C-7. This hypothesis was confirmed by the ^{13}C NMR spectrum (DMSO-d_6) which showed six signals at δ 60.5, 69.4, 72.9, 76.2, 77.0 and 99.7 for the sugar moiety and by acid hydrolysis which yielded D-glucose and chrysin (**1**). The β-configuration of glucose was suggested by permethylation of **2**. In fact the ^{1}H NMR spectrum ($CDCl_3$) of the permethylated derivative showed a doublet (1H) at δ 4.95 (J = 8 Hz) attributable to the anomeric proton with a β-configuration [5]. Therefore, **2** was identified as chrysin 7-β-D-glucopyranoside.

The UV spectrum of **3** was similar to that of **2**, suggesting again the presence of a free hydroxyl group at C-5. The ^{1}H NMR and ^{13}C NMR spectra showed, in the aromatic region, similar signals to those of **2** and more signals in the sugar region suggesting that the sugar moiety linked to the hydroxyl group of chrysin at C-7, was more complex than in **2**. Partial acid hydrolysis of **3** yielded **1**, **2** and D-glucose, while complete hydrolysis yielded **1** and glucose. The β-configuration of both glycosidic linkages was evident from the presence of two doublets at δ 5.32 and 5.42 (1H, each, J = 8 Hz) in the ^{1}H NMR spectrum (DMSO-d_6) of permethylated **3**. Acid

hydrolysis of permethylated **3** yielded 2,3,4,6-tetramethylglucose and 2,3,4-trimethylglucose, which were identified by TLC comparison with authentic samples. Moreover the hydrogen peroxide oxidation of **3** yielded, β-gentiobiose. From these data, **3** was identified as the new 7-[6-(β-D-glucopyranosyl)-β-D-glucopyranosyl] derivative of chrysin (chrysin 7-gentiobioside).

Compounds **1–3** were tested in the *Hordeum vulgaris* root growth bioassay [6]. Water solutions (100 ppm) of **1–3** showed an increase of root growth, of 20% for **1**; 30% for **2** and 40% for **3**. Recently, Nicollier *et al.* [7] observed an activity of chrysin on radish root, whose growth was reduced by 20% at 100 ppm.

EXPERIMENTAL

Plant material and general methods. Flowers of *Spartium junceum* L. were collected near Naples (Pozzuoli) in June. Plant material was verified by comparison with a voucher specimen deposited in the herbarium (NAP) of the Istituto Botanico, Facoltà di Scienze, University of Naples. Mps were uncorr. MS were recorded at 70 eV. Sephadex LH-20 (Pharmacia) and Kieselgel 60 (Merck) were used for chromatography. Precoated Si gel 60 F_{254} (Merck) was used for prep. TLC and TLC in: (1) $CHCl_3$–MeOH (19:1); (2) Me_2CO–*n*-BuOH–H_2O (17:2:1); (3) Me_2CO–*n*-BuOH–H_2O (8:1:1); (4) *n*-BuOH–pyridine–H_2O (8:2:1); (5) Me_2CO–MeOH–H_2O (5:4:1); (6) CH_2Cl_2–EtOH (19:1); (7) $CHCl_3$–MeOH (9:1); and (8) *n*-BuOH–EtOH–H_2O (5:1:4, upper phase). Compounds were detected in UV light and by spraying with echtrotsalz B (Fluka). Sugars and sugar derivatives were detected with aniline phosphate. Commercial *Hordeum vulgaris* seeds were used for bioassay.

Extraction and isolation. The flowers (260 g dry wt) of *S. junceum* were extracted with Me_2CO (× 3), the extract was concd *in vacuo* and the H_2O residue extracted, with Et_2O and then *n*-BuOH, giving on concn a dark brown oil (5.9 g) and an amorphous solid (9.5 g) respectively. The Et_2O extract, chromatographed on a Si gel column eluted with $CHCl_3$–MeOH (49:1), gave **1** (340 mg) with R_f 0.5 on TLC in solvent 1. The *n*-BuOH extract (0.95 g) was triturated with MeOH–H_2O (4:1), filtered and chromatographed on Sephadex LH-20 (2.5 × 100 cm) column using MeOH–H_2O (4:1) as eluent. Fractions (10 ml) were collected at 150 ml/hr and investigated by TLC with solvent 2. Compound **3** (70 mg) appeared in fractions 15–22, and **2** (180 mg) in fractions 28–35, with R_fs on TLC in solvent 2 of 0.15 and 0.80 respectively.

Chrysin 7-glucoside (**2**). Mp 213–216° (EtOH aq.). UV λ_{max} nm: MeOH 269, 306(i); NaOMe 278, 375; $AlCl_3$ 282, 325, 384; $AlCl_3$–HCl 282, 325, 384. ^{1}H NMR (DMSO-d_6): δ 3.20–4.20 (*ca* 7 H, *m*, H-Glc), 6.52 (1H, *d*, *J* = 2 Hz, H-6), 6.95 (1H, *d*, *J* = 2 Hz, H-8), 7.05 (1H, *s*, H-3), 7.70 (3H, *m*, H-3′, H-4′ and H-5′) 8.10 (2H, *m*, H-2′ and H-6′). ^{13}C NMR (DMSO-d_6): δ 181.8 (*s*, C-4) 163.4 (*s*, C-5), 162.9 (*s*, C-2), 160.8 (*s*, C-7), 156.8 (*s*, C-9), 131.9 (*d*, C-4′), 130.3 (*s*, C-1′), 128.8 (2*d*, C-3′ and C-5′), 126.2 (2*d*, C-2′ and C-6′), 105.2 (*d* + *s*, C-3 and C-10), 99.8 (*d*, C-1″), 99.5 (*d*, C-6), 94.8 (*d*, C-8), 77.0 (*d*, C-3″), 76.2 (*d*, C-5″), 72.9 (*d*, C-2″), 69.4 (*d*, C-4″), 60.5 (*t*, C-6″).

Chrysin 7-gentiobioside (**3**). Mp 185–188° (EtOH). UV λ_{max} nm: MeOH 270, 306(i); NaOMe 277, 370; $AlCl_3$ 283, 327, 386; $AlCl_3$–HCl 283, 327, 386. ^{1}H NMR (DMSO-d_6): δ 3.20–4.20 (*ca* 14H, *m*, H-Glc), 6.50 (1H, *d*, *J* = 2 Hz, H-6), 6.92 (1H, *d*, *J* = 2 Hz, H-8), 7.05 (1H, *s*, H-3), 7.65 (3H, *m*, H-3′, H-4′ and H-5′), 8.06 (2H, *m*, H-2′ and H-6′). ^{13}C NMR (DMSO-d_6): δ 182.0 (*s*, C-4), 169.7 (*s*, C-5), 162.9 (*s*, C-2), 162.7 (*s*, C-7), 157.3 (*s*, C-9), 131.5 (*d*, C-4′), 130.4 (*s*, C-1′), 128.7 (2*d*, C-3′ and C-5′), 125.9 (2*d*, C-2′ and C-6′), 115.9 (*d*, C-8), 105.4 (*d* + *s*, C-3 and C-10), 100.0 (*d*), 99.8 (*d*), 99.6 (*d*, C-6), 76.9 (*d*), 76.3 (*d*), 76.1 (*d*), 73.7 (*d*), 72.9 (2*d*), 69.5 (2*d*), 63.1 (*t*, C-6″), 60.5 (*t*, C-6‴).

Hydrolysis of **2** *and* **3**. Compounds **2** (15 mg) and **3** (10 mg) were refluxed with 2 N HCl (3 ml) for 2 hr. The reaction mixtures were evaporated *in vacuo* and analysed on TLC with solvents 1, 3 and 4. Both mixtures gave **1** and glucose.

Partial hydrolysis of **3**. Compound **3** (10 mg) was refluxed with 1 N HCl (3 ml) for 30 min; the concd mixture was analysed on TLC with solvents 1–4 giving **1**, **2** and glucose.

H_2O_2 *oxidation of* **3**. A soln of **3** (10 mg) in 36% H_2O_2 (10 ml) was kept at room temp. for 1 week. In order to decompose the excess of H_2O_2, 5% Pd–C was added; after filtration of catalyst and concn *in vacuo* of filtrate, β-gentiobiose was recovered and identified on TLC with solvents 3 and 5, by comparison with an authentic sample.

Permethylation. Permethylation of **2** (30 mg) and **3** (30 mg) was carried out with MeI and Ag_2O in DMF. The products were purified on prep. TLC with solvent 1 to give permethylated **2** (24 mg) and permethylated **3** (26 mg).

Permethylated **2**. Mp 187–190° (MeOH). ^{1}H NMR ($CDCl_3$): δ 3.40 (3H, *s*, OMe), 3.57 (3H, *s*, OMe), 3.67 (6H, *s*, OMe), 3.96 (3H, *s*, OMe), 4.95 (1H, *d*, *J* = 8 Hz, H-1″), 6.53 (1H, *d*, *J* = 2 Hz), 6.68 (1H, *s*), 6.73 (1H, *d*, *J* = 2 Hz), 7.50 (3H, *m*), 7.86 (2H, *m*).

Permethylated **3**. Mp 173–178° (MeOH), ^{1}H NMR (DMSO-d_6): δ 3.38 (9H, *s*, OMe), 3.46 (3H, *s*, OMe), 3.56 (3H, *s*, OMe), 3.57 (6H, *s*, OMe), 3.86 (3H, *s*, OMe), 5.32 (1H, *d*, *J* = 8 Hz, H-1‴), 5.42 (1H, *d*, *J* = 8 Hz, H-1″), 6.59 (1H, *d*, *J* = 2 Hz), 6.80 (1H, *s*), 6.94 (1H, *d*, *J* = 2 Hz), 7.59 (3H, *m*), 8.04 (2H, *m*).

Hydrolysis of permethylated **2** *and permethylated* **3**. The hydrolyses were performed as for **2** and **3**. The sugar derivatives were identified on TLC in solvents 6–8 by comparison with authentic samples.

Bioassay. The root growth bioassay technique was essentially that of ref. [6] in which samples were dissolved in 0.5 ml EtOH or MeOH and diluted with H_2O at 100 ppm. The soln was poured into 10 Petri dishes, with Whatman grade 113 paper as support and 10 *H. vulgaris* seeds placed on each plate. After 4 days at 20° in the dark, the length of the main root was measured and compared with that of control plants.

Acknowledgements—We thank Mr. A. De Giulio for skilful assistance during the experimental work, Mr. C. Di Pinto for the ^{13}C NMR spectra and Centro Metodologie Chimico-Fisiche, Università di Napoli, for the ^{1}H NMR spectra. We are also grateful to Mrs. M. R. Vaccaro for typing the manuscript.

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