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ISOSCOPARIN-2″-*O*-GLUCOSIDE FROM *PASSIFLORA INCARNATA*

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Key Word Index—*Passiflora incarnata*; Passifloraceae; *C*-glycosylflavone; isoscoparin 2″-*O*-glucoside.

Abstract—In an investigation of herbal material of *Passiflora incarnata* the flavone *C*-glycoside, isoscoparin-2″-*O*-glucoside, was detected and isolated together with eleven known flavonoids. This glycoside was found for the first time in Passifloraceae.

INTRODUCTION

Passiflora incarnata L. (Passifloraceae) is widely used as a sedative. The determination of the flavonoid composition is useful for standardization of the crude drug as well as of preparations.

In HPLC investigations of Hb. Passiflorae of different origins a compound (**1**) not found previously in this drug was detected along with the known flavone-*C*-glycosides; vicenin-2, lucenin-2, isoorientin 2″-*O*-glucoside, schaftoside, isoschaftoside, iso-orientin, orientin, isovitexin 2″-*O*-glucoside, vitexin, isovitexin and swertisin. The substance occurred in 15 out of 18 commercial samples, ranging from 0.3 to 2.85% of the total flavonoid content [1]. This paper presents the isolation and structural elucidation of the *C*-glycoside (**1**) from *P. incarnata* by use of UV-VIS, mass and NMR spectroscopy.

RESULTS AND DISCUSSION

From a methanolic extract of the herb **1** was isolated by CC on Polyamide and Sephadex-LH 20, with further purification by PC and HPLC. In the HPLC-system previously described, **1** eluted between isovitexin 2″-*O*-glucoside and vitexin [1]. Its R_f on paper suggested that **1** was a flavonoid diglycoside, which appeared purple under UV (366 nm) and yellow when exposed to NH_3–UV and after detection with Naturstoffreagens A. Acid hydrolysis yielded glucose and a mixture of two isomeric *C*-glycosylflavones (Wessely–Moser rearrangement [2]). Diagnostic UV-VIS shifts showed the presence of free 5-, 7- and 4′-hydroxyl groups and the absence of a free 3′,4′-dihydroxy group: A bathochromic shift of 5 nm of band II with NaOAc and a minor change of band I with H_3BO_3–NaOAc indicated the presence of a free 7-hydroxyl and the absence of an *ortho*-dihydroxy group, respectively. The $AlCl_3$ spectrum showed the presence of a free 5-hydroxyl group. With NaOH, the increase in intensity of band I along with a pronounced bathochromic shift to 411 nm, indicated a free 4′-hydroxyl group. These shifts correlate with those described for isoscoparin 2″-*O*-glucoside [3]. From the high resolution mass spectrum (HRMS), the molecular formula of **1** was concluded to be $C_{28}H_{32}O_{16}$ (see Experimental). The NI-DCI mass spectrometry data gave, in addition to radical NI, a molecular ion at *m/z* 624, and fragment ions at *m/z* 504 [M–120], *m/z* 534 [M–90), *m/z* 462 [M–162] and *m/z* 342 [M–120–162]. The base peak at *m/z* 504 and the fragment ion at *m/z* 534 indicated the presence of a *C*-hexose. The [M − 162] ion gave evidence for an *O*-linked second hexose (glucose) and the fragment ion at *m/z* 300 [M − 162 − 162] indicated the presence of chrysoeriol as result of the elimination of two hexose units. The ^{1}H NMR spectrum in MeOH-d_4 showed two singlets due to one proton each at δ 6.62 and 6.45 for H-3 and H-8, respectively, and pointed to the absence of a proton at position 6 of the A-ring [4]. In isovitexin 2″-*O*-glucoside and isoorientin 2″-*O*-glucoside the H-8 proton appears at δ 6.42 and 6.45, respectively [5, 6].

According to the substitution pattern of ring B, the doublet for H-2′ (δ = 7.48) occurred with a small coupling constant of 2.2 Hz, while the one for H-5′ (δ = 6.93) had a large coupling constant of 8.2 Hz and the proton H-6′ (δ = 7.51) occurred as a double doublet. Furthermore, the signal of a methoxyl group was observed as a singlet at δ 3.96.

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In the range of δ = 4.30–5.00 three signals occurred, two of which (δ = 4.94, *d*, H-1″ and δ = 4.38, *d*, H-1‴) were attributed to the anomeric protons. The coupling constants of 8.5–10 Hz indicated the β-configuration of the glycosidic linkage. The proton H-2 of the *C*-glucosyl moiety occurred as a broad singlet at δ 4.55 and showed a downfield shift due to its linkage to the *O*-glucosyl moiety. Eleven signals for the protons H-2 to H-6 of the sugar residues were observed in the range of δ 2.90–3.90. A complete assignment of the proton resonances was performed by the ^{1}H,^{1}H-COSY spectrum. Comparison with data obtained from isovitexin 2″-*O*-glucoside confirmed the structure [5, 6]. The NMR data (DMSO-d_6) correlated with that of isoscoparin [7]. The results of these investigations confirmed the structure of **1** as isoscoparin 2″-*O*-glucoside, which was first reported in *Oryza sativa* [3] and is reported from *P. incarnata* for the first time.

EXPERIMENTAL

Plant material. Herba Passiflorae (Ch-B.: KL-5432) was provided by Dr Peithner KG, Vienna, Austria.

Extraction and isolation of **1**. Powdered plant material (100 g) was extracted with 40% MeOH (4 × 1 l). After evapn under red. pres., the residue was dissolved in a small vol of 20% MeOH and applied to CC on polyamide, initiated with 20% MeOH and gradually increased to pure MeOH. The obtained frs were further sepd on Sephadex LH-20 using 20% MeOH, with gradually increasing portions of MeOH as mobile phase. Further purification was performed by 1D-PC (Whatman chromatography paper, 3 MM Chr, 46 × 57 cm) using BAW (*n*-BuOH–HOAc–H_2O 4:1:5, upper phase), 15% HOAc and (BEW) (*n*-BuOH–EtOH–H_2O, 4:1:2,2) as solvent systems, and by HPLC (Nucleosil RP 18, 5 μm, 4.0 × 250 mm) with MeOH–H_2O linear gradient elution (40 + 60, increasing to 58 + 42 within 15 min). Prior to NMR-analysis, compound **1** was purified over Sephadex LH-20 using first H_2O and then MeOH.

Hydrolysis procedures. Acid hydrolysis: Compound **1** was hydrolysed with 80% MeOH–2 M HCl (1:2) at 100° for 60 min. The hydrolysate was extracted first with EtOAc and then *iso*-amylalcohol. The latter yielded scoparin and isoscoparin. R_fs on PC scoparin 0.33 (BAW); 0.12 (15% HOAc) and isoscoparin 0.54 (BAW); 0.40 (15% HOAc). The sugar component was identified by 1D-PC with BAW, BEW, TBPW (toluene–*n*-BuOH–pyridine–H_2O, 2:5:3:3) and phenol (phenol–H_2O, 4:1) for 36 hr against a standard mixture of sugars [2].

Isoscoparin 2″-O-glucoside (**1**). PC R_f 0.41 (BAW); 0.74 (15% HOAc); 0.47 (BEW). UV: λ_{max}^{MeOH} nm: 252 sh, 272, 345; +NaOAc: 277, 317, 368; +NaOAc + H_3BO_3: 274, 347; +$AlCl_3$: 260sh, 276, 296sh, 357, 383; +$AlCl_3$ + HCl: 259sh, 276, 295sh, 353, 380; +NaOH: 268, 278, 339sh, 411. HR-PI-FAB MS (MAT 95, glycerol–MeOH): *m/z*: Calcd. for $C_{28}H_{33}O_{16}$ $[M+H]^+$. 625,1769. Found: 625,1787. NI-DCI MS (NH_3): *m/z* [% rel.int.]: 624 [M; 7], 606 [M − 18; 2], 534 [M − 90; 1], 504 [M − 120; 100], 462 [M − 162; 4], 444 [M − 162 − 18; 34], 342 [M − 120 − 162; 15], 300 [M − 162 − 162; 4]. ^{1}H NMR (400 MHz, MeOH-d_4, 24°): δ 2.90 (1H, *m*, H-5‴), 3.07 (1H, *pt*, *J* = 8.5 Hz, H-2‴), 3.16 (1H, *pt*, *J* = 8.5 Hz, H-4‴), 3.24 (1H, *pt*, *J* = 8.5 Hz, H-3‴), 3.37 (2H, *m*, H-6a‴ + 6b‴), 3.40 (1H, *m*, H-5″), 3.51 (1H, *pt*, *J* = 9.2 Hz, H-4″), 3.66 (1H, *pt*, *J* = 9.2 Hz, H-3″), 3.71 (1H, *dd*, *J* = 12.2 Hz, H-6b″), 3.85 (1H, *br d*, *J* = 12.4 Hz, H-6a″), 3.96 (3H, *s*, —OCH_3), 4.38 (1H, *d*, *J* = 8.5 Hz, H-1‴), 4.55 (1H, *br s*, H-2″), 4.94 (1H, *d*, *J* = 10 Hz, H-1″), 6.45 (1H, *s*, H-8), 6.62 (1H, *s*, H-3), 6.93 (1H, *d*, *J* = 8.2 Hz, H-5′), 7.48 (1H, *d*, *J* = 2.2 Hz, H-2′), 7.51 (1H, *dd*, *J* = 8.2 and 2.2 Hz, H-6′) ^{1}H NMR (400 MHz, δ in DMSO-d_6, 70°): 2.70–4.70 (sugar protons), 3.90 (3H, *s*, —OCH_3), 4.70 (1H, *d*, *J* = 10 Hz, H-1″), 6.37 (1H, *s*, H-8), 6.71 (1H, *s*, H-3), 6.93 (1H, *d*, *J* = 8.8 Hz, H-5′), 7.49 (1H, *d*, *J* = 2.4 Hz, H-2′), 7.50 (1H, *dd*, *J* = 8.8 and 2.4 Hz, H-6′)

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