Phytochemistry, Vol. 26, No 3, pp. 859–860, 1987.
Printed in Great Britain.

0031 9422.87 $3.00 + 0.00

LUTEOLIN 6-*C*-β-RISTOBIOSIDE FROM *POA ANNUA*

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(*Received* 15 *May* 1986)

Key Word Index—*Poa annua*; Gramineae; flavone *C*-glycosides; alditol; luteolin 6-*C*-β-ristobioside; 2″-*O*-α-D-mannopyranosylisoorientin; orientin; isoorientin; tricin; mannitol.

Abstract—A new glycoflavone, luteolin 6-*C*-(2″-*O*-α-D-mannosyl-β-D-glucoside), was characterized from whole plants of *Poa annua*.

Poa is a cosmopolitan genus of the Gramineae that comprises some 200 species. Several introduced and native *Poa* species grow in Argentina but little is known of their chemical components. We have previously reported compounds from *Poa huecu* [1, 2], an Argentinian plant toxic to cattle.

In this paper we describe the identification of a new *C*-glycosylflavone: luteolin 6-*C*-(2″-*O*-α-D-mannopyranosyl-β-D-glucopyranoside) (luteolin 6-*C*-β-ristobioside or 2″-*O*-α-D-mannopyranosylisoorientin) (1), which was isolated together with tricin, orientin, isoorientin and mannitol from whole plants of *Poa annua*. Tricin [3], orientin [4] and the triterpenoid friedelinol [5] have been reported previously from this species growing in other countries.

As far as we know this is the first report of luteolin 6-*C*-β-ristobioside and there are no reports of the disaccharide, 2-*O*-α-D-mannopyranosyl-β-D-glucopyranoside, either in synthetic form or from a secondary metabolite. However, this sugar moiety is part of the glycopeptide ristomycin A that belongs to the vancomycin group of antibiotics [6]. It has been called ristobiose due to its presence in the ristotetrose unit, a tetrasaccharide associated with the antibiotic mentioned above [6].

The UV spectrum of **1** with maxima at 269 and 347 nm, was similar to those reported for flavones. The bathochromic shifts of the UV bands of **1** with sodium methoxide ($\Delta\lambda_I$ = 58 nm) and sodium acetate ($\Delta\lambda_{II}$ = 6 nm) suggested the presence of free 4′- and 7-hydroxyl groups, respectively. Moreover, the bathochromic shifts with aluminum chloride ($\Delta\lambda_I$ = 78 nm) and $AlCl_3$/HCl ($\Delta\lambda_I$ = 23 nm) showed the presence of a free 5-hydroxyl and two free *ortho* hydroxyl groups on the B ring. These results suggested the aglycone was luteolin.

Acid hydrolysis of **1** yielded mannose but no aglycone was obtained suggesting the presence of a *C*-glycoside because of its resistance to hydrolysis.

The ^{1}H NMR spectrum of **1** in DMSO-d_6 showed two singlets of one proton each at δ6.50 and δ6.69 attributed to H-8 and H-3, respectively. The absence of a signal at δ6.30 due to H-6, normally unaffected by *C*-glycosidation in other positions, indicated the possibility of a 6-*C*-glycoside. Moreover, two sugar moieties were present that gave two anomeric proton signals: one doublet at δ4.30 with an *equatorial–equatorial* coupling ($J_{1,2} = J_{ee}$ = 2 Hz) that was in agreement with that of α-methyl mannopyranoside (β-methyl mannopyranoside: $J_{1,2} = J_{aa}$ = 4 Hz); the second doublet appeared at δ4.70 with an *axial–axial* coupling ($J_{1,2} = J_{aa}$ = 8 Hz) indicating a β-configuration.

The MS of permethylated **1** (M$^+$ *m/z* 764) exhibited the fragmentation pattern typical for a 2″-*O*-hexosyl-6-*C*-hexosylflavone: absence of M − 15 and M − 31 peaks and a base peak at *m/z* 529 (ion S) [7].

The 1‴ → 2″ interglycosidic linkage was further confirmed by the ^{1}H NMR spectrum of peracetylated **1**. The acetyl protons at position 2 of tetraacetyl-*C*-glucosylbenzene were reported to be shielded by 0.3 ppm in relation to the other acetyl protons [8]. Therefore, the absence of proton signals in the region δ1.70–1.80 of peracetyl **1** indicated that the 2″-hydroxyl was substituted. In fact the acetyl sugar protons appeared at δ1.97–2.05 and those of the phenolic acetyls at δ2.35–2.50. Thus, D-mannose was linked to the 2″-hydroxyl of the *C*-hexosyl moiety. The aglycone was determined by ^{13}C NMR data due to its resistance to hydrolysis. Thus, the ^{13}C NMR spectrum of **1** in DMSO-d_6 exhibited a signal at 93.3 ppm due to C-8 of luteolin and a signal at 108.0 ppm assigned to C-6 shifted to lower field (+8 to +10 ppm) due to *C*-glycosidation [9]. Furthermore, C-5 and C-7 of luteolin were slightly protected (0.1–2 ppm) due to 6-*C*-glycosidation [10]. The glycosidation effect on C-2″ was *ca* +10 ppm, this signal appearing at 80.0 ppm. The anomeric carbon of the *O*-mannopyranosyl unit was observed at 102.3 ppm and the other sugar carbons in the region 61.3–81.3 ppm that included another anomeric carbon from the *C*-glucosyl unit. The glucose was β-linked to the aglycone on the basis of ^{1}H NMR data (δ4.70; $J_{1,2} = J_{aa}$ = 8 Hz) and ^{13}C NMR values (δ_c = 71.3: β-*C*-glucopyranosyl bonded to an aromatic ring).

The α configuration of the anomeric carbon of mannose was further established by enzymatic hydrolysis with α-mannosidase which gave D-mannose and isoorientin.

From these data we conclude that **1** is luteolin 6-*C*-(2″-*O*-α-D-mannopyranosyl-β-D-glucopyranoside).

Other 2″-*O*-glycopyranosyl-*C*-glycopyranosides such

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as spinosin (2"-*O*-β-D-glucopyranosylswertisin) [11] have been reported from other families. However, as far as we know, this is the first report of *O*-mannopyranose attached to a *C*-glycoside.

EXPERIMENTAL

Plant material. Whole plants of *Poa annua* were collected in Ciudad Universitaria, Buenos Aires, Argentina. A voucher specimen (BAFC 1343) was deposited in the Herbarium of Departamento de Biologia, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina.

Extraction and isolation of the components. Dried ground whole plants were defatted with petrol (60–80°) and further extracted in a Soxhlet with MeOH. The MeOH extract was conc to a brown residue (6.0% rel. to dry plant) that was successively percolated on polyamide with $CHCl_3$, H_2O and MeOH. The MeOH fraction was chromatographed on a Sephadex LH-20 column using MeOH as eluent giving three main fractions. The first was further chromatographed on a Sephadex LH-20 column to yield mannitol (mmp, GLC with standards) as a white ppt and **1**, the second gave orientin and isoorientin, and the third, tricin.

Luteolin 6-C-β-ristobioside (**1**). Mp. 216–220° (Me_2CO); $[\alpha]_D^{25}$ +24° (*c* 0.2, Py). UV λ_{max}^{MeOH} (nm): 255, 269, 291 (sh), 347; + NaOMe: 268, 277 (sh), 335 (sh), 405; + $AlCl_3$: 276, 301 (sh), 425; + $AlCl_3$/HCl: 263 (sh), 277, 296 (sh), 370; + NaOAc: 275, 324 (sh), 405; + NaOAc/BO_3H_3: 272, 425. ^{1}H NMR (100 MHz; DMSO-d_6) ppm: δ 3.00–3.60 (*m*, sugar protons), 4.30 (*d*, 1H, $J_{1''}$ = 2 Hz, H-1'''), 4.70 (*d*, 1H, $J_{1''}$ = 8 Hz, H-1''), 6.50 (*s*, 1H, H-8), 6.69 (*s*, 1H, H-3), 6.92 (*d*, 1H, J_o = 8 Hz, H-5'), 7.42 (*m*, 2H, H-2' and H-6'). ^{13}C NMR (25.2 MHz, DMSO-d_6) ppm: 61.3 (C-6'''), 64.3 (C-6''), 66.7 (C-4'''), 70.2 (C-4'' and C-3'''), 71.3 (C-1''), 72.2 (C-2'' and C-5'''), 78.5 (C-3''), 80.0 (C-2''), 81.3 (C-5''), 93.3 (C-8), 102.3 (C-1'''), 102.4 (C-3), 104.8 (C-10), 108.0 (C-6), 112.8 (C-2'), 115.9 (C-5'), 118.6 (C-6'), 120.9 (C-1'), 145.7 (C-3'), 149.9 (C-4'), 156.2 (C-9), 160.7 (C-5), 163.2 (C-2 and C-7), 181.3 (C-4).

Permethylation of **1**. A soln of **1** in DMF was permethylated with NaH and MeI in the usual manner. MS of permethyl **1**: *m/z* (%) 765 (M + 1, 0.2), 764 (M, 1.2), 545 (SO, 75.3), 543 (SO − 2, 6.5), 529 (S, 100.0), 497 (S − 32, 19.0), 385 (*i*, 21.2), 371 (*j*, 95.7), 369 (*j* − 2H, 17.1), 355 (*k*, 46.9).

Acid hydrolysis of **1**. **1** (2 mg) in MeOH–H_2O (1:1) was heated with 7% HCl in a sealed tube for 1 hr at 100°. The aq. layer was neutralized with K_2CO_3 and extracted with *n*-BuOH to give isoorientin, orientin and other decomposition products. Mannose was identified from the desalted aq. layer by co-TLC (cellulose F; *n*-BuOH–pyridine–H_2O, 6:4:3; R_f mannose: 0.58) and as the alditol acetate (reduction of mannose with $NaBH_4$ at pH 9 followed by acetylation; GC: 3% ECNSS-M, 1.8 m length, 180°, isothermal: R_t peracetylmannitol: 27.65 min).

Enzymatic hydrolysis of **1**. **1** (2 mg) in citric acid–citrate buffer (pH 4.60) (0.2 ml) was incubated at 37° with 0.1 ml of α-mannosidase of *Canavalia ensiformis* (5 mg/ml) (Boehringer). Total hydrolysis was achieved after 48 hr giving D-mannose and isoorientin. TLC (silica gel; EtOAc–pyridine–H_2O–MeOH, 8:2:1:0.5) R_f: isoorientin: 0.59; orientin: 0.65; **1**: 0.10; hydrolysate of **1**: 0.57. HPLC (H_2O–MeOH–HOAc, 55:45:0.1; 1.7 ml/min) R_t (min): isoorientin: 3.2; orientin: 3.6; hydrolysate of **1**: 3.2. HPLC (H_2O–MeOH–HOAc, 7:3:0.1; 1.7 ml/min) R_t (min): **1** 4.8.

Peracetylation of **1**. **1** (3 mg) was acetylated with Ac_2O–pyridine in the usual manner. ^{1}H NMR of peracetyl **1** (100 MHz, $CDCl_3$) ppm: δ 1.98–2.10 (*m*, 21 H, seven sugar acetyls), 2.30–2.60 (*m*, 12 H, four phenolic acetyls), 4.42 (*d*, 1H, $J_{1''}$ = 2 Hz, H-1'''), 4.86 (*d*, 1H, $J_{1''}$ = 6 Hz, H-1''), 6.41 (*s*, 1H, H-3), 6.44 (*br s*, H-8), 6.52 (*d*, 1H, J_o = 7 Hz, H-5') (H-2' and H-6' are overlapped with the $CHCl_3$ signal).

Acknowledgements—We are indebted to Lic. Enrique M. Zallocchi (Departamento de Biologia, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina) for identification of the plant material; UMYMFOR-CONICET (Argentina) for recording spectra, and CONICET (Argentina) for financial support. One of us (R.D.R.) thanks CIC (Argentina) for a research fellowship.

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