Polyphenolic Compounds from *Plantago lagopus* L.

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In the present study we report the isolation of a phenylpropanoid glucoside, plantamajoside, together with rosmarinic acid, chlorogenic acid and luteolin-7-O-monoglucoside. This is the first report of these compounds from *Plantago lagopus.*

Polyphenolic compounds constitute one of the most characteristic classes of compounds in higher plants. Cinnamic acid derivates (e.g. caffeic, ferulic and p-coumaric acid) occur widely in higher plants as ester or glycosides. Quinic acid and monocaffeates, especially chlorogenic acid, are the most widely present ester derivates. Besides, a large number of other simple esters and glycosides (mainly with glucose and rhamnose as sugar moieties) of these phenylpropanoid acids have been described. Only a few phenylpropanoid ester glycosides which contain more than one sugar unit and a substituted phenylethanol moiety are known to date. Caffeic acid sugar esters have been shown to have antibacterial, antifungal, antiviral activity and inhibit 5-lipoxygenase (from the leucotriene biosynthesis). These compounds may also be interest in plant pathology as natural plants protective agents and as repellent against herbivores (Ravn and Brimer, 1988).

Moreover, rosmarinic acid and caffeic acid have shown a strong antimicrobial effect aginst selected plant pathogenic bacteria and fungi (Petersen *et al.*, 1991). Rosmarinic acid, has been shown to inhibit 5-lipoxygenase, 3 α-hydroxysteroid dehydrogenase, and lipid peroxidation and to have antinflammatory activity (Nakazawa and Ohsawa, 1998).

On the other hand, *Plantago* plants have been used since ancient times as diuretic, an anti-inflammatory and an asthmatic drug in Asia and Europe (Nishibe *et al.*, 1995), but the biologically active principles are still unknown. "Plantaginis herba" is listed in Japanese Pharmacopeia XI as an important crude drug and an aqueous extract is also used as a medicine (Nippon Koteisho Kyokai, 1986).

In the course of a search for biologically active substances from Spanish plants, the phytochemical analysis of the aerial parts of *Plantago lagopus* L. revealed that it is a rich source of polyphenols of therapeutic interest, and a part of the possible biological activity of this species could be due to the polyphenolic compounds.

In previous papers, we have reported the isolation and structure elucidation of four iridoid glucosides from *P. lagopus* (Velázquez *et al.*, 2000). Continuing our work on the constituents of this species, this paper deals with both the isolation and structure elucidation of plantamajoside (**1**), luteolin-7-O-monoglucoside (**2**), rosmarinic acid (**3**) and chlorogenic acid (**4**) from the aerial parts of the same plant. The compounds isolated were identified by means of spectral (UV, NMR) and chemical (acid and alkaline hydrolysis) evidence.

Material and Methods

The aerial parts of *Plantago lagopus* (Plantaginaceae) were collected in 1991 at Jaén (Spain) and identified by Prof. C. Bartolomé Esteban, Department of Vegetal Physiology, Faculty of Sciences, University of Alcalá de Henares, Madrid (Spain). A voucher specimen was deposited (M. PV 91) in the Laboratory of Pharmacognosy, Faculty of Pharmacy, University of Alcalá de Henares, Madrid, Spain.

The aerial parts of *P. lagopus* (800 g) were powdered and extracted with CH_3OH/H_2O (80:20 v/v) at room temperature. The 80% CH_3OH extract

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was concentrated to dryness *in vaccuo* to afford a residue (120 g). The residue (40 g) was chromatographed on polyamide (MN-Polyamide SC 6, Macherey-Nagel, Düren, Germany, 1 kg, 60×9.5 cm) and eluted successively with H_2O (6 l), 50% CH_3OH (11 l), CH_3OH (5 l), CH_3COCH_3-H_2O (50:50 v/v, 3 l), CH_3COCH_3-H_2O (70:30 v/v, 14 l), 0.5% HCl (4 l) and 1% HCl in 70% CH_3COCH_3, to afford seventeen fractions (F_1–F_{17}).

Fraction F_8 (0.7 g/elution with 50% CH_3OH) was submitted to Medium Pressure Liquid Chromatography (MPLC) on Lichroprep RP 18 (200 g, 26–40 μ, Merck) using the following linear gradient: CH_3OH-H_2O (20:100 v/v, 4 l) to yield pure compound **1** (20 mg, 30% CH_3OH).

Fraction F_{12} (400 mg/elution with 50% CH_3OH) was chromatographed by CC on polyamide (MN-polyamide SC 6<0.07 mm, 40 g, 33×2.5 cm) using a linear gradient of CH_3CH_2OH-H_2O (30–100%, 700 ml), CH_3COCH_3-H_2O (50:50 v/v and 70:30 v/v, 300 ml each one) and 1% HCl in CH_3COCH_3-H_2O (70:30 v/v, 200 ml) affording **2** (50% CH_3CH_2OH, 33 mg) and **3** (60% CH_3CH_2OH, 15 mg).

Fraction F_{14} (150 mg/ elution with CH_3OH) was subjected to column chromatography on cellulose (10 g, 45×1.5 cm) using as eluent system t-BuOH/HOAc/H_2O (3:1:1 v/v/v), compound **4** (37 mg) was obtained.

Identification and assignment of the isolated compounds were performed by acid and alkaline hydrolysis, TLC, UV, ^{1}H NMR and ^{13}C NMR in comparison with literature data (Mabry *et al.*, 1970; Sasahara *et al.*, 1992; Ravn and Brimer, 1988; Markham *et al.*, 1978) or by direct comparison with authentic samples. This compounds have been isolated for the first time from *Plantago lagopus*.

2 mg of compound **2** were treated with HCl 10% (2 ml) in a sealed tube at 100 °C for 4 h. The aglycone was extracted with Et_2O, then the aqueous layer was neutralized with N,N-methyl-dioctylamine (Merck) (10% in $CHCl_3$) and freeze-dried. The aglycone was detected in the organic layer, while the sugar was identified in the aqueous layer. TLC analysis of the sugar was performed in precoated Si gel plates (Silicagel G-60 F_{254}, 0.2 mm, Merck) using as solvent system AcOEt/CH_3OH/H_2O/CH_3CO_2H (65:15:15:25 v/v/v/v). The plates were developed with naphthoresorcinol phosphoric reagent (Merck) by heating at 110 °C.

Compound **4** (2 mg) was heated with 2 N NaOH (2 ml) in a sealed tube at 100 °C for 1 h. The hydrolized compound was neutralized with 2 N HCl and extracted with Et_2O, then the aqueous layer was extracted with AcOEt. The AcOEt fraction was analyzed by TLC in precoated Si gel plates (Silicagel G 60 F_{254}, 0.2 mm, Merck) using as solvent system toluene/CH_3OH/HOAc/H_2O (30:50:10:2 v/v/v/v) and developed with fast blue salt reagent: 0.5 gr fast blue salt B was dissolved in 100 ml water. [Fast blue salt B = 3,3'-dimethoxy-biphenyl-4-4'-bis(diazonium)-dichloride]. The plate was sprayed with 6–8 ml, dried and inspected visually. Spraying could be repeated, using 0.1 M NaOH, followed again by inspection.

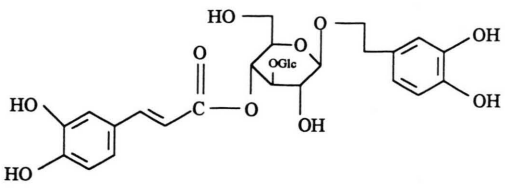
Results and Discussion

The 80% MeOH extract of the aerial parts was subjected to repeated column chromatography on reverse phase RP_{18}, polyamide and cellulose affording compounds **1**–**4** which were identified by acid and alkaline hydrolysis, UV, ^{1}H and ^{13}C NMR. Structures of the compounds are showed in Figure **1**.

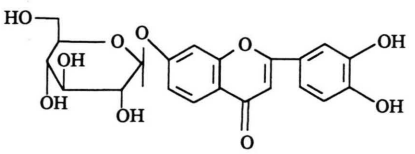
The results obtained from analysis of the compounds isolated were:

Plantamajoside (**1**)

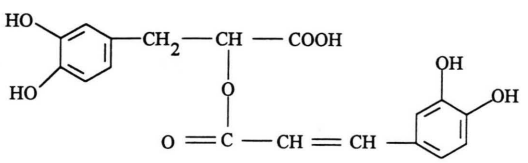
$C_{29}H_{36}O_{16}$. Amorphous powder. Mp: 158–162° (uncorr.). $[\alpha]_D^{192}$ −42.47, $[\alpha]_D^{24.8}$ −43.88 (MeOH; c 0.47), UV λ_{max}^{MeOH} nm: 220.4, 247.6, 292.4, 332.4, IR λ_{max}^{KBr} 3350 cm^{-1} (br OH) 1685 cm^{-1} (conjugated C=O), 1625 cm^{-1} [C(7')=C(8')], 1600 cm^{-1}, 1515 cm^{-1} (arom. ring), 850, 810 cm^{-1} (1, 3, 4-tri. subst. arom. ring) , FABMS *m/z* 641 $[M+H]^+$, ^{1}HMNR (in $CDCl_3$): δ 2.80 and δ 3.80 (both 2H, t, H-7, H-8 phe), δ 4.45 (1H, d, *J*=12 Hz ~H-1 glu-i), δ 4.59 (1H, d, *J*=12 Hz H-1 ~ glu), δ 6.34 (1H, d, *J*=16 Hz ~ H-8'), δ 7.61 (1H, d, *J*=16 Hz ~ H-7'), δ 6.52 [1H, d, *J*=2 Hz ~ H-2 (phe)], δ 6.65 [1H, d, *J*=10 Hz ~ H-5 (phe)], δ 6.72 [1H, d, *J*=9 Hz ~ H-6 (phe)], δ 6.68 (1H, d, *J*=10 Hz ~ H-5'), δ 7.03 (1H, dd, *J*=2 Hz; *J*=10 Hz ~ H-6'). ^{13}CNMR (in $CDCl_3$): 130.3 (C-1), 114.0 (C-2), 144.8 (C-3), 143.4 (C-4), 115.8 (C-5), 120.1 (C-6), 36.5 (C-7), 73.8 (C-8), 126.5 (C-1'), 114.9 (C-2'), 145.5 (C-3'), 148.4 (C-4'), 117 (C-5'), 122.0 (C-6'), 146.4 (C-7'), 115.5 (C-8'), 167.4 (C-9'), 102.7 (C-1 glu-i), 74.8 (C-2 glu-i), 83.1 (C-3 glu-i), 69.7 (C-4 glu-i), 74.5



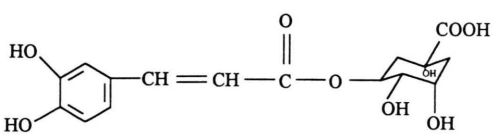
Plantamajoside



Luteolin-7-O-monoglucoside



Rosmarinic acid



Chlorogenic acid

Fig. 1. Structures of compounds isolated.

(C-5 glu-i), 61.2 (C-6 glu-i), 104.5 (C-1 glu), 71.0 (C-2 glu), 76.4 (C-3 glu), 70.0 (C-4 glu), 76.6 (C-5 glu), 61.1 (C-6 glu).

Luteolin-7-O-β-D-glucopyranoside (**2**)

$C_{20}H_{16}O_{11}$. Pale yellow powder. UV_{max}^{MeOH} nm: 256, 267sh, 350; + NaOMe: 261, 416; + $AlCl_3$: 272, 365, 426; + $AlCl_3$/HCl: 236, 420; + NaOAc: 266, 410; + NaOAc/H_3BO_3: 266, 389. ^{13}CNMR (in CD_3OD): 164.8 (C-2), 104.2 (C-3), 184.0 (C-4), 162.9 (C-5), 101.2 (C-6), 164.8 (C-7), 96.1 (C-8), 158.9 (C-9), 104.2 (C-10), 123.5 (C-1'), 114.3 (C-2'), 147.1 (C-3'), 151.2 (C-4'), 116.8 (C-5'), 120.5 (C-6'), 104.8 (C-1"), 74.7 (C-2"), 78.4 (C-3"), 71.5 (C-4"), 77.9 (C-5"), 62.5 (C-6").

Rosmarinic acid (**3**)

$C_{18}H_{15}O_8$. White powder. TLC: silicagel G 60 F_{254}, AcOEt/HCO_2H/AcOH/H_2O (100:11:11:27 v/v/v/v), Rf: 56; $CHCl_3$/AcOEt/HCO_2H (5:4:1 v/v/v), Rf: 50; n-BuOH/EtOH/H_2O (4:1:2 v/v/v), Rf: 79; cellulose 0.5 mm, n-BuOH/AcOH/H_2O (4:1:5 v/v/v, upper phase), Rf: 69; AcOH 15%, Rf: 80. UV λ_{max}^{MeOH} nm: 296, 319, 338; + NaOH: 260, 314, 380.

Chlorogenic acid (**4**)

$C_{16}H_{18}O_9$. White powder. TLC: silicagel G 60 F_{254}, AcOEt/HCO_2H/AcOH/H_2O (100:11:11:27 v/v/v/v), Rf: 83; cellulose 0.5 mm, n-BuOH/AcOH/H_2O (4:1:5 v/v/v, upper phase), Rf: 60; AcOH 15%, Rf: 85. UV λ_{max}^{MeOH} nm: 218, 244sh, 304, 329; + NaOH: 262, 318sh, 375. ^{13}CNMR (in CD_3OD): δ 6.15 (1H, d, *J*=15.9 Hz H-2'), δ 7.43 (1H, d, *J*=15.9 Hz H-3'), δ 6.78 (2H, d, *J*=8 Hz H-8'), δ 7.04 (2H, d, *J*=2 Hz H-5'), δ 6.98 (2H, dd, H-9').

The UV spectra indicated that **1** was a phenylpropanoid glucoside, **2** was a flavonoid glucoside and that **3** and **4** were rosmarinic acid and chlorogenic acid, respectively.

Acid hydrolysis of **2** gave glucose as sugar moiety. Luteolin was identified as the aglycone by TLC comparison. Alkaline hydrolysis of **4** gave quinic and caffeic acid as moieties.The ^{1}H-NMR spectrum of **4** is identical to that reported in the literature.

The ^{13}CNMR spectrum of **2** led to the identification of the sugar moiety and its site of linkage by comparison with literature data (Markham *et al.*, 1978). The C-6 signal of 101.2 ppm indicates the linkage between the glucose and the aglycone.

The ^{13}CNMR data of **1**, were comparable with those described by Ravn and Brimer (1988), thus proving the presence of the dihydroxy-β-phenethyl moiety. The attachment of the caffeoyl and the outer glucose moieties at the C-4 and C-3 carbons, respectively, is supported by the ^{13}CNMR. Only signals corresponding to two monosaccharides were found. The aromatic protons of **1** are

resolved in two ABX systems, one belonging to the caffeic acid moiety substitution, the other to the 3,4-dihydroxy-phenethyl part.

The H-7'proton and the H-8'proton from the caffeoyl moiety gave two doublets at δ 7.61 and 6.34, respectively (J=16 Hz) proving a *trans* configuration around the C=C. The two anomeric protons (H-1 inner glucose, δ 4.45) and (H-1 outer glucose, δ 4.59) are both doublets with J=12 Hz and hence in accordance with a β-configuration in both cases.

Based of the above evidence the structures of **1**–**4** were elucidated as: 3,4-dihydroxy-β-phenethyl-O-β-D-glucopyranosyl(1→3)-4-O-caffeoyl-β-D-glucopyranoside, luteolin-7-O-β-D-glucopyranoside, rosmarinic acid and chlorogenic acid. These compounds have been isolated for the first time from *Plantago lagopus*.

Acknowledgements

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