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RESEARCH ARTICLE

Iridoid and Megastigman Glucosides from *Plantago lagopus* L.

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Quantitative Determination of Citalopram and its Metabolite Desmethycitalopram in Plasma by High Performance Liquid Chromatography

Summary

Two iridoid glucosides, catalpol and aucubin, one chlorinated iridoid aglycon, rehmaglutin D were isolated from the iridoid fraction of the aerial parts of Plantago lagopus L. together with the megastigman glucoside, phlomuroside. The structures of the isolated compounds were identified by means of spectroscopic (UV, HR-ESI, ^{1}H-NMR, ^{13}C NMR , HMQC, HMBC, COSY) methods. This is the first report for the first isolation of rehmaglutin D and phlomuroside from the genus Plantago.

Key Words: *Plantago, iridoid glucoside, rehmaglutin D, phlomuroside, catalpol, aucubin.*

***Plantago lagopus* L.' dan İzole Edilen İridoit ve Megastigman Glukozitleri**

Özet

Plantago lagopus L. bitkisinin toprak üstü kısımlarından hazırlanan iridoit fraksiyonunda; iki iridoit glukoziti (katalpol ve okubin), bir klorlu iridoit aglikonu (rehmaglutin D) ve bir de megastigman glukoziti (filomurozit) olmak üzere toplam 4 maddenin izolasyonu gerçekleştirilmiştir. İzole edilen bileşiklerin yapı tayinleri spektroskopik yöntemler (UV, HR-ESI, ^{1}H-NMR, ^{13}C NMR , HMQC, HMBC, COSY) kullanılarak aydınlatılmıştır. Rehmaglutin D ve filomurozit Plantago türlerinden ilk kez bu çalışma ile izole edilmiştir.

Anahtar Kelimeler: *Plantago türleri, iridoit glukoziti, rehmaglutin D, filomurozit, katalpol, okubin.*

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INTRODUCTION

The genus *Plantago* is represented by 21 species in Turkish flora two of them are endemic (1). *Plantago* species are known not only as a food plant, but also an old medicinal plant that has been known for centuries. *P. major* and *P. lanceolata* are the most widespread species in Turkey and mainly the leaves of *P. major* are used externally to treatment of wound, abscess and acnes, internally to treatment of diabetes, urinary infections and cancer as a decoction, common cold and viral infections as infusion in Anatolia (2,3).

Previous phytochemical studies showed that iridoid glucosides, phenylethanoid glycosides, terpenoids, flavons and flavon glycosides have been isolated from *Plantago* species (4,5). In this study; *Plantago lagopus* L which commonly exists in Marmara

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and Eagean regions in Turkey was investigated for its phytochemical content. In our previous studies, acteoside and calceorioside were isolated from *P. lagopus* by bioactivity guided isolation method and both compounds were found strong radical scavenging and cytotoxic activities (6). In a continuation of our studies on *Plantago* species, iridoid fraction of *P. lagopus* were investigated for its phytochemical content and we report here the isolation and structure determination of two iridoid glucosides, one chlorinated iridoid aglycone and one megastigman glucoside from *P. lagopus*.

MATERIAL AND METHODS

Plant Material

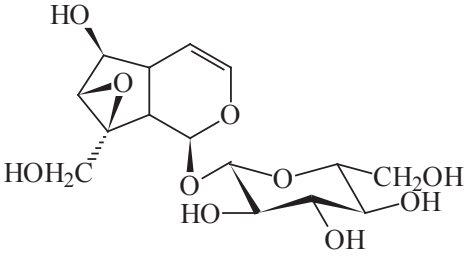
Plantago lagopus L. (Plantaginaceae) was collected from Antalya, Düden cascade in May 2009. A voucher specimen has been deposited in the Herbarium of Pharmacognosy Department, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey (HUEF 09325).

Extraction and Isolation

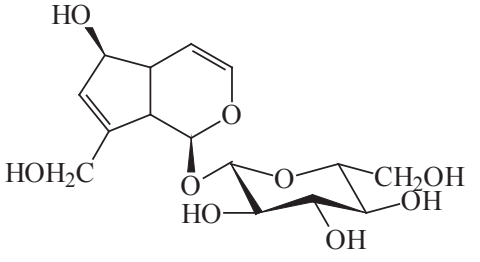
The air dried and powdered aerial parts of plant (118g) were extracted with methanol at 40 °C three times. Methanolic extracts were combined and evaporated under vacuum. Methanolic extract was dissolved in distilled water and extracted with petroleum ether to remove non-polar compounds such as chlorophylls. Water extract was applied to the polyamide column using 0-100% methanol as a solvent system to give five main fractions (Fr.A-E). Fraction A which is aqueous fraction were found to be rich in iridoids in thin layer chromatography (TLC). It was dissolved in water and extracted with *n*-butanol to remove sugars. *n*-Butanol phase was evaporated under vacuum and applied to medium pressure liquid chromatography (MPLC). Six main fractions were collected from MPLC and 25% MeOH fraction was determined as a pure compound (PL-1). 50% MeOH fraction of MPLC column was applied to silicagel column chromatography using chloroform:methanol as solvent system (100:0, 95:5, 90:10, 85:15, 80:20, 75:25). Silica gel column chromatography resulted in isolation of two pure compounds PL-2 and PL-3. The another fraction from MPLC (70% MeOH) was applied to silicagel column chromatography to give PL-4 as a pure form (Figure).

RESULTS AND DISCUSSION

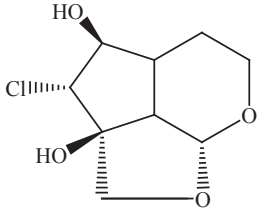
The water soluble part of the methanolic extract prepared from the aerial parts of *P. lagopus* was subjected to successive column chromatography (polyamide, normal/reverse phase silica gel, and Sephadex LH 20) to give compounds 1-4 in pure form (Figure). PL-1 and PL-2 were obtained as colorless and amorphous powder. Their UV spectra showed



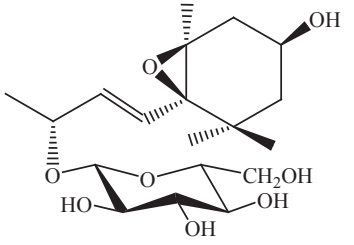
Catalpol (PL-1)



Okubin (PL-2)



Rehmaglutin D (PL-3)



Phlomuroside (PL-4)

Figure: Isolated compounds (PL 1-4)

an absorption peak (λmax 205 nm) characteristic of a 4-nonsubstituted iridoid enol ether system for both compounds. The molecular formula of PL-1 was established as $C_{15}H_{22}O_{10}$ by the evaluation of molecular ion peak at 385.1110 $[M+Na]^+$ and 747.2344 $[2M+Na]^+$ in HR ESI-mass spectrum and ^{13}C NMR results. The ^1H-NMR signal at δ_H 4.76 for PL-1 and δ_H 4.67 for PL-5 (d, *J*=7.9 Hz) was assigned to the anomeric proton and their *J* – coupling (7.9 Hz) indicated *β* – glucopyranose structure as a sugar unit. Five carbon signals between δ_C 62.71-78.59 ppm and proton signals between δ_H 3.21-3.90 ppm and the evaluation of their COSY and HMQC spectra in addition to above data confirm the presence of glucopyranose unit for both structures (Table 1 and 2).

^1H-NMR signals of PL-1 at δ_H 6.34, 5.07, 5.03, 3.90, 3.44, 2.53 and 2.27 showed the presence of 7 methyne groups in cyclopentan-pyran structure. Signals at δ_H 4.13 and 3.79 (J_{AB}=13.1Hz) belong to an AB system indicated the presence of hydroxymetyhlen group. HMBC correlations between H_2-10/C-7, H_2-10/C-8, ve H_1-10/C-9 were confirmed that C-8 was quarterner and hydroxymetyhlen group was located on C-8. Detailed examination of COSY correlations between H-1 signal (δ_H 5.03) to δ_H 2.53 (1H, dd, *J* = 9.7/7.6 Hz, H-9); H-9 proton signal to δ_H 2.27 (1H, m, H-5); H-5 proton signal to δ_H 5.07 olefinic proton (1H, dd, *J* = 5.8/4.6 Hz, H-4) and δ_H 3.90 oxymethyne proton (1H, H-6) and last H-4 proton signal (δ_H 5.07) to δ_H 6.34 olefinic proton (1H, dd, *J* = 6.1/1.8 Hz, H-3)

Table 1. ^1H-NMR Spectral Data of PL 1-4 (500 MHz, CD3OD)

H	PL-1	PL-2	PL-3	PL-4
Aglycone				
1	5.03 d (9.7)	4.95 d (7.3)	5.30 d (5.1)	
2				1.22 dd (10.9/2.6) 1.54 dd (12.6/2.6)
3	6.34 dd (6.1/1.8)	6.30 dd (6.2/2.0)	3.86 dd (12.0/2.8) 3.52 dd (10.6/4.3)	3.73 m
4	5.07 dd (5.8/4.6)	5.09 dd (6.1/4.0)	1.65 m 1.77 m	1.59 dd (9.2/14.2) 2.25 dd (4.8/14.0)
5	2.27 m	2.65 m	2.14 m	
6	3.90†	4.43 m	3.81 t (10.3)	
7	3.44 d (0.9)	5.76 t (1.5)	4.06 d (8.6)	5.95 d (16.0)
8				5.71 dd (16.0/6.8)
9	2.53 dd (9.7/7.6)	2.89 t (7.5)	2.29 dd (10.4/5.2)	4.41 t (6.3)
10	4.13 d (13.1) 3.79 d (13.1)	4.34 gd (15.2) 4.16 dd (15.2/0.9)	3.42 d (9.3) 4.38 d (9.3)	1.27 d (6.2)
11				1.18 s
12				1.11 s
13				0.96 s
β – glucopyranose				
1′	4.76 d (7.9)	4.67 d (7.9)		4.34 d (8.0)
2′	3.24 dd (9.4/7.9)	3.21 dd (9.1/7.9)		3.17 dd (8.0/9.1)
3′	3.40 t (9.1)	3.37 t (9.0)		3.20 m (9.0)
4′	3.27 dd (9.5/7.0)	3.27†		3.33 dd (3.4/6.3)
5′	3.31 m	3.27†		3.33
6′	3.90 dd (12.1/1.8) 3.63 dd (11.9/6.4)	3.85 dd (11.9/1.8) 3.64 dd (11.9/5.5)		3.81 dd (2.5/11.6) 3.67 dd (5.1/12.0)

† Signal patterns are unclear due to overlapping.

Table 2. ^{13}C NMR Spectral Data of PL 1-4 (125 MHz, CD_3OD)

C	PL-1	PL-2	PL-3	PL-4
Aglycone				
1	95.26	97.78	101.44	35.96
2				48.68
3	141.78	141.63	56.51	64.57
4	103.99	105.76	22.03	41.60
5	39.08	46.34	36.73	68.05
6	79.57	82.90	76.23	71.20
7	62.52	130.30	74.15	127.75
8	66.18	148.07	85.23	137.14
9	43.55	47.99	45.92	76.93
10	61.58	61.46	72.73	21.00
11				20.22
12				30.12
13				25.14
β – glucopyranose				
1′	99.68	99.98		102.64
2′	74.82	74.97		75.30
3′	77.67	77.95		77.95
4′	71.74	71.62		71.40
5′	78.59	78.33		78.13
6′	62.89	62.71		62.57

revealed the cyclopentan-piran ring system with double bond between C-3 and C-4. On the other hand, HMBC correlation between H-1′/C-1 showed that *β*–glucose is substituted from C-1 of cyclopentan-pyran. After the complete interpretation of the NMR data based on the ^1H-^1H COSY, ^1H-^{13}C HMQC, and HMBC experiments, and comparison of these data with those reported in the literature, PL-1 was determined to catalpol (7,8).

^1H and ^{13}C NMR signals of cyclopentan-pyran ring for PL-2 were very similar to those of catalpol. The presence of three olefinic proton signals at δ_H 6.30, 5.76 ve 5.09 and the absence of oxymethyne proton at δ_H 3.44 in ^1H-NMR spectrum indicated than one more double bound between C-7 and C-8 for PL-2. Signals at δ_H 4.34 and δ_H 4.16 (J_{AB}=15.2Hz) belong to an AB system were assigned to the protons of CH_2OH, located on C-8. No proton signal of C-8 in ^1H-NMR confirmed the presence of olefinic bound at C-7 and C-8. The anomeric proton signal at δ_H 4.67 (d, *J*=7.9 Hz) and long-range correlation between the anomeric proton and C-1 (97.78) assigned to the location of the sugar unit, which attached to C-1 of the aglycone. These data suggested that the structure of PL-2 was the same as aucubin and this was confirmed by the comparison of its spectral data with those reported in the literature (9,10).

The third compound PL-3 was also isolated from the water fraction of polyamide column. Its absorption peak at 202 nm in UV spectrum indicated the 4 non-substituted iridoid enol ether system. The ^{13}C NMR

and ^{1}H-NMR spectra of compound exhibited no signal for anomeric carbon and proton. In addition, absence of any significant signal between δ_H 3.20-3.90 ppm indicated the nonglycosidic structure for PL-3. 9 carbon resonances in DEPT spectrum belonging to one quarterner (C), five methyne (CH) and three methylene (CH_2) confirmed that PL-3 was a non-glucosidic iridoid compound with 9 carbon members. The hemiacetal signal at δ_H 5.30 (H-1, d, *J*=5.1 Hz) was observed in same spin system with all the protons except for the CH_2 protons located on C-10 in the structure in COSY spectrum. Additionally; signals at δ_H 3.42 and δ_H 4.38 (J_{AB}=9.3Hz) belong to an AB system were assigned to the protons of hydroxymetyhlene group. Long range correlation between δ_C 72.73 (CH_2, C-10) with δ_H 4.06 (d, *J* = 8.6 Hz, H-7) and δ_H 2.29 (dd, *J* = 10.4/5.2 Hz, H-9) in HMBC spectrum indicated that hydroxymetyhlene was attached to the C-8 of the cyclopentan pyran ring. HMBC correlations of carbon resonance at δ_C 101.44 (C-1) with hydroxymetyhlene protons [H-10, 3.42 and 4.38 (J_{AB}=9.3Hz)] indicated the presence of ether chain between C-1 and C-10 and tricyclic structure for PL-3 (Table 1 and 2).

On the other hand, about 10 ppm downfield shift for C-7 (δ_C 74.15) comparing the hydroxy derivatives (C-7; δ_C 84.6) indicated the presence of chlorine substitution instead of hydroxyl at C-7 (7,8). Chlorine substitution was confirmed with the characteristic molecular ion in HR-ESI mass spectrum of the compound. After the comparison of these data with those reported in the literature, the structure of PL-3 was identified as rehmaglutin D by using COSY, HMQC, HMBC and HR ESI-mass spectrums which were superimposable to those of the data published for rehmaglutin D (11). Rehmaglutin D was isolated from the genus *Plantago* for the first time in this study. In addition isolation of chlorine derivative iridoid structure is also reported here first time. Previously C-7 chlorinated iridoid glucosides were isolated from *Rehmannia*, *Veronica, Myoporum* and *Verbascum* species from the Schrophulariaceae family (7,12,13). Isolation of Rehmaglutin D from *Plantago* species is very remarkable from the view point of chemotaxonomic researches on Plantaginaceae and Scrophulariaceae families.

PL-4 was obtained as colorless and amorphous powder. The molecular ion peak at m/z 389 $[M+1]^+$ in the EI-mass spectrum and ^{13}C NMR values indicated the molecular formula of PL-4 as $C_{19}H_{32}O_8$. The anomeric proton signal at δ_H 4.34 (d, *J*=8.0 Hz) and signals between δ_H 3.17-3.81 ppm indicated the presence of *β*-glucopyranose moiety. 19 carbon resonances were observed in ^{13}C NMR spectrum of PL-4. Carbon signals were determined as three quaternary (C), nine methyne (CH), three methylen (CH_2) and 4 methyl (CH_3) from the examination of DEPT 135 spectrum and 13 carbon signals were attributed to aglycon. Three tertiary metyhl groups at δ_H 1.18 (s, H_3-11), 1.11 (s, H_3-12) and 0.96 (s, H_3-13) and one secondary methyl group at δ_H 1.27 (d, *J*=6.2 Hz, H_3-10) were observed in ^{1}H NMR spectrum of PL-4. Long range correlations of tertiary methyl group in HMBC spectrum showed that tertiary methyl groups were located on the C-1 and C-5 of aglycon. The ^{1}H-NMR signals at δ_H 5.95 (d, *J*=16.0 Hz), 5.71 (dd, *J*=16.0/6.8 Hz) and ^{13}C NMR signals at δ_C 127.75 (C-7), 137.14 (C-8) indicated the presence of *trans* double bond between C-7 and C-8 carbons. Observation of the same spin system for δ_H 1.22 and 1.54 methylens with δ_H 3.73 (1H, m, H-3) and H-3 with δ_H 1.59 and δ_H 2.25 methylens, the correlations of δ_H 5.95 (d, J = 16.0, H-7) with δ_H 5.71 (dd, J = 16.0/6.8, H-8), H-8 with δ_H 4.41 (t, *J* = 6.3, H-9) and H-9 with δ_H 1.27 (d, *J* = 6.2, H-10) revealed megastigman structure for PL-4. On the other hand HMBC correlation between anomeric proton (δ_H 4.34 d, *J*=8.0 Hz) and δ_C 76.9 (CH, C-9) indicated the substitution of glucose unit from the 9th position of the aglycon. From the above results, the structure of PL-4 was determined as phlomuroside and this was confirmed by the comparison its data with published for phlomuroside (14,15). This is the first report for the isolation of megastigman glucoside from the genus *Plantago*.

Phytochemical studies on iridoidic fraction of *Plantago lagopus* which is widely distributed Aegean and Mediterranean area of Turkey were resulted to the isolation of catalpol (PL-1), aucubin (PL-2), rehmaglutin D (PL-3) and phlomuroside (PL-4). While aucubin and catalpol are characteristic iridoid glucosides for Schrophulariaceae and Plantaginaceae family such as *Veronica*, *Verbascum* and *Plantago*

species, rehmaglutin D and phlomuroside were isolated from the genus *Plantago* for the first time in this study (16,17). Their isolation is very significant for the chemotaxonomical researches on *Plantago* species and Plantaginaceae family. Our studies on different secondary metabolites from *Plantago* species have been continuing.

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