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Amino Acid, Flavonoid and Neolignan Glucosides From *Astragalus melanophrurius*

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Introduction

Astragalus genus (Leguminosae) is represented by approximately 380 species in the flora of Turkey[1] among which *A. mirocephalus* is used primarily for the production of the economically important gum, traganth[2]. The roots of several *Astragalus* species represent a very old and well-known drug in traditional medicine for its usage in the treatment of nephritis, diabetes, leukemia, uterine cancer and as an antiperspirant, diuretic and tonic[3]. Our earlier investigations on *Astragalus* species resulted in the isolation of a series of cycloartane-type triterpenic saponins[4-14], flavonol, lignan and simple phenolic glycosides[15,16]. In previous study, the isolation of eight cycloartane saponins from the roots of an endemic species *A. melanophrurius* which were found to stimulate lymphocyte transfer in vitro were reported[5]. Continuing our studies on the constituents of *Astragalus* species, we investigated the aerial parts of *A. melanophrurius*. In this study we report the isolation and structure elucidation of an amino acid, tryptophan **(1)**, a flavonol glucoside, isorhamnetin 3-*O*-β-D-glucopyranoside **(2)** and a neolignan, dehydrodiconiferyl alcohol-4-*O*-β-D-glucopyranoside **(3)** from the aerial parts of *A. melanophrurius*.

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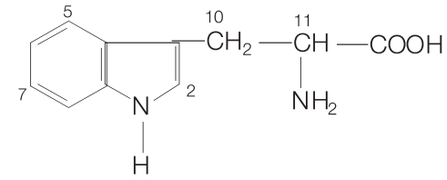
Material and Methods

General experimental procedures: UV spectra were recorded on a Hewlett-Packard 5500/hp compaq spectrophotometer. IR spectra were measured on a Bruker Vector 22IR (Opus Spectroscopic Software Version 2.0) spectrophotometer. NMR spectra were recorded on a Bruker AMX 600 and 300 spectrometers in methanol-d_4 (^{1}H: 300 and 600 MHz; ^{13}C: 75 and 150 MHz). HR-FAB-MS spectra were obtained on an Ion Spec Ultima FTMS spectrometer. For vacuum liquid chromatography (VLC), reversed phase material LiChroprep C_{18} was used. Column chromatography was carried out on silica gel (Kieselgel 60, 60-230 mesh) and sephadex LH-20 were used. For thin layer chromatography (TLC), pre-coated Kieselgel 60 F_{254} aluminum sheets were used. Compounds were detected by UV fluorescence and 1 % vanilin/H_2SO_4 followed by heating at 100^{0}C for 5 min.

Plant material: *A. melanophrurius* Boiss. was collected from Ahlatlıbel, Ankara in May 2002. Voucher specimens (94-003) have been deposited at the Herbarium of the Faculty of Pharmacy, Hacettepe University.

Extraction and isolation: Air dried overground parts of the plant (400 g) were extracted with methanol (2x2 l). The methanolic extract was evaporated under vacuum. The residue (51 g) was dissolved in water and then extracted with chloroform. The water extract (24 g) was subjected to VLC (Vacuum Liquid Chromatography) using reversed phase material LiChroprep C_{18} with H_2O (500 ml), H_2O-MeOH (9:1, 200 ml; 8:2, 200 ml; 7:3, 200 ml; 5:5, 200 ml) and MeOH (500 ml) to yield eleven fractions (A-K). Fr. F (106 mg) eluted with H_2O-MeOH (70:30) was further subjected to Si gel column with $CHCl_3$-MeOH (9:1, 8:2) and $CHCl_3$-MeOH-H_2O (80:20:1 60:40:4) solvent systems to give ten fractions ($F_{1\text{-}10}$). $F_{5\text{-}9}$ (24 mg) was rechromatographed over Si gel column using $CHCl_3$-MeOH-H_2O (80:20:1 70:30:3) solvent system to give **1** (7.3 mg). Fr. I (1 g) eluted with H_2O-MeOH (50:50) from VLC was rechromatographed over Si gel column with $CHCl_3$-MeOH-H_2O (80:20:2 70:30:3) solvent system to give thirteen fractions ($I_{1\text{-}13}$). Fr. I_2 (87 mg) was further subjected to Sephadex LH-20 column with MeOH to give **2** (26 mg). Fr. I_3 (74 mg) was subjected to Sephadex LH-20 column with MeOH and rechromatographed over Si gel column with $CHCl_3$-MeOH-H_2O (80:20:2) solvent system to give **3** (6 mg).



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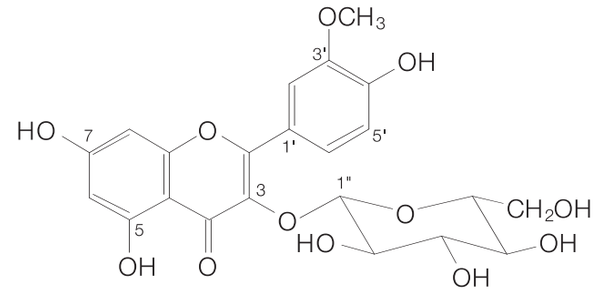

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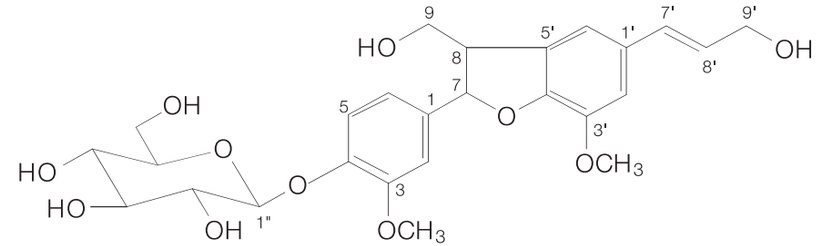

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Figure 1
Compounds isolated from *Astragalus melanophrurius*

Results

Tryptophan (1): $C_{11}H_{12}O_2N_2$

UV λ_{max} (MeOH) nm: 212, 280, 289; IR ν_{max} (KBr) cm^{-1}: 3450 (NH), 3400 (OH), 1620 (C=C), 1600 (arom. ring); ^{1}H-NMR (CD_3OD, 600 MHz): Table I.

TABLE I

^{1}H- NMR Data of triptophan (**1**) (CD_3OD).

H	δ (ppm)	J (Hz)
2	7.22 s	
5	7.73 d	(7.8)
6	7.08 t	(7.8)
7	7.15 t	(7.8)
8	7.39 d	(7.8)
10	3.18 dd	(9.6/15.0)
	3.55 dd	(3.6/15.0)
11	3.89 dd	(3.6/9.6)

Isorhamnetin 3-*O*-β-D-glucopyranoside (2): $C_{22}H_{22}O_{12}$

UV λ_{max} (MeOH) nm: 256, 267 (sh), 352; IR ν_{max} (KBr) cm^{-1}: 3400 (OH), 1655 (C=O), 1606, 1508 (arom. rings); ^{1}H- (CD_3OD, 600 MHz) and ^{13}C-NMR (CD_3OD, 150 MHz): Table II.

Dehydrodiconiferylalcohol-4-*O*-β-D-glucopyranoside (3):

$C_{26}H_{32}O_{11}$

UV λ_{max} (MeOH) nm: 211, 274; IR ν_{max} (KBr) cm^{-1}: 3400 (OH), 1599, 1512 (arom. rings); ^{1}H- (CD_3OD, 300 MHz) and ^{13}C-NMR (CD_3OD, 75 MHz): Table 3. HR-FAB-MS: (m/z) 543.1842 $[M+Na]^+$.

TABLE II

^{1}H- and ^{13}C-NMR Data of Isorhamnetin 3-*O*-β-D-glucopyranoside (**2**) (CD_3OD).

C/H	δ_C (ppm)	δ_H (ppm)	J (Hz)	HMBC (H→C)
2	158.6			
3	135.3			
4	180.0			
5	163.0			
6	99.4	6.22 d	(2.0)	C-8
7	166.9			
8	94.7	6.41 d	(2.0)	C-2, C-6, C-7,C-10
9	158.0			
10	105.4			
1'	122.8			
2'	113.9	7.95 d	(2.0)	C-2, C-3', C-4',C-6'
3'	148.1			
4'	150.6			
5'	116.0	6.93 d	(8.0)	C-1', C-3', C-4'
6'	123.8	7.62 dd	(8.0/2.0)	C-2, C-2', C-4'
1''	103.5	5.43 d	(7.8)	C-3
2''	75.5			
3''	77.6	3.28-3.50*		
4''	71.1			
5''	78.4			
6''	62.1	3.60 dd	(12.0/4.2)	
		3.77 dd	(12.0/2.0)	
OCH_3	56.8	3.97 s		

*Signal pattern unclear due to overlapping

TABLE III

^{1}H- and ^{13}C-NMR Data of Dehydrodiconiferyl alcohol-4-*O*-β-D- glucopyranoside (**3**) (CD_3OD).

C/H	DEPT	δ_C (ppm)	δ_H (ppm)	J (Hz)
1	C	138.0		
2	CH	111.1	7.02 d	(1.8)
3	C	150.9		
4	C	147.7		
5	CH	117.9	7.15 d	(8.3)
6	CH	119.4	6.92 dd	(8.3/1.8)
7	CH	88.8	5.58 d	(5.9)
8	CH	55.4	3.50*	
9	CH_2	64.9	3.85*	
			3.79*	
3-OCH_3	CH_3	56.7	3.83 s	
1′	C	130.0		
2′	CH	112.1	6.95*	
3′	C	145.5		
4′	C	149.0		
5′	C	132.7		
6′	CH	116.2	6.95*	
7′	CH	132.7	6.54 d	(15.8)
8′	CH	127.6	6.23 dt	(15.8/5.7)
9′	CH_2	63.8	4.20 dd	(5.7/1.0)
3′ -OCH_3	CH_3	56.6	3.88 s	
1″	CH	102.7	4.89*	
2″	CH	74.9		
3″	CH	77.8	3.39-3.50	
4″	CH	71.3		
5″	CH	78.2		
6″	CH_2	62.5	3.85*	
			3.67 dd	(11.9/5.0)

*Signal pattern unclear due to overlapping

Discussion

Compound **1** was obtained as an amorphous powder. ^{1}H-NMR spectrum of **1** showed the presence of typical aromatic signals belonging to the C-3 substituted indol derivative (Table I). The aliphatic proton signals, a methine δ_H 3.89 (dd, J= 3.6/9.6 Hz) and two neighboring geminal methylene protons δ_H 3.18 (dd, J= 9.6/15.0 Hz), δ_H 3.55 (dd, J= 3.6/15.0 Hz) respectively were attributed to the side chain attached at C-3 of the indol unit. Thus compound **1** was identified as tryptophan by comparison of its spectral data with literature values[8].

Compound **2** was obtained as a yellow amorphous powder. The IR and UV spectral data of the compound showed its phenolic structure. The UV absorbtions (UV λ_{max} 256, 267 (sh), 352) suggested **2** to be a flavonol. In the ^{1}H-NMR spectrum of **2** the two meta-coupled doublets at δ_H 6.22 (J= 2.0 Hz, H-6) and δ_H 6.41 (J= 2.0 Hz, H-8) in the aromatic region which showed a correlation with carbons at δ_C 99.4 and δ_C 94.7 in the HSQC spectrum were consistent with a 5,7-dihydroxy A ring of the flavonoid (Table 2). The ^{1}H-NMR resonances at δ_H 7.95 (d, J= 2.0 Hz), δ_H 6.93 (d, J= 8.0 Hz) and δ_H 7.62 (dd, J= 8.0/2.0 Hz) were attributed to H-2′, H-5′ and H-6′, respectively. Additionaly, the singlet at δ_H 3.97 in the ^{1}H-NMR spectrum was indicative for the presence of a methoxyl group which showed a correlation with the carbon resonance at δ_C 148.1 (C-3′) in the HMBC spectrum. According to the findings based on 1D and 2D NMR spectra, aglycone was found to be isorhamnetin[16]. The remaining proton and carbon resonances were indicative for the presence of a β-D-glucose unit. The anomeric proton signal at δ_H 5.43 (d, J= 7.8 Hz) which shows long-range correlation with C-3 of the aglycone (δ_C 135.3) indicated the site of the glycosidation. Thus compound **2** was identified as isorhamnetin 3-*O*-β-D-glucopyranoside[17,18].

Compound **3** was obtained as an amorphous powder. The high resolution (HR)-FAB-MS spectrum of the compound exhibited a pseudomolecular ion peak at m/z 543.1842 $[M+Na]^+$ which is compatible with the molecular formula $C_{26}H_{32}O_{11}$. The signals observed in the ^{1}H- and ^{13}C-NMR spectra of compound **3** suggested a dehydrodiconiferyl alcohol type lignan[19-21] (Table 3). The ^{1}H-NMR spectrum of **3** revealed the signals for five aromatic protons. The 2H resonance at δ_H 6.95 indicated the presence of a tetrasubstituted aromatic moiety. The proton resonances

at δ_H 6.92 (dd, J= 8.3/1.8) Hz), 7.02 (d, J= 1.8 Hz) and 7.15 (d, J= 8.3 Hz) observed as an ABX system in the ^{1}H-NMR spectrum were indicative for the presence of a additional trisubstituted aromatic moiety. The signals at δ_H 6.54 (d, J= 15.8 Hz) and 6.23 (dt, J= 15.8/5.7 Hz) belonging to two trans olefinic protons were attributed to the side chain of coniferyl alcohol moiety. Additionaly the methylene protons at δ_H 3.85, 3.79 (δ_C 64.9), two methine protons at δ_H 3.50 (δ_C 55.4) and δ_H 5.58 (d, J= 5.9 Hz) (δ_C 88.8) suggested the dehydrofuran ring and its side chain. The signals at δ_H 4.89 and δ_C 102.7 arrising from the anomeric proton and carbon resonances were consistent for a β-linked glucose moiety indicating the monoglucosidic structure of **3**. The carbon resonances attributed to the methylene groups of the side chain of dehydrofuran (δ_C 64.9, C-9) and the side chain of coniferyl alcohol (δ_C 63.8, C-9′) showing no downfield shifts revealed that glycosidation was not at these locations. Therefore, the downfield shift resonance at δ_C 147.7 assigned to C-4 was found to be the site of the glycosidation. Consequently, the structure of compound **3** was identified as dehydrodiconiferyl alcohol-4-*O*-β-D-glucopyranoside[20,21].

In our previous studies, in addition to cycloartene-type triterpenic saponins[4-14], an indol derivative, achillamide (*N*-[3-hydroxy-3-methyl-glutaroyl]-tryptophan)[8], flavonol (isorhamnetin glycosides)[16], lignan [(+)-neo-olivil 4-*O*-β-apiofuranosyl-(1→2)- β-glucopyranoside] and simple phenolic glycosides[15] were reported. This study resulted in the isolation of an amino acid, tryptophan, a flavonol glucoside, isorhamnetin 3-*O*-β-D-glucopyranoside and a neolignan, dehydrodiconiferyl alcohol-4-*O*-β-D-glucopyranoside. Therefore, *Astragalus* species were found to be worthy to be investigated in point of cycloartane derivatives as well as indol derivatives, flavonoids, lignan and neolignan glycosides.

Summary

An amino acid, tryptophan, a flavonol glucoside, isorhamnetin 3-*O*-β-D-glucopyranoside and a neolignan, dehydrodiconiferyl alcohol-4-*O*-β-D-glucopyranoside were isolated from the aerial parts of *Astragalus melanophrurius* and their structures were identified by spectroscopic (UV, IR, ^{1}H-^{13}C-NMR) methods.

Key Words: Astragalus melanophrurius, Leguminosae, Amino acid, Flavonoid, Neolignan, Tryptophan, Isorhamnetin 3-*O*- β-D-glucopyranoside, Dehydrodiconiferyl alcohol-4-*O*-β-D-glucopyranoside

Özet

Astragalus melanophrurius'un Amino Asit, Flavonoit ve Neolignan Glikozitleri

Astragalus melanophrurius'un toprak üstü kısımlarından bir amino asit, triptofan, bir flavonol glukoziti, izoramnetin 3- *O*-β-D-glukopiranozit ve bir neolignan, dehidrodikoniferil alkol-4-*O*-β-D-glukopiranozit elde edilmiş ve yapıları spektroskopik yöntemler (UV, IR, ^{1}H-^{13}C-NMR) yardımıyla aydınlatılmıştır.

Anahtar Kelimeler: Astragalus melanophrurius, Leguminosae, Amino asit, Flavonoit, Neolignan, Triptofan, İzoramnetin 3-*O*- β-D-glukopiranozit, Dehidrodikoniferil alkol-4-*O*-β-D-glukopiranozit

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