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Research Article / Araştırma Makalesi

FLAVONOIDS FROM ORNITHOGALUM UMBELLATUM L.

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Özet: Bu çalışmada, Edirne civarında yetişen *Ornithogalum umbellatum* L.bitkisinin içerdiği flavonoid bileşikleri incelenmiştir. Bitkinin etanolik ekstraktı kromatografik teknikler yardımı ile ayrılmış ve saflaştırılmıştır. İzole edilen bileşiklerin kimyasal yapıları UV, IR, NMR ve EI kütle spektroskopileri kullanılarak aydınlatılmıştır. Isovitexin **(1)**, Saponarin **(2)**, Isoorientin **(3)** olmak üzere üç C-glikozil flavonoid *Ornithogalum umbellatum L.*bitkisinin toprak üstü kısımlarında tespit edilmiştir.

Anahtar Kelimeler: C-glikozid flavonoidler,*Hyacinthaceae*, *Liliace*, *Ornithogalum.*

Ornithogalum Umbellatum L. Bitkisindeki Flavonoidler

Absract: In this study, the flavonoids in *Ornithogalum umbellatum L.* grows around Edirne have been investigated. Ethanolic extracts of the plant were seperated and purificated by chromatographic techniques. The chemical structures of the isolated compounds were elucidated by UV, IR, NMR and EI and FAB mass spectroscopies. Three C-glycosylflavones were identified in the aerial parts of *Ornithogalum umbellatum L.*; Isovitexin **(1)**, Saponarin **(2)**, Isoorientin **(3)**.

Key words: C-glycosyl flavonoids, *Hyacinthaceae*, *Liliace*, *Ornithogalum.*

Introduction

Flavonoids are widely distributed group of plant substances that are also important for normal plant growth, development and defence against infection and injury. The compounds obtained from natural sources are the active ingredients of many folk medicines, still used in various parts of the world (Sakushima et.al.,1989).

Aerial parts of the *Ornithogalum umbellatum L.* were studied in order to determine the flavonoid compounds. Three flavonoids were found in the arial parts of *O. umbellatum*. These flavonoids were identified as isovitexin (1), saponarin (2) and isoorientin (3). The occurence of isovitexin was previously noted from *O. kochii*, *O. algerience* and *O. gussonei* Ten. Saponarin was also found in *O. Gussonei* and isovitexin-7-X"-di-O-glycoside was found in *O. umbellatum* and *O. Kochii* (Azzioui and Braemer,1989). In this study, we also found isoorientin not detected previously. All of the flavonoids were present as glycosides. The chemical structure of these compounds were elucidated by UV, IR, NMR and EI mass spectrscopies (Bhatia et.al., 1966).

Experimental

Materials:

General: ^{1}H NMR spectras were recorded at Bruker AC 400 MHz in DMSO-d_6. IR spectras (Shimadzu IR-470 spectrometer) were recorded in MeOH. UV (Shimadzu UV 160 A) were recorded in MeOH. EI mass data were obtained at VG-zab spectrometer and FAB MS at ZabSpec. Sephadex LH-20(Fluka 22181) was used for column chromatography and silicagel 60 F 254 (Merck) precoated plates for TLC. Spots were detected under a UV lamp or by heating after spraying with ceric sulphate solution. Whatmann 3MM chromatograpic paper (46x57) was used for two dimensional paper chromatography. In paper chromatography NA reagent*(Naturstoffreagenz A: Diphenilboricacid β-amino ethyl ester)* was used for determination of the color reactions of the compounds.

Plant material: *Ornithogalum umbellatum L*. was collected from Edirne in 1995 and identified by Assoc. Prof. G. Dalgıç; a voucher specimen is deposited in the Herbarium of the Biology Department, Faculty of Science-Arts, University of Trakya, EDTU 1923.

Method

Extraction and Isolation of the Compounds: The arial parts of the dried plants (338 g) were extracted with 80% EtOH in a soxhlet apparatus. The extract was evaporated under vacuum. The residue was suspended in water and extracted with Et_2O, EtOAc and BuOH respectively. About 0.2-0.3 g residue from the BuOH extract was dissolved in MeOH. Then this solution was spotted using a pipette on the lower right-hand corner of a sheet Whatmann 3MM chromatographic paper about 4 cm in diameter. Two dimensional paper chromatogram was developed descendingly in the long direction in a chromatocab using ter-butanol:acetic acid:water (TBA) (3:1:1). When the solvent front reach to lower edge of the paper(after25 hr), the chromatogram removed from the cabinet. The dry chromatogram was folded along along the edge adjacent to the band containing flavonoids and then developed descendingly in the second direction with AcOH (acetic acid:water) (15:85) solvent systems. After 4 hr for completion of the run The dried two dimensionally developed chromatogram was viewed in UV light alone and in the precence of ammonia fumes then by spraying NA reagent (Mabry, 1970). All spots were detected by this procedure and the R_f (TBA and AcOH) values for each of the spots were calculated. It was clear from the chromatogram that there were three spots

which indicated the flavonoids compounds. These spots were called as 1, 2 and 3. The R_f values and the colour reactions of the spots were shown in Table-1.

Two dimensional paper chromatography was also used for the isolation of the compounds which detected as flavonoids. That chromatographic procedure was repeated 30 times without using ammonia fumes and NA reagent. After detection of the spots under UV light, they were circled with a lead pencil. These spots were cut out from the paper by scissors. The compounds could isolated by extraction of the pieces of paper so each pieces cut in to small sections. Same spots combined together in to in a erlenmeyer flask. Then each compound was solved in MeOH. After filtration and pump vacuum evaporation of the extract yields the flavonoid compounds. For the final purification of the compounds Sephadex LH-20 was used. Elution system was MeOH.

Table 1. The R_f values of the compound **(1)**, **(2)**, **(3)**

Compound	*TBA R_f*	*AcOH R_f*	*UV*	*UV/NH_3*	*UV/NA*
1	0.54	0.55	Deep purple	Yellow	Yellow
2	0.30	0.70	Deep purple	Yellow	Yellow
3	0.41	0.37	Deep purple	Yellow	Orange

Results

Three flavonoid compounds, **Isovitexin (1)**(10 mg), **Saponarin (2)**(12 mg), **Isoorientin (3)** (15 mg), were isolated in the arial parts of *Ornithogalum umbellatum* L..The chemical structures of the isolated compounds were elucidated by spectroscopic methods. The obtained spectral results were shown in the Table2 and Table3.

Table 2. UV, IR and EI mass spectral data for **Isovitexin(1)**, **Saponarin(2)** and **Isoorientin(3)**

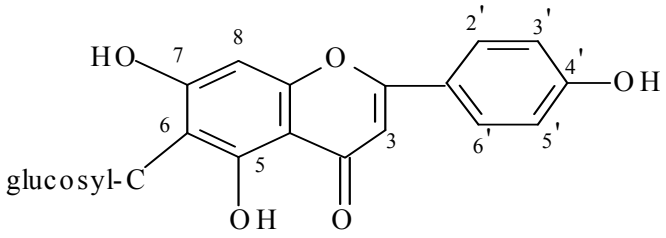
		Isovitexin (1)	**Saponarin (2)**	**Isoorientin (3)**
UV λ_{max} (MeOH) nm	MeOH	271,335	271,336	256,270,349
	NaOMe	279,326,397	271,389	278,388,406
	$AlCl_3$	278,304,352,382	277,301,352,381	275,318,424
	$AlCl_3$/HCl	280,302,344,379	279,300,344,378	276,363,381
	NaOAc	279,303,385	271,350,392	277,326,401
	NaOAc/H_3BO_3	274,346	271,341	267,382
IR γ_{max} (MeOH) cm^{-1}		3440,1660,1620,1523, 1475,1017,848,805,	3440,1660,1625,1520, 1462,1020,840,805	3376,1660,1628, 1561, 1446,1030,845, 800
[#]EI-MS (m/z) (rel.int.)		281.4(10) [A^+], 93.8(53), 72.9(32), 83(20), 59.9(10), 98(10), 111.1(5), 121(5)	279.5(11) [A^+], 69.1(100), 97.1(87), 83.1(86), 111.1(48), 125.1(20), 149(19), 191.3(12), 163.2(10)	
FAB-MS (m/z) (rel.int.)				448.3(6) [$M]^+$, 298.2(16) [A^+],176(32), 214(13), 284.3(12)

[#]The molecular ion peaks were not observed in the EI mass spectrum of compounds **(1)**, **(2)**

Table 3. ^{1}H NMR data for **Isovitexin(1)**, **Saponarin(2)** and **Isoorientin(3)** measured at 400 MHz in DMSO-d_6

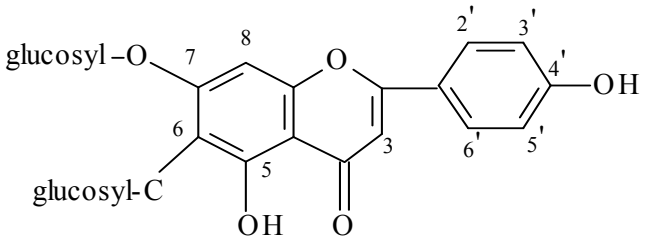
H	**Isovitexin (1)**	**Saponarin (2)**	**Isoorientin (3)**
H-3	6.25(1H, s)	6.23 (1H, s)	6.25 (1H, s)
H-8	6.50 (1H, s)	6.23 (1H, s)	6.75(1H, s)
H-2'	7.62 (2H, d, J=8 Hz)	7.86 (2H, d, J=8 Hz)	7.80 (m)
H-3'	6.84 (2H, d, J=8 Hz)	6.85 (2H, d, J=8 Hz)	
H-5'	6.84 (2H, d, J=8 Hz)	6.85 (2H, d, J=8 Hz)	6.80 (1H, d, J=9 Hz)
H-6'	7.62 (2H, d, J=8 Hz)	7.86 (2H, d, J=8 Hz)	7.80 (m)
Sugar protons	3.5-4.7 (m)	3.5-4.9 (m)	3.5-4.8 (m)
O-glucosyl H-1"		5.27 (1H, d, J=7 Hz)	
C-glucosyl H-1"	4.88 (1H, d, J=8 Hz)		4.93 (1H, d, J=7 Hz)
C-glucosyl H-1"'		4.98 (1H, d, J=6.5 Hz)	

Discussion



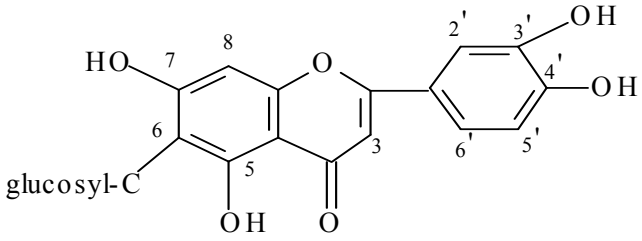
(1)

The UV spectrum of the compound (1) was an indication of hydroxyl groups at C-4', C-5 and C-7 because of the presence of a bathochromic shift with NaOMe, NaOAc and $AlCl_3$/HCl in its UV spectrum. The IR spectrum of the compound (1) showed absorptions at 3440 cm^{-1}(OH), 1660 cm^{-1} (C=O), 1620 cm^{-1} (C=C), 1523, 1475 cm^{-1} (Aromatic group), 1017 cm^{-1} (C-O). The ^{1}H NMR spectrum of the compound (1) showed six aromatic proton resonances from δ 6-8. A set of four AA'BB'proton signals at δ 7.62 (2H, d, J=8 Hz, H-2'and H-6') and 6.84 (2H, d, J=8 Hz, H-3', H-5') located in ring B. H-8 and H-3 protons were observed as singlets at δ 6.5 (1H) and 6.25 (1H) respectively. The anomeric proton showed a doubled at 4.88 ppm (1H, d, J=8 Hz, glucosyl H-1"). Sugar proton signals overlapped at 3.5-4.7 ppm. In the EI mass spectrum of the compound (1), aglycon peak $[A]^+$ was observed at m/z 281.4. This ion was formed by the loss of $C_5H_{10}O_5$ from the molecular ion. On the basis of UV, IR, NMR, EI mass data, compound (1) has been identified as isovitexin.



(2)

The bathochromic shifts observed in UV spectrum with NaOMe and $AlCl_3$/HCl was an indication of the presence of hydroxyl groups at C-4' and C-5 in compound **(2)**. Its IR spectra showed the absorptions at 3440 (OH), 1660 (C=O), 1625 (C=C), 1520, 1462, 840, 805 (Aromatic group), 1020 (C-O). The ^{1}H NMR spectrum of the compound **(2)** correlated with the suggested structure: δ 7.86 ppm (2H, d, J=8 Hz, H-2', H-6'), 6.85 ppm (2H, d, J=8 Hz, H-3', H-5'), 6.23 ppm (2H, s, H-3 and H-8 overlapped), 5.27 ppm (1H, d, J=7 Hz, O-glucosyl H-1"), 4.98 ppm (1H, d, J=6.5 Hz, C-glucosyl H-1'"), 3.5-4.9 ppm (12 sugar proton signals overlapped). The EI mass spectrum of the compound **(2)** showed an aglycon peak $[A]^+$ at m/z 279.5 which indicates C-glycoside and O-glycoside moeity eliminated from flavon. Molecular ion peak couldn't observed in this spectrum. All the above evidence showes that compound **(2)** is saponarin.



(3)

In the UV spectrum of the compound **(3)**, bathochromic shifts with NaOMe, NaOAc and $AlCl_3$/HCl was observed. That observation in UV spectrum was an indication of the presence of hydroxyl groups at C-4', C-5 and C-7. Compound **(3)** showed hydroxyl (3376 cm^{-1}), carbonyl (1660 cm^{-1}) and aromatic groups (1561, 1446, 845, 800 cm^{-1})absorptions in its IR spectrum. The ^1H NMR spectrum of the compound **(3)** indicated that B ring protons, H-2'and H-6' gave multiplet at δ 7.80 and H-5' proton signal was observed at 6.8 ppm as a dublet(J=9 Hz). H-8 and H-3 protons were at δ 6.75 (1H, s) and δ 6.25 (1H, s) respectively. Anomeric proton was observed at δ 4.93 (1H, d, J=7 Hz, C-glucosyl H-1") and sugar proton signals overlapped at δ 3.5-4.8. In the FAB(+) mass spectrum of the compound **(3)**, aglycon peak $[A]^+$ was observed at m/z 298.2. This ion was formed by the loss of $C_5H_{10}O_5$ from the $[M]^+$ ion peak which was observed at *m/z* 448.3 . From the above results the structure of the compound **(3)** was determined as isoorientin.

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