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THE CONSTITUENTS OF THE AERIAL PARTS OF *GLAUCIUM GRANDIFLORUM* VAR. *GRANDIFLORUM*

GLAUCIUM GRANDIFLORUM VAR. *GRANDDIFLORUM*'UN TOPRAK ÜSTÜ KISIMLARININ KİMYASAL BİLEŞENLERİ

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The constituents of Glaucium grandiflorum var. grandiflorum collected from Doruksaray (Erzincan) have been investigated and the major alkaloids of the aerial parts were identified to be allocryptopine and protopine. In addition (-)-α-N-methylcanadine, (-)-β-N-methylcanadine, (+)-reticuline and berbithine have been isolated as minor alkaloids. The occurrence of berbithine in Papaveraceae family and of (+)-reticuline in G.grandilorum var. grandiflorum species have been shown for the first time. In addition to these alkaloids, in the quaternary base portion, the presence of ferulic acid amide (ferulamide) was also shown which is the first case of the presence of ferulamide as a natural substance.

Bu çalışmada Glaucium grandiflorum var. grandiflorum türünün Doruksaray (Erzincan) dan toplanan örneğinin kimyasal bileşenleri incelenmiştir. Bitkinin toprak üstü kısımlarından allokriptopin ve protopin ana alkaloitler, (-)-α-N-metilkanadin, (-)-β-N-metilkanadin, (+)-retikulin, berbithin minör alkaloitler olarak izole edilmiştir Berbithin'in Papaveraceae familyasında, (+)-retikulin'in ise G.grandiflorum var. grandiflorum türünde varlığı ilk defa bu çalışma ile ortaya konmaktadır. Bu alkaloitlere ilave olarak katerner alkaloid fraksiyonundan ferulik asit amidi (ferulamid) elde edilmiştir. Ferulamid doğal kaynaklardan ilk defa bu çalışma ile elde edilmiş olmaktadır.

Keywords: *Glaucium grandiflorum var. grandiflorum; Isoquinoline alkaloids; Ferulamide*

Anahtar Kelimeler: *Glaucium grandiflorum var. grandiflorum; İzokinolin alkaloidleri; Ferulamid*

Introduction

Glaucium grandiflorum Boiss. et Huet. (Papaveraceae) is a perennial herb indigenous to various regions of the Middele East extending from the eastern Mediterranean to Iran (1). In the Flora of Turkey, *G. grandiflorum* has two varieties as var. *grandiflorum* and var. *torquatum*, of which the latter is indigenous to Turkey (2). The fruits of *G. grandiflorum* have been used as folk medicine in Turkey for the purification of blood and in the treatment of ophthalmic diseases (3). It is also reported that the sap of this plant is

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being used widely in Iran for the same purposes(4).

Previous investigations on the alkaloids of *G. grandiflorum* revealed the existence of protoberberine (berberine, (+)-tetrahydrojatrorrhizine, (+)-tetrahydropalmatine), protopine (allocryptopine, protopine), aporphine [(+)-glaucine, glauvine, isoboldine, isocorydine, oxoglaucine, thalicmidine], benzophenanthridine [(+)-8-acetonyldihydrochelerythrine, dihydrochelerythrine, (-)-norchelidonine, sanguinarine] type alkaloids (5, 6). Prior findings on the alkaloids of Turkish samples of *G. grandiflorum* were as follows: *G. grandiflorum* var. *torquatum*: Protoberberine (canadine methochloride), protopine (allocryptopine, protopine), aporphine (corydine, (+)-glaucine, isocorydine) types(7). *G. grandiflorum* var. *grandiflorum*: Protoberberine (N-methylcanadine), protopine (protopine, allocryptopine), aporphine (isocorydine, corytuberine, corydine) types(8).

In the present work the constituents of the aerial parts of *G. grandiflorum* var. *grandiflorum* collected from other parts of Anatolia have been investigated.

Material and Methods

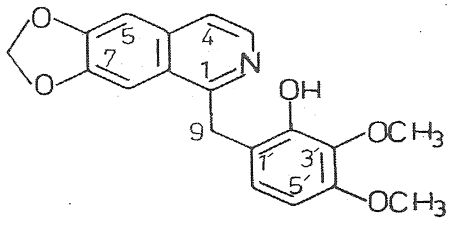
Plant material

The plant material used in this study was collected from Doruksaray near Erzincan (Eastern Anatolia) in June 1995. Voucher specimens were retained in the Herbarium of the Faculty of Pharmacy, Istanbul University (ISTE 68132).

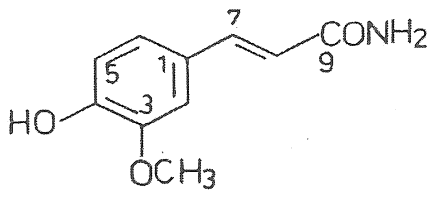
Extraction, isolation and identification

The dried and powdered aerial parts (2.6 kg) were extracted by percolation with EtOH. Total tertiary alkaloids (14.17 g) and quaternary alkaloids (0.427 g) were then obtained following the reported methods(9). It was observed that quaternary alkaloids (-)-α-N-Methylcanadine, and (-)-β-N-methylcanadine passed partly into chloroform, even with anions different from iodide (probably with chloride) when isolated in the described manner; thus they passed into tertiary alkaloid fraction from where they have been obtained and were separated from the nonquaternary base present in this fraction.

Separation of allocryptopine (3150 mg), protopine (376 mg), (-)-α-N-methylcanadine(10 mg), (-)-β-N-methylcanadine (8 mg), (+)-reticuline (4 mg), berbithine (2 mg) was achieved by column chromatography on silica gel (200 g) using $CHCl_3$ and $CHCl_3$: MeOH (9:1, 1:1) as the elution solvents. 136 fractions of 50 ml each were collected. Preparative TLC was then used for further separation and purification on silica gel with systems C_6H_6: Me_2CO: NH_3 (8:2:0.05), toluene: Me_2CO: MeOH: NH_3 (13:13:5:1), toluene: Me_2CO: EtOH: NH_3 (45:45:7:3). Separation of ferulamide (5.8 mg) was by preparative TLC on silica gel using the system $CHCl_3$: MeOH: NH_3 (15:5:1). The structures of the alkaloids were confirmed by spectral (UV, IR, ^{1}H-NMR, ^{13}C-NMR and EIMS) and physical ($[\alpha]^{20}_D$, m.p.) methods (7-15). The spectral data of berbithine: UV (MeOH): λ_{max} 278, 316, 328 nm, EIMS: m/z (%)= 306(12), 292(24), 163(20), 277(22), 264(84), 263(62), 262(26), 248(14), 247(24), 235(13), 234(23), 224(53), 220(20), 207(23), 206(29), 205(23), 194(49), 187(100), 177(66). CIMS: m/z = 338(M-1), 340(M+1). ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 3.73 (s, OCH_3), 3.84 (s, OCH_3), 4.33 (2H, s, H-9), 6.04 (OCH_2O), 6.31 (d, J = 8.48 Hz, H-5'), 6.84 (d, J=8.48, H-6'), 7.00 (s, H-5), 7.31 (d, J = 5.73 Hz, H-4), 7.55 (s, H-8), 8.15 (d, J = 5.72, H-3). The spectral data of ferulamide is presented here for the first time: UV (MeOH): λ_{max} = 217, 232, 292, 317 nm; (MeOH + NaOH): λ_{max} = 239, 250, 306, 364 nm. IR ($CHCl_3$): ν = 3416, 2918, 2849, 1662, 1589, 1515, 1465, 1430, 1399, 1278, 1160, 1126, 1031, 980, 938, 817 cm^1. EIMS: m/z (%) = 193(100), 177(63), 163(20), 148(36), 133(30), 117(26), 105(24). The NMR data have been included in Table.



Berbithine



Ferulamide

Table. ^{1}H-NMR, ^{13}C-NMR and HMBC spectral data for ferulamide. Coupling constants Hz in paranthesis.

Position	δ H ($CDCl_3$)	δ H (DMSO)	δ C (DMSO)	HMBC
1	-	-	126.4	-
2	7.00 d (1.8)	6.76 d (1.7)	110.0	C-4, C-6, C-7
3	-	-	147.4	-
4	-	-	148.2	-
5	6.83 d (7.9)	6.58 d (8.1)	115.3	C-1, C-3
6	7.01 dd (1.8, 8)	6.72 dd (1.7, 8.2)	121.8	C-2, C-4, C-7
7	7.48 d (15.7)	7.20 d (15.7)	140.9	C-2, C-6, C-9
8	6. 32 d (15.7)	6.15 d (15.7)	117.5	C-1
9	-	-	168.1	-
OCH_3-3	3.86 s	3.62 s	55.5	C-3
OH-4	-	5.90 br s	-	-
NH_2	3.50 br s	2.83 br s	-	-

Results and Discussion

In the present communication the major alkaloids of the aerial parts of *G. grandiflorum* var. *grandiflorum* collected from Doruksaray (Erzincan) have been proven to be protopine (allocryptopine, protopine) type. (-)-α-N-methylcanadine, (-)-β-N-methylcanadine (tetrahydroprotoberberine), (+)-reticuline (benzyltetrahydroisoquinoline), berbithine (benzylisoquinoline) have been isolated as minor alkaloids. The occurrence of berbithine in Papaveraceae family and of (+)-reticuline in *G. grandiflorum* var. *grandiflorum* species has been shown for the first time. Berbithine was previously found in *Berberis actinacantha* Mart. Ex Schult. (Berberidaceae)(15). In addition to these alkaloids, in the quaternary base portion, the presence of ferulic acid amide (ferulamide) was also shown. Ferulamide, the bile flow stimulating effect of which was previously determined(16), was derived from a natural source for the first time.

Ferulamide was obtained as a colourless compound. The base peak in the mass spectrum of this compound corresponded to the molecular ion and was at m/e 193. An intense peak was also found at m/e 177 due to the loss of NH_2 group from the molecular ion. The IR spectrum indicated the presence of a carbonyl group at 1662cm^{-1} and a phenolic hydroxyl group at 3416 cm^{-1}. The ^{1}H-NMR spectrum (Table) showed the presence of downfield doublets at 6.15 and 7.20 with a coupling constant 15.7 1 Hz, conforming the presence of' a double bond (vinilic protons) with a trans- configuration. The aromatic protons were observed at δ 6.72(dd), 6.76(d), 6.58(d) as expected. In the

aliphatic region a singlet was observed at 65.90 and assigned to a phenolic hydroxyl group. The methoxyl protons appeared as a singlet at 3.62. A broad two proton singlet at δ 2.83 showed the presence of the $CONH_2$ group. The ^{13}C-NMR spectrum (Table) confirmed the structure with resonances attributable to a carbonyl group (δ 168.1), two deshielded oxygen bearing quaternary carbon, five methine carbons and a shielded aromatic quaternary carbon. Further assignments in both the ^{1}H and ^{13}C-NMR spectra were achieved by means of HMBC study (Table 1) in which ^{3}J heteronuclear interactions were observed. Long range coupling was noted between the methoxyl proton signals and the signal at 147.4 (C-3). Long range couplings were also observed between the 5-H signal at 6.58 ppm and ^{13}C signals at 126.4 (C-1), 147.4 (C-3). These data provided us to identify the position of the CH_3O group which was at C-3.

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