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Isoquinoline Alkaloids from *Fumaria bastardii*

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Abstract – The extract of the aerial parts of *Fumaria bastardii* Bor. afforded 12 alkaloids belonging to the skeletally six different groups of the isoquinoline alkaloids. In this publication, the isolation and identification of protopine (**1**), corydaldine (**2**), oxyhydrastinine (**3**), (-)-fumaritine (**4**), (+)-fumariline (**5**), (-)-*O*-methylfumarophycine (**12**), (+)-bicuculline (**10**), (-)-β-hydrastine (**7**), (-)-corlumine (**11**), (+)-tetrahydropalmatine (**8**), (-)-stylopine (**6**), and (+)-juziphine (**9**) are described. Their structures have been determined by using extensive spectroscopic techniques. This is the first report of the occurrence of these alkaloids in *Fumaria bastardii* of Turkish origin.

Key words – *Fumaria bastardii*, Fumarioideae, isoquinoline alkaloids, spectral data.

Introduction

Turkey has a very rich flora and a long history of herbal medicine practice. The identification of the active compounds within herbal mixtures becomes an important area of research directed towards the development of leads for novel medicinal agents. Unfortunately, the nature produces the active compounds in low concentrations. In order to prepare these molecules in large quantities, plant cell culture techniques have been used. Continuing our studies on the bioactive compounds from Turkish medicinal plants, a project has been initiated to develop stable plant cell culture lines of *Taxus* and *Fumaria* species growing in Turkey. Our investigations, involving 13 *Fumaria* (Sener, 1981) and 6 *Corydalis* (Sener, 1988) species, have shown that these plants contain the highest concentrations of the interesting alkaloids. From these, protopine has shown strong antiplatelet activity (Saeed *et al.*, 1990).

The role of antiplatelet drugs in the control of cardiovascular diseases continues to be of prime importance. It is well recognized that platelet-vessel wall interactions are important in the development of thrombosis and atherosclerosis. Thus, inhibition of platelet function may be a promising approach for the prevention of thrombosis. Although many agents have been reported to have *in vitro* antiplatelet effects, only a few of them are clinically useful in antithrombotic therapy. Therefore, it is very important to continue the search for new antiplatelet drugs for this purpose. The effect of some Turkish medicinal plants against human platelet aggregation induced by arachidonic acid (AA), collagen and platelet activated factor (PAF) have been examined. Our preliminary biological activity evaluation of ethanolic extracts of some Turkish

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medicinal plants used in folk medicine were the most potent inhibitors with minimal effective concentrations. Among them, *Fumaria* species showed complete inhibition of platelet aggregation caused by AA and collagen inhibitors of thromboxane formation. Bioassay-directed fractionation of the ethanolic extract of *Fumaria* species resulted in the isolation of protopine (Sener, 1994).

There are 15 *Fumaria* species growing in Turkey (Davis, 1965) and 13 of them have been investigated in terms of alkaloid content. In order to find an additional source for protopine, alkaloids from the aerial parts of the previously not reported species, *Fumaria bastardii* Bor. have been investigated for the first time and presented in this publication.

Experimental

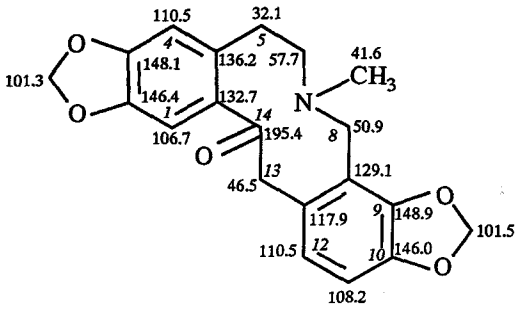
Plant material – The aerial parts of *Fumaria bastardii* Bor. (Fumarioideae) were collected from Kusadasi near Söke (Turkey) during the flowering stage. The plant was identified by Prof. Dr. B. Sener and a voucher specimen No. BS 1353 was kept in the Herbarium of the Faculty of Pharmacy, Gazi University, Ankara-Turkey.

Extraction, isolation and purification – The air-dried and powdered form of the plant material (850 g) was extracted with EtOH (70%) by percolation at room temperature and the extract was concentrated to a crude gum (30 g). The gum was acidified with 5% HCl (100 ml), extracted with chloroform (400 ml) and the chloroform extracts dried with anhydrous sodium sulphate (fraction A, 2.34 g). The acidic phase was made basic with 10% NH_4OH and extracted with chloroform. The combined chloroform extracts were dried over anhydrous sodium sulphate. Thereafter, the solution was evaporated to dryness *in vacuo* leaving a yellowish-brown residue (fraction B, 4.10 g). The large quantity of protopine (**1**) found in the fractions A and B was obtained as white crystals (1.95 g) from MeOH. The mother liquors of fraction A and B, after removal of protopine, were evaporated and subjected to column chromatography over silica gel. Elution was achieved using chloroform-methanol mixtures with increasing polarity such as 9 : 1, 8 : 2 and 7 : 3 to afford A-1, A-2, A-3, B-1, B-2 and B-3 subfractions. Final purification was achieved using preparative tlc. These procedures resulted in the isolation of the following alkaloids:

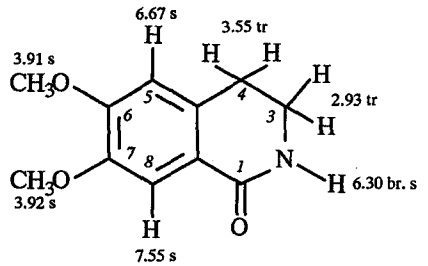
Fraction A-1 when chromatographed on prep. tlc using petroleum ether: acetone (8 : 2) afforded two alkaloids (**2** and **3**).

Corydaldine (**2**): Obtained as a colorless amorphous solid (24 mg). $[\alpha]_D^{26}=0°$ (c. 0.3, MeOH), UV (MeOH): λ_{max} 223, 261, 295 nm. IR ($CHCl_3$): 1710 (C=O) cm^{-1}, ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: summary of signals shown on structure **2**. EIMS m/z (rel. int.): 207(M^+, 37), 178(80), 150(44), 104(12), 57(100).

Oxyhydrastinine (**3**): Obtained as a colorless amorphous solid (36 mg). $[\alpha]_D^{26}=0°$ (c. 0.3, MeOH), UV (MeOH): λ_{max} 218, 263, 302 nm. IR ($CHCl_3$): 1640 (C=O) cm^{-1}, ^{1}H-NMR, ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: summary of sig-



Protopine (**1**)



Corydaldine (**2**)

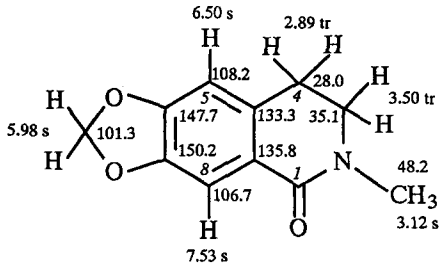
nals shown on structure **3**. EIMS m/z (rel. int.): 205(M^+, 80), 162(46), 134(97), 104(16), 57(100).

Fraction A-2 when chromatographed on prep. tlc using petroleum ether: acetone (6: 4) afforded two alkaloids (**1** and **4**).

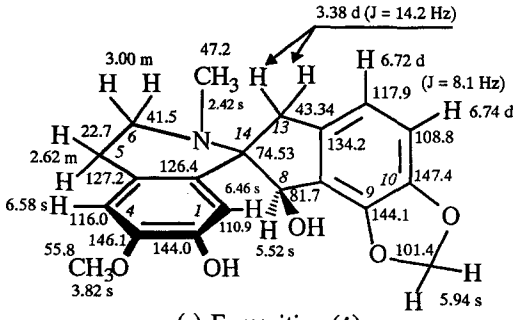
Protopine (**1**): Obtained as a white crystalline compound (236 mg). $[\alpha]_D^{26}=0°$ (c. 0.03, MeOH), UV (MeOH): λ_{max} 221, 292 nm. IR ($CHCl_3$): 1665 (C=O) cm^{-1}, ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: summary of signals shown on structure 1. EIMS m/z (rel. int.): 353(M^+, 10), 338(15), 322(2), 190(11), 163(19), 148(100).

(-)-Fumaritine (**4**): Obtained as a colorless amorphous solid (57 mg). $[\alpha]_D^{26}=-29.6°$ (c. 0.03, MeOH), UV (MeOH): λ_{max} 212, 289 nm. IR ($CHCl_3$): 3550 (OH) cm^{-1}, ^{1}H-NMR ($CDCl_3$, 400 MHz) and ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: summary of signals shown on structure **4**. EIMS m/z (rel. int.): 355(M^+, 28), 340(16), 324(40), 192(100), 177(9), 164(6).

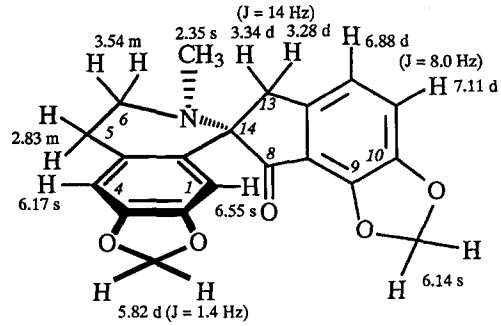
Fraction A-3 when chromatographed on prep. tlc using petroleum ether: acetone: diethylamine (8: 1.5: 0.5) afforded three alkaloids (**5, 6** and **7**).



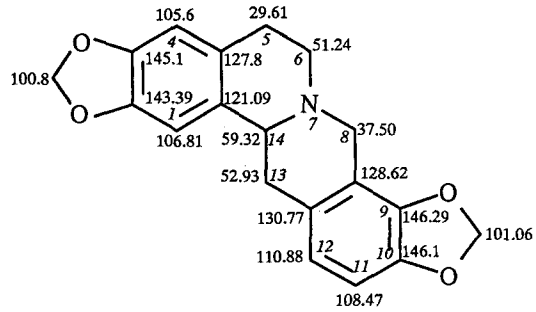
Oxyhydrastinine (**3**)



(-)-Fumaritine (**4**)



(+)-Fumariline (**5**)



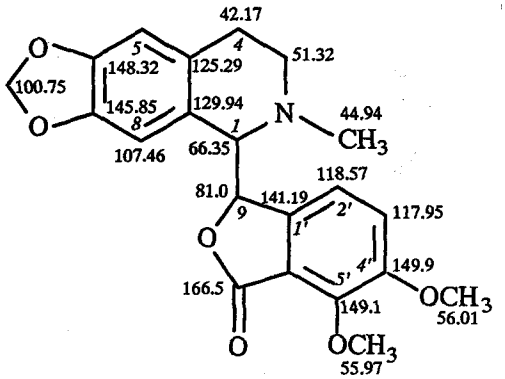
(-)-Stylopine (**6**)

(+)-Fumariline (**5**): Obtained as a colorless amorphous solid (9 mg). $[\alpha]_D^{26}=+68.6°$ (c. 0.03, MeOH), UV (MeOH): λ_{max} 209, 235, 266, 293, 356 nm. IR ($CHCl_3$): 1705 (C=O) cm^{-1}, ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: summary of signals shown on structure **5**. EIMS m/z (rel. int.): 351(M^+, 40), 336(28), 322(100), 190(60).

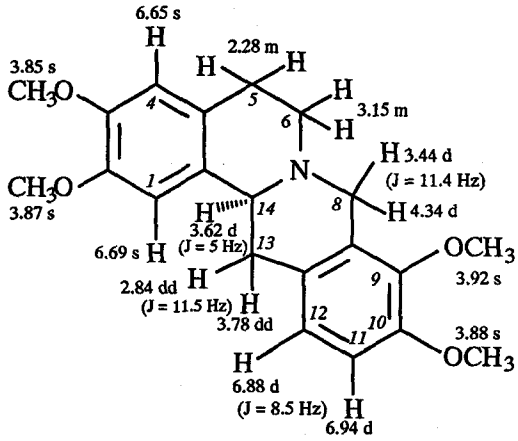
(-)-Stylopine (**6**): Obtained as a pale yellow crystalline compound (33 mg). $[\alpha]_D^{26}=-339°$ (c. 0.2, MeOH), UV (MeOH): λ_{max} 212, 291 nm. IR ($CHCl_3$): 3400, 2780, 1600, 1460 cm^{-1}, ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: summary of signals shown on structure **6**. EIMS m/z (rel. int.): 323(M^+, 35), 322(25), 174 (18), 148(100).

(-)-β-Hydrastine (**7**): Obtained as a white crystalline compound (36 mg). $[\alpha]_D^{26}=-66°$ (c. 0.02, MeOH), UV (MeOH): λ_{max} 227, 297 nm. IR ($CHCl_3$): 1760 (C=O) cm^{-1}, ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: summary of signals shown on structure **7**. EIMS m/z (rel. int.): 383(M^+, 10), 353(10), 190(22), 178(22), 190(100).

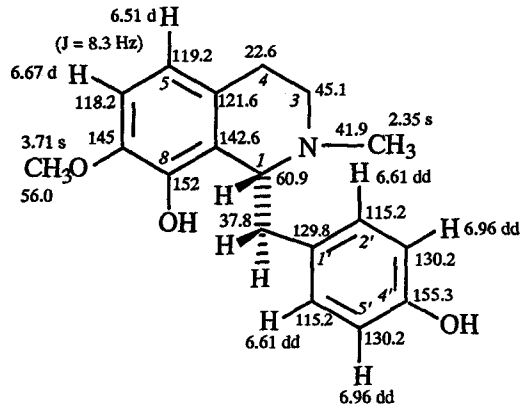
Fraction B-1 when chromatographed on prep. tlc using petroleum ether: acetone: diethylamine (6: 3.5: 0.5) afforded two al-



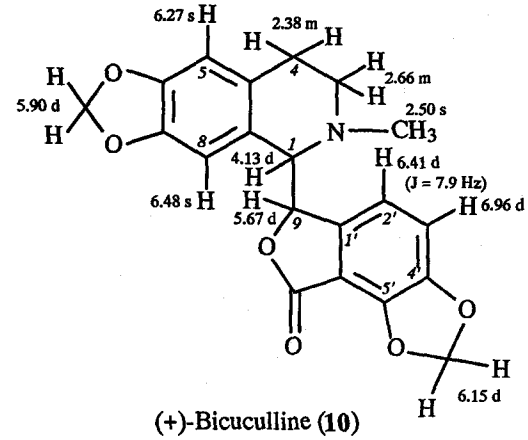
(-)-β-Hydrastine (7)



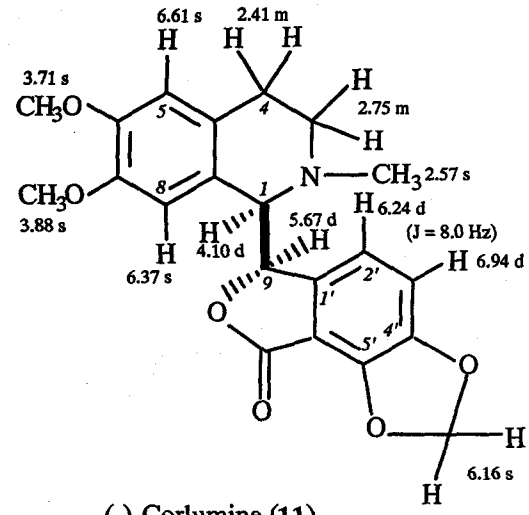
(+)-Tetrahydropalmatine (8)



(+)-Juziphine (9)



(+)-Bicuculline (10)



(-)-Corlumine (11)

kaloids (**8** and **9**).

(+)-Tetrahydropalmatine (**8**): Obtained as a colorless amorphous solid (10 mg). $[\alpha]_D^{26}$= +290° (c. 0.3, MeOH), UV (MeOH): λ_{max} 201, 284, 335 nm. IR ($CHCl_3$): 1600 (C=C) cm^{-1}, ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: summary of signals shown on structure **8**. EIMS m/z (rel. int.): 355(M^+, 28), 354(12), 340(10), 323(9), 190(6), 149(20).

(+)-Juziphine (**9**): Obtained as a colorless amorphous solid (12 mg). $[\alpha]_D^{26}$=+18.4° (c. 0.04, MeOH), UV (MeOH): λ_{max} 225, 279 nm. IR ($CHCl_3$): 3400 (OH), 2800, 1600 (C=C) cm^{-1}, ^{1}H-NMR ($CDCl_3$, 400 MHz) and ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: summary of signals shown on structure **9**. EIMS m/z (rel. int.): 299(M^+, 8), 284(7), 192(99), 107(10).

Fraction B-2 when chromatographed on

prep. tlc using petroleum ether: acetone: diethylamine (5:4.5:0.5) afforded two alkaloids (**10** and **11**).

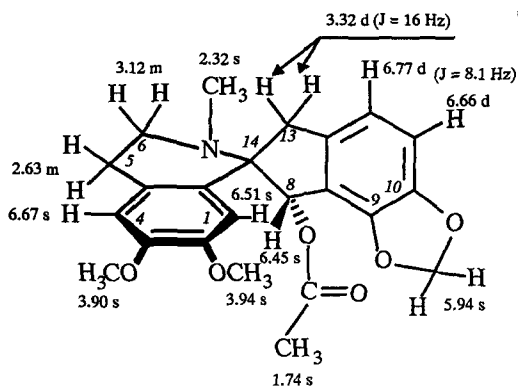
(+)-Bicuculline (**10**): Obtained as a colorless amorphous solid (14 mg). $[\alpha]_D^{26}$=+113° (c. 0.06, MeOH), UV (MeOH): λ_{max} 227, 297, 322 nm. IR ($CHCl_3$): 1760 (C=O) cm^{-1}, ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: summary of signals shown on structure **10**. EIMS m/z (rel. int.): 367(M^+, 6), 352(5), 190(100), 177(28), 149(36).

(-)-Corlumine(**11**): Obtained as a dark yellow amorphous solid (18 mg). $[\alpha]_D^{26}$= − 73° (c. 0.8, MeOH), UV (MeOH): λ_{max} 220, 236, 294, 322 nm. IR ($CHCl_3$): 1760 (C=O) cm^{-1}, ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: summary of signals shown on structure **11**. EIMS m/z (rel. int.): 383(M^+, 11), 353(12), 190(50), 148(12), 135(5).

Fraction B-3 when chromatographed on prep. tlc using petroleum ether: acetone: diethylamine (8:2.5:0.5) afforded one alkaloid (**12**).

(-)-*O*-Methylfumarophycine (**12**): Obtained as amorphous solid (23 mg). $[\alpha]_D^{26}$= − 51° (c. 0.05, $CHCl_3$), UV (MeOH): λ_{max} 205, 289 nm. IR ($CHCl_3$): 1720 (C=O) cm^{-1}, ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: summary of signals shown on structure **12**. EIMS m/z (rel. int.): 411(M^+, 4), 396(2), 367(3).

Instrumentation – The UV spectra were recorded in MeOH on a 8450 UV/VIS Hewlett-Packard spectrophotometer. The optical rotations were measured in $CHCl_3$ on a Polartronic Universal Australian Standard K-157 spectropolarimeter. The IR spectra were taken in $CHCl_3$ on a Perkin-Elmer 281 B infrared spectrophotometer. The ^{1}H-NMR spectra were taken at 400 MHz on a Bruker AM-400 NMR spectrometer, ^{13}C-NMR spectra were recorded at 100 MHz on the same instrument. The mass spectra were obtained on V.G. Micromass 2 AB-HF 9Q spectrometer, coupled to a V.G. 11/250 Data System.



(-)-O-Methylfumarophycine (**12**)

Results and Discussion

Fumaria species are an invaluable source of isoquinoline alkaloids many of which possess a wide diversity of structures and biological activities. The genus *Fumaria* is represented by 15 species in Turkey (Davis, 1965). They are all small herbs. Some of them are used in folk medicine in the treatment of eczema, rheumatism, stomach ache and dysentery. The aerial parts of 13 *Fumaria* species yielded 34 isoquinoline alkaloids (Sener *et al.*, 1991). Among them, protopine is one of the major alkaloids and it showed complete inhibition on human platelet aggregation caused by AA and collagen inhibitors of thromboxane formation. Protopine also inhibits human platelet aggregation induced by PAF (Sener *et al.*, 1991). Since PAF is an important mediator of inflammation, thrombosis and asthma, it can be deduced that protopine may be a useful compound possessing anti-PAF properties.

In this publication, the alkaloids of *Fumaria bastardii* Bor. growing in Turkey were investigated.

According to the procedure described in the experimental section, 12 isoquinoline alkaloids have been isolated using column chromatography and preparative tlc. Of these, protopine (**1**) is also found as a major alkaloid such as in other *Fumaria* species. The other alkaloids representing the skeletally five different groups have been obtained for the first time in *Fumaria bastardii*.

Their structures were established on the basis of their spectral data given in the experimental part and the data reported in the literature. They are summarized as follows:

i. corydaldine (**2**) (Shamma, 1972) and oxyhydrastinine (**3**) (Israilov *et al.*, 1975) were identified as isoquinolone-type alkaloids.

ii. (-)-fumaritine (**4**) (Sener, 1981), (+)-fumariline (**5**) (Sener, 1981) and (-)-*O*-methylfumarophycine (**12**) (Sener, 1994) were characterized as spirobenzylisoquinoline-type alkaloids.

iii. (-)-stylopine (**6**) (Sener, 1981) and (+)-tetrahydropalmatine (**8**) (Sener, 1981) were described as protoberberine-type alkaloids.

iv. (-)-β-hydrastine (**7**) (Israilov *et al.*, 1975), (+)-bicuculline (**10**) (Sener, 1981) and (-)-corlumine (**11**) (Sener, 1994) were determined as phthalide isoquinoline derivatives.

v. (+)-juziphine (**9**) (Shamma, 1972) was identified as a benzylisoquinoline-type alkaloid.

From these findings, it can be reported that *Fumaria bastardii* Bor. has also been described as a new source for protopine.

Acknowledgement

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