FUMAFLORINE, A NEW 1-BENZYLISOQUINOLINE ALKALOID FROM *FUMARIA DENSIFLORA*

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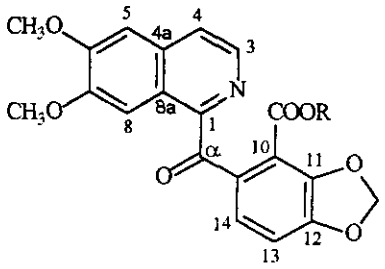
Abstract - Fumaflorine (**1**), a new 1-benzylisoquinoline alkaloid, was isolated from the aerial parts of *Fumaria densiflora*. Its structure was determined by spectroscopic methods.

About 40 species belong to the genus *Fumaria* (Fumariaceae).[1] The plants of this genus are used mainly in folk medicine to treat gastrointestinal disorders and certain skin diseases. *Fumaria densiflora* DC. is an annual plant indigenous to southeast Europe and the Middle East. Previously, we have reported the isolation of adlumidiceine, coptisine, cryptopine, densiflorine, palmatine, protopine, and sinactine from the aerial parts of *F. densiflora* collected in Bulgaria.[2] Continuing our phytochemical studies on this genus, we reinvestigated *F. densiflora* of Bulgarian origin. The present paper deals with the isolation and structure elucidation of the new alkaloid fumaflorine (**1**).

Fraction I, consisting of strongly polar alkaloids from *F. densiflora*, yielded (-)-*N*-methylstylopinium iodide, (+)-corytuberine hydroiodide, and the crystalline compound(**1**)(17.7 mg). In the EIMS of **1**, a weak molecular cation-radical ion at *m/z* 381 was found to lose 45 amu (COOH) giving a base peak at *m/z* 336 ($C_{19}H_{14}NO_5$). The ^{1}H-NMR spectrum (Table 1) contained two aromatic methoxy groups, one methylenedioxy group, two aromatic proton singlets, and two pairs of *ortho*-oriented protons (J_{vic}=5.5 and

8.0 Hz). On the basis of the observed long-range couplings and NOESY experiments, both methoxyls were assigned in the same aromatic ring as the *para*-protons (proved by a cross-peak in the delay-COSY spectrum). The last-mentioned protons were in turn long-range coupled to the protons at 8.29 and 7.71 ppm (with a mutual coupling of J=5.5 Hz), assigned to the protons α- and β- to the nitrogen atom of the pyridine ring.[3] With the exception of two methoxyl and one -OCH_2O- carbon, all carbons were sp^2-hybridized (Table 1). Two of these (resonating at 167.88 and 195.93 ppm) could be assigned to carbonyls. The sum of aromatic/olefinic carbons was odd; this indicated the presence of one -C=N bond. Therefore, the molecule of **1** contained an isoquinoline nucleus joined by a benzene ring carrying -COOH and -OCH_2O- groups through a carbonyl that was consequently conjugated. This structure was supported by the observation of complementary ions at *m/z* 193 and 188 in the EIMS, corresponding to a splitting between the isoquinoline ring and the carbonyl group. On the basis of the above-mentioned spectroscopic studies, the benzylisoquinoline structure (**1**) was assigned to this alkaloid. This is the first alkaloid with completely aromatic isoquinoline ring found in the Fumariaceae.



1: R = H
2: R = CH_3

Fraction B afforded coptisine and the crystalline compound (**2**) (10.6 mg) of molecular formula $C_{21}H_{17}NO_7$. In the EIMS the M^+ ion at *m/z* 395 lost CH_3O, CH_3CO and $COOCH_3$, leading to a base peak at *m/z* 336. According to its ^{13}C-NMR data (Table 1), only four of the twenty one carbon signals were of the sp^3-type: three methyls and one oxymethylene group. The remaining carbons represented six -CH= units, nine sp^2-hybridized carbons, one conjugated ketone (196.05 ppm), and one C=O group resonanting at 165.67 ppm (ester or amide). The sum of all olefinic and aromatic carbons was again odd. This implied the presence of a -C=N bond. The ^{1}H-NMR spectrum of **2** (Table 1) contained three methoxyl groups singlets and one methylenedioxy group singlet. The nature of one methyl (^{1}H, δ 3.36, ^{13}C, 52.65 ppm) was not clear and could be ascribed to either a =NCH_3 group or a -OCH_3 group. However, the later alternative was supported from the ^{13}C-NMR data (Table 1). Six aromatic protons observed in the ^{1}H-NMR spectrum formed three systems: two singlets (*para*-coupling detected in the delay-COSY), two

Table 1. ^{1}H- and ^{13}C-NMR data of fumaflorine (**1**) and its O-methyl ester (**2**).

	1[a]		**2**[b]	
	^{1}H	^{13}C	^{1}H	^{13}C
1		147.77 s		147.84 s
3	8.29 d	140.03 d	8.31 d	140.13 d
4	7.71 d	123.23 d	7.69 d	123.19 d
4a		134.31 s		133.93 s
5	8.11 s	105.52 d	8.20 s	104.93 d
6		154.01 s		153.72 s
7		153.03 s		152.30 s
8	7.22 s	105.52 d	7.19 s	126.45 d
8a		135.19 s		134.91 s
α		195.93 s		196.05 s
9		123.85 s		123.63 s
10		117.24 s		114.88 s
11		152.05 s		151.81 s
12		152.28 s		151.94 s
13	7.16 d	126.40 d	7.26 d	110.38 d
14	6.95 d	110.02 d	6.99 d	105.35 d
6-OCH_3	4.04 s	56.65 q	4.07 s	56.56 q
7-OCH_3	4.07 s	56.65 q	4.08 s	56.51 q
10-COOR		167.88 s		165.67 s
10-$COOCH_3$			3.36 s	52.65 q
11,12-OCH_2O	6.19 s	103.43 t	6.19 s	103.34 t

[a] Measured in CD_3OD. [b] Measured in $CDCl_3$.

ortho-protons (J=8.0 Hz), and one pair of heterocyclic *ortho*-protons (7.69 and 8.31 ppm, J=5.5 Hz). The carbons directly coupled to the last-mentioned protons resonated at 123.19 and 140.13 ppm and their $^{1}J_{CN}$ (164.67 and 181.0 Hz) values indicated that they were adjacent to nitrogen. According to the long-range couplings and NOESY experiments observed, both singlet protons were found to be next to a methoxyl group. Furthermore, they were also long-range coupled to the pyridine protons, thus completing

isoquinoline moiety. The second aromatic nucleus carries the $-OCH_2O-$ and $-COOCH_3$ units. The conjugated ketone therefore joined both parts, leading to the structure(**2**). Methylation of fumaflorine (**1**) led to the methyl ester identical according to ^{1}H-NMR and EIMS spectra with compound (**2**) which was probably formed from **1** during the isolation process.

EXPERIMENTAL PART

Melting points were determined on a Mettler FP-51 instrument and are uncorrected. IR and UV spectra were recorded on Ati Matson Genesis FT-IR and Perkin-Elmer PE-552 spectrophotometers, respectively. NMR spectra were measured on a Varian VXR-400 spectrometer (400 MHz for ^{1}H, 100 MHz for ^{13}C) at 25°C. Chemical shifts in ppm are given with respect to TMS, and coupling constants are expressed in Hz. Carbon signal multiplicities determined using an Attached Proton Test (APT) or DEPTGL experiments. MS was performed on a Finnigen MAT-90 double-focusing instrument. High-resolution experiments were carried out by the peak matching method using Ultramark 1600F as internal standard. The device was tuned to a resolution of approximately 10,000 (10% valley).

Plant material. Flowering aerial parts of *F. densiflora* were collected in the Galata area (near Varna, Bulgaria) in May 1994. A voucher specimen of the plant was deposited at the Institute of Medical Chemistry, Palacký University.

Extraction and isolation. The dried and powdered plant (2.7 kg) was extracted exhaustively with 5000 mL of MeOH for 24 h at 60°C. The extract was evaporated in *vacuo*. The residue obtained (12.06 g) was dissolved with 1% H_2SO_4 and then separated into fractions L, A, B, and I.[4] Crystallization of fraction I (2.05 g) from MeOH afforded (-)-*cis*-*N*-methylstylopinium iodide (36.5 mg). The mother liquors were separated by column chromatography on silica gel. From fractions eluted with $CHCl_3$ a new alkaloid(**1**) (17.7 mg) was obtained. Further elution with the mixture $CHCl_3$-MeOH (40:1) gave (+)-corytuberine (10.2 mg). From fraction B (1.25 g) an orange red compound(**2**)(10.6 mg) and coptisine (9.1 mg) were obtained by crystallization from MeOH.

1-(2-Carboxy-3,4-methylenedioxyphenylcarbonyl)-6,7-dimethoxyisoquinoline (1). Yellowish needles (17.7 mg); mp 218-220°C (MeOH); UV (MeOH) λ_{max} (log ε): 236 (4.69), 330 (3.98) nm; IR (KBr) ν_{max} : 1505, 1590, 1617, 1661, 2360, 2923, 3425 cm^{-1}; ^{1}H- and ^{13}C-NMR (Table 1); EIMS: *m/z* (rel. int.): M^+ 381 (4), 365 (8, found 365.0901, calcd for $C_{20}H_{15}NO_6$ 365.08999), 364 (11), 352 (21, found 352.0817, calcd for $C_{19}H_{14}NO_6$ 352.0821), 337 (28), 336 (100, found 336.0874, calcd for $C_{19}H_{14}NO_5$ 336.0872), 322 (13), 308 (11), 193 (8), 188 (3).

1-(2-Methoxycarbonyl-3,4-methylenedioxyphenylcarbonyl)-6,7-dimethoxyisoquinoline (2). Red needles (10.6 mg); mp 184-186°C (MeOH); UV (MeOH) λ_{max} (log ε): 213 (4.62), 236 (4.54), 282-290sh (4.03-4.01), 334 (4.07) nm; ^{1}H- and ^{13}C-NMR (Table 1); EIMS: *m/z* (rel. int): M^+ 395 (1, found 395.1008, calcd for $C_{21}H_{17}NO_7$ 395.1005), 364 (5, found 364. 0822, calcd for $C_{20}H_{14}NO_6$ 364.0821), 353 (8), 352 (37, found 352.0820, calcd for $C_{19}H_{14}NO_6$ 352.0821), 337 (22), 336 (100, found 336.0870, calcd for $C_{19}H_{14}NO_5$ 336.0872), 320 (5), 292 (3), 207 (12), 188 (2), 164 (3), 45 (3). The compound (**2**) was also obtained by methylation of **1**: Concentrated H_2SO_4 (0.1 mL) was added to a solution of **1** (10 mg) in MeOH (2 mL) and the mixture was refluxed with stirring for 1 h, neutralized with $NaHCO_3$ and extracted with CH_2Cl_2. The methyl ester of **1** was obtained (5 mg). Its ^{1}H-NMR and EIMS were identical with those of compound (**2**).

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