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ALKALOIDS OF *FUMARIA OFFICINALIS*

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Key Word Index—*Fumaria officinalis*; Fumariaceae; alkaloid; structure elucidation; *N*-methylsinactine; dihydrofumariline.

Abstract—*N*-Methylsinactine and dihydrofumariline have been found in a methanol extract of *Fumaria officinalis* along with a number of other alkaloids previously found in this plant material.

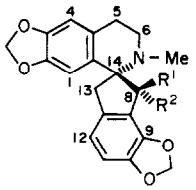
INTRODUCTION

The alkaloids of *Fumaria officinalis* have been the subject of several investigations [1–8]. From these studies the following alkaloids have been identified: protopine [1, 3, 7, 8], cryptopine [1, 3, 8], (±)-stylopine [1, 7], (±)- and (−)-scoulerine (aurotensine) [1, 2, 7], sinactine [1, 3, 7], fumaricine [1, 7, 9], fumaritine [1, 2, 9], fumariline [2, 9], fumarofine [1, 7, 10, 11], fumarophycine [3, 12], cryptocavine [7], *N*-methylhydrasteine [4], oxo-*N*-methylhydrasteine [4, 5] and *N*-methylhydrastine [5].

Here we report an investigation of the alkaloid content of *F. officinalis*‡ of Bulgarian origin purchased from a herb shop. We have observed the presence of several previously reported alkaloids, namely, (−)-sinactine, (+)-fumariline, protopine, cryptopine, (−)-fumaricine, fumarofine and fumaritine *N*-oxide [13]. We have also isolated a new quaternary tetrahydroprotoberberine alkaloid which has been shown to be *N*-methylsinactine and an apparently new dihydrofumariline (see below).

RESULTS AND DISCUSSION

The *N*-methylsinactine crystallized from chloroform as colorless needles melting at 318–320° presumably as the quaternary hydroxide. The mass spectrum had a M^+ peak at *m/z* 354 corresponding in composition to $C_{21}H_{24}NO_4$. There were fragment ions at *m/z* 338, 336, 190 and 148. The ions at *m/z* 190 ($C_{11}H_{12}NO_2$) and 148 ($C_9H_8O_2$) provided the first clue to the structure of the new alkaloid since such ions are characteristic of protoberberine systems. An explanation for their formation as well as for the formation of ions at *m/z* 338 and 336 is provided in Scheme 1. The data suggested that the alkaloid was *N*-methylsinactine.



Fumariline, $R^1+R^2=O$

Dihydrofumariline-1: $R^1=H$, $R^2=OH$

Dihydrofumariline-2: $R^1=OH$, $R^2=H$

The structure assigned to the new alkaloid was supported by its ^1H NMR spectrum recorded at 80 MHz in DMSO-d_6. There were sharp singlets at δ 2.89 (3H, N–Me), 3.81 and 3.84 (6H, 2 × OMe). There was also a signal centred at δ 6.14 for a methylenedioxy group that was split into an apparent doublet and a multiplet of area 4H centred at δ 6.98 attributed to aromatic protons. The aliphatic protons of the ring were poorly resolved but there were three protons in the region δ 4.8–5.3, two in a multiplet centred near δ 4.0 and another signal at δ 3.28. The signal of the ninth proton was apparently hidden beneath the solvent peak or the *N*-methyl or *O*-methyl signals. The UV spectrum of the compound showed peaks in ethanol at 242 and 290 nm, log ε = 4.05 and 3.86, respectively.

The UV and NMR data support the conclusion that the new alkaloid is *N*-methylsinactine. The structure was confirmed by the methylation of sinactine with methyl iodide in methanol. The product had an NMR and UV spectrum identical with the natural material.

A dihydrofumariline has been previously reported (in *F. schrammii*) [14]. However, the melting point was somewhat lower than that of the compound isolated in this study. Accordingly we have prepared dihydrofumariline by reduction of fumariline with sodium borohydride and have found two products of reduction apparently epimeric at C-8. The faster moving component on TLC, designated dihydrofumariline-1, melting at 191–193°, is identical with the alkaloid isolated in this study by comparison of its melting point and IR spectrum. The slower moving component is designated dihydrofumariline-2. It melts at 135–137° and has distinct differences in its NMR spectrum from that of its epimer (see Experimental); it has properties similar to those reported earlier for dihydrofumariline [9].

The NMR of dihydrofumariline-2 has a signal at δ 5.52 assigned to the proton at C-8 geminal to the OH

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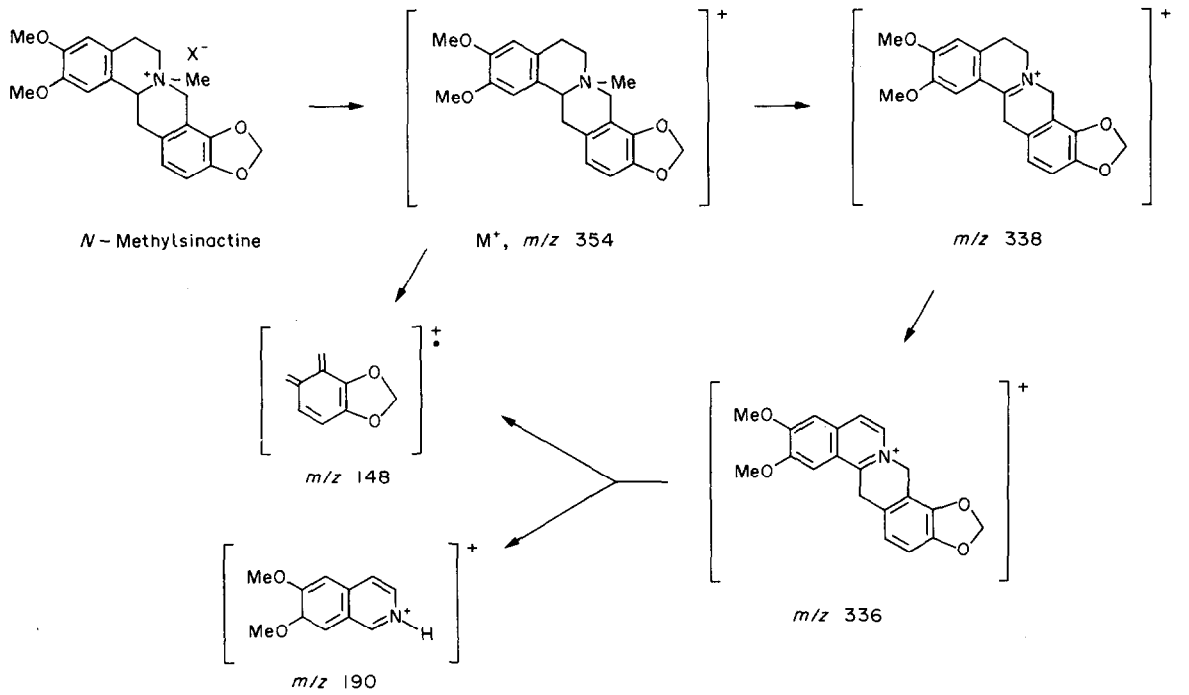
‡Aqueous extracts of the herb (prepared from one spoonful in 300 ml of water per day) are used in Bulgaria to treat digestive problems (colitis, gastritis, etc).



Scheme 1. Possible pathway for fragmentation of the *N*-methylsinactine.

group. The chemical shift of this signal is similar to that of the corresponding signals of fumaricine and fumaritine in which it has been shown that the C-8 hydrogen is *cis* to the N–Me group in the five-membered ring [9]. This signal is broad and sharpens on addition of D_2O as reported for the similar signal in fumaricine and fumaritine [9]. Dihydrofumariline-1 has the signal of the C-8 hydrogen at δ 4.82 and must be epimeric at C-8 with dihydrofumariline-2 having the hydrogen at C-8 *trans* to the N–Me group. The chemical shift of this hydrogen is similar to that of the hydrogen at C-13 in ochrobirine [15], which has been shown to be *trans* to the N–Me group in the five-membered ring.

The dihydrofumariline of Popova *et al.* [14] has a chemical shift for the hydrogen at C-8 similar to that of dihydrofumariline-2. However, its reported melting point of 184–187° is much higher than we have observed. It is possible that dihydrofumariline-2 may exist in two crystalline modifications but this matter has not been satisfactorily resolved.

It is of interest that this plant material contains both sinactine, its *N*-methyl derivative and the related spirobenzylisoquinoline alkaloids. It has been suggested that the spirobenzylisoquinolines are rearranged protoberberine systems [16, 17] and this is perhaps the first instance where the protoberberine, its metho salt and the rearranged protoberberine (i.e. the spirobenzylisoquinoline system) of the same substitution pattern are found in the same extract.

EXPERIMENTAL

General. The IR of alkaloids were recorded in $CHCl_3$ or KBr. The MS were measured using a probe inlet at 70 eV. ^{1}H NMR spectra were run either at 80 MHz on a Fourier transform spectrometer or at 60 MHz on a continuous wave instrument. UV were recorded in EtOH and the optical rotation measured in H_2O. Mps are uncorr. Al_2O_3 (Brockman II) was used for CC; TLC was carried out on Si gel G (Merck).

Isolation of alkaloids. Air dried plant material (20 kg) was extracted with MeOH, the extract concd nearly to dryness and then treated with 2% aq. HCl. The resulting acid soln was extracted with Et_2O until the extract was nearly colorless and then it was made basic with aq. NH_3. The alkaline soln was extracted firstly with Et_2O yielding, (after removal of solvent) fraction A (60 g) and secondly with $CHCl_3$ yielding fraction B (15 g).

Separation of fraction A. The crude alkaloids (60 g) were chromatographed on Al_2O_3 (2 kg). The column was eluted first with C_6H_6 followed by C_6H_6–MeOH. From the C_6H_6 fraction (−)-sinactine* (0.7 g) was obtained mp 183–184°, after recrystallization from EtOH. The fraction (3.0 g) eluted with C_6H_6 containing 1% MeOH was comprised mainly of (+)-fumariline. It crystallized from EtOH as yellow prisms mp 150–151°. Further elution gave a fraction which showed two spots on TLC. The less polar component corresponded to fumariline. The more polar component (10 mg), separated from fumariline by prep. TLC, had mp 191–193° and proved to be identical with dihydrofumariline-1 prepared by $NaBH_4$ reduction of fumariline (see below).

The eluate with C_6H_6 containing 5% MeOH contained two alkaloids that were separated by fractional crystallization of the mixture from EtOH. The alkaloids were identified as protopine (4.09 g, mp 205–207°) and cryptopine (0.05 g, mp 220–221°). Further elution gave (−)-fumaricine (2.5 g), mp 179–182°, after recrystallization from EtOH. In some fractions the fumaricine was accompanied by minor amounts of a second alkaloid which was separated by prep. TLC. It proved to be fumaritine *N*-oxide.

*Previously reported alkaloids were identified by spectroscopic comparison with authentic samples and by their mp, mmp and TLC behaviour.

With 15% MeOH in C_6H_6, fumarofine (0.8 g) was eluted and had mp 258–260° after recrystallization from EtOH.

Separation of fraction B. The crude alkaloid mixture (15 g) was chromatographed on Al_2O_3 (400 g). The column was eluted first with $CHCl_3$ and then with $CHCl_3$–MeOH. From the eluate with $CHCl_3$–MeOH (97:3), *N*-methylsinactine (0.35 g) was obtained. It was recrystallized from EtOH–$CHCl_3$ (4:1) yielding colorless needles mp 318–320°. $[\alpha]_D^{20} = -260°$ (H_2O; *c* 0.11) IR ν_{max}^{KBr} cm^{-1}: 2950, 1600, 1045 and 930.

Methylation of sinactine. Sinactine (100 mg), dissolved in MeOH (15 ml), was treated with excess MeI and the mixture allowed to stand for 24 hr. The solvent was evaporated yielding colorless crystals, which were recrystallized from MeOH-$CHCl_3$ (35 mg, mp 308–309°). The mother liquors yielded a second crop of yellow crystals (15 mg) mp 295–296°. The TLC behaviour and spectroscopic properties of both compounds were identical suggesting that they are two crystalline forms of *N*-methylsinactine iodide.

Reduction of fumariline. Fumariline (100 mg) was reduced with $NaBH_4$ as previously described [9]. The product, showing two spots on TLC (EtOH–C_6H_5Me–NH_3, 9:40:1), was chromatographed on Al_2O_3 using C_6H_6 as eluant yielding two products. The less polar product was dihydrofumariline-1 (20 mg), mp 191–193° and the more polar product, dihydrofumariline-2 (55 mg), mp 135–137°. Their IR spectra were very similar but there were distinct differences in their ^1H NMR spectra (Table 1).

Table 1. 60 MHz ^1H NMR spectra of dihydrofumariline-1 and dihydrofumariline-2 in chloroform

Protons	Chemical shifts (δ-units)	
	Dihydrofumariline-1	Dihydrofumariline-2
H-1	6.23 (*s* 1H)	6.35 (*s* 1H)
H-4	6.51 (*s* 1H)	6.55 (*s* 1H)
H-8	4.82 (*s* 1H)	5.52 (*s* 1H)
H-11, H-12	6.70 (*s* 2H)	6.68 (*s* 2H)
H-13	3.29 (*s* 2H)	3.24 (*d* 2H): $J = 16.0$ Hz
2.3 OCH_2O	5.78 (*s* 2H)	5.78 (*s* 2H)
9.10 OCH_2O	5.94 (*s* 2H)	5.94 (*s* 2H)
–NMe	2.41 (*s* 3H)	2.37 (*s* 3H)
$-CH_2-CH_2-$	3.18–2.54 (*m*)	3.04–2.55 (*m*)

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