Fumaria densiflora DC. ALKALOIDS

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Received January 31, 1996
Accepted March 10, 1996

Twenty isoquinoline alkaloids were identified in the aerial part of *Fumaria densiflora* DC. In addition to bicuculline, densiflorine, fumaritine, fumariline, scoulerine, coptisine, cryptopine, parfumine, protopine, and sinactine which have been found previously, the following alkaloids were isolated for the first time from this species: adlumine, fumaricine, fumaritridine, fumarophycine, fumarofine, corytuberine, *cis-N*-methylstylopinium iodide, stylopine, and two new 1-benzylisoquinoline alkaloids, viz. fumaflorine and fumaflorine methyl ester. The cytoprotectivity of extracts of *F. densiflora* and *F. officinalis*, and that of protopine, cryptopine, and parfumine was tested on primary cultures of rat hepatocytes intoxicated with carbon tetrachloride. The cytoprotective effect of the extracts is associated with the alkaloids.

Key words: *Fumaria densiflora* DC.; Isoquinoline alkaloids; Cytoprotective effect.

Fumaria densiflora DC. (*F. micrantha*) is an annual plant wildly growing in southeastern Europe and in the Middle East. It is one of the 40 species of the genus *Fumaria* (Fumariaceae), which have been employed for centuries in folk medicine and some of which are components of phytotherapeutic preparations exerting choleretic and spasmolytic effects[1,2]. The following isoquinoline alkaloids have been identified in *F. densiflora*: fumaramine[3,4], adlumidiceine, densiflorine, coptisine, cryptopine, palmatine, protopine, sinactine[5], adlumidine, bicuculline fumadensine, fumaridine, fumariline, fumaritine, hydrastine, isosalutaridine, *N*-methylhydrasteine, parfumidine, parfumine, and scoulerine[6]. In the context of a phytochemical investigation of the genus *Fumaria* we reexamined *F. densiflora* of Bulgarian origin. The plant was collected at the same site as in 1983 (ref.[5]). The current paper deals with the isolation and characterization of alka-

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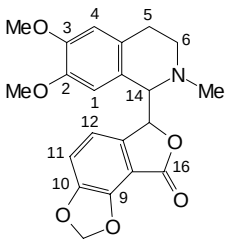
loids, with the cytoprotective activity of *Fumaria* extracts and of three major alkaloids of this plant, using a model of rat hepatocytes.

EXPERIMENTAL

The melting points were measured on a Mettler FP51 instrument and are not corrected. Thin layer chromatography (TLC) was performed on TLC plates coated with $60F_{254}$ silica gel (Merck) using the following systems: cyclohexane–diethylamine 9 : 1 (S1), cyclohexane–chloroform–diethylamine 7 : 2 : 1 (S2), methanol–25% ammonia 200 : 1 (S3), and methanol–water–25% ammonia 15 : 3 : 1 (S4). The alkaloids were detected either with UV radiation at 235 or 326 nm or by spraying with potassium iodoplatinate(IV). The UV and IR spectra were scanned on Perkin–Elmer PE-552 and ATI MATTSON Genesis FT IR spectrometers, respectively. NMR spectra were measured on a Varian VXR-400 spectrometer (400 MHz for ^{1}H NMR, 100 MHz for ^{13}C NMR) at 25 °C. The chemical shifts are expressed in ppm with respect to tetramethylsilane, the spin–spin interaction constants are in Hz. The carbon signal multiplicity was measured by APT and DEBT procedures. The mass spectra were obtained on a Finnigan MAT 90 instrument. Alkaloid standards used for identification were obtained from collections of the Institute of Medical Chemistry in Olomouc and Institute of Biochemistry in Brno. Hepabene® is a preparation of Merckle GmbH.



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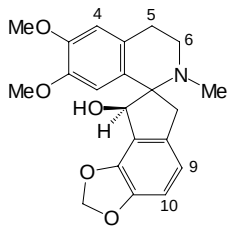

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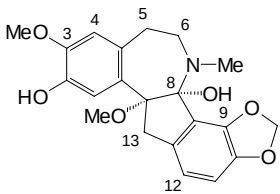

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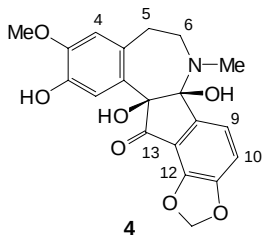

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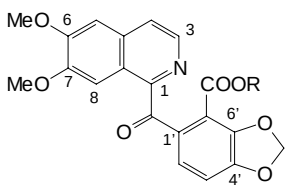

5, R = Me
6, R = H

Plant Material

The flowering aerial parts of *F. densiflora* DC. were collected in the surroundings of Galata (near Varna, Bulgaria) in May 1994 and identified by Dr M. E. Popova (Institute of Pharmacognosy, Varna University). A herbal specimen is deposited with the Institute of Medical Chemistry, Faculty of Medicine, Palacky University in Olomouc.

Extraction and Isolation of Alkaloids

The plant was dried at room temperature. The amount of 2.7 kg of the dry drug was then ground and extracted with methanol at 60 °C. The volume of the extract was reduced by evaporation in a vacuum and dissolved in 1% aqueous sulfuric acid. The lipoid fraction was removed by extraction with petroleum ether, and the L-fraction was separated off by extraction with ether[7]. The aqueous layer was made alkaline with 40% aqueous NaOH to a pH > 12, and this fraction B was extracted. The remaining aqueous fraction was made acidic to pH 4 with 1 M aqueous H_2SO_4, and a saturated solution of potassium iodide was added. The strongly polar alkaloids (fraction I) were extracted into chloroform. Fraction A was dissolved in 1% H_2SO_4 and made alkaline with NaOH, and the majority of non-phenolic tertiary bases (A_1) was extracted into ether. The aqueous phase was adjusted to pH 6 and alkalinized with ammonia. The phenolic bases (A_2) were separated by extraction into ether.

Fraction A_1

Protopine. Obtained as a crystalline substance (1.46 g); m.p. 219–221 °C (MeOH) (ref.[8]: 222–225 °C); the UV, ^{1}H NMR, and ^{13}C NMR spectra were identical with those of the authentic sample.

Cryptopine. Obtained as a crystalline substance (0.37 g), m.p. 187 °C (Me_2CO) (ref.[8]: 185–186 °C); the UV, ^{1}H NMR, and ^{13}C NMR spectra were identical with those of the authentic sample.

Stylopine. Obtained as a crystalline substance (7.2 mg), m.p. 203–205 °C (EtOH) (ref.[8]: 204 °C); the UV, ^{1}H NMR, and ^{13}C NMR spectra were identical with those of the authentic sample.

Sinactine. Obtained as a crystalline substance (12.1 mg), m.p. 168–170 °C (EtOH) (ref.[8]: 169–170 °C); the UV, ^{1}H NMR, and ^{13}C NMR spectra were identical with those of the authentic sample.

Adlumine (**1**). Obtained as a crystalline substance (8.5 mg), m.p. 181–182 °C (MeOH) (ref.[8]: 178–180 °C), $[\alpha_D]$ +42° ($CHCl_3$). UV spectrum (MeOH), λ_{max}, nm (log ε): 288 (3.97), 324 (3.99). FAB-MS, *m/z* 384 (M + H)$^+$. EI-MS, *m/z* (%): 206 (100), 190 (10), 177 (3). ^{1}H NMR spectrum ($CDCl_3$): 2.46 ddd, 1 H, *J* = 15.4, 3.2, 3.0 (H-5a); 2.56 ddd, 1 H, *J* = 11.0, 10.9, 3.0 (H-6a); 2.65 s, 3 H (NMe); 2.78 ddd, 1 H, *J* = 15.4, 11.0, 4.2 (H-5b); 3.08 ddd, 1 H, *J* = 10.9, 4.2, 3.2 (H-6b); 3.76 s, 3 H (3-OMe); 3.82 s, 3 H (2-OMe); 4.08 d, 1 H, *J* = 3.4 (H-14); 5.69 dd, 1 H, *J* = 3.4, 0.9 (H-13); 6.08 s, 2 H (–OCH_2O–); 6.38 s, 1 H (H-4); 6.70 s, 1 H (H-1); 6.88 d, 1 H, *J* = 7.9 (H-11); 7.17 dd, 1 H, *J* = 7.9, 0.9 (H-12).

Fumariline. Obtained as a crystalline substance (28.7 mg), m.p. 137 °C (MeOH) (ref.[8]: 138 °C or 144 °C); the UV, ^{1}H NMR and ^{13}C NMR spectra were identical with those of the authentic sample.

Fumaricine (O-methylfumaritine) (**2**). Obtained as a crystalline substance (13.0 mg), m.p. 173–176 °C (MeOH) (ref.[8]: 177 °C); $[\alpha_D]$ –31° ($CHCl_3$). UV spectrum, λ_{max}, nm (log ε): 212 (4.56), 290 (3.83). EI-MS, *m/z* (%): 369 ($C_{21}H_{23}NO_5$, 36, M$^+$), 354 (73), 338 (16), 336 (6), 322 (12), 206 (100), 190 (9), 153 (4), 136 (5), 89 (6), 77 (10). ^{1}H NMR spectrum ($CDCl_3$): 2.41 s, 3 H (NMe); 2.57 m, 1 H (H-5a); 2.97–3.06 m, 2 H (H-6a, H-5b); 3.28 dd, 1 H, *J* = 15.6, 0.9 (H-13a); 3.34 dd, 1 H, *J* = 15.6, 1.2 (H-13b); 3.51 s, 3 H (2-OMe); 3.78 m, 1 H (H-6b); 3.84 s, 3 H (3-OMe); 4.56 brs, 1 H (H-8); 5.94 d, 1 H, *J* = 1.4 (–OCH_2O–); 5.97 d, 1 H, *J* = 1.4 (–OCH_2O–); 6.41 s, 1 H, (H-1); 6.61 s, 1 H (H-4); 6.72 ddd, 1 H, *J* = 7.8, 1.2, 0.9 (H-12); 6.76 dd, 1 H, *J* = 7.8, 0.8 (H-11). ^{13}C NMR spectrum ($CDCl_3$): 23.03 t (C-5), 38.15 q (NMe), 43.64 t (C-13), 47.53 t (C-6), 55.52 q (2-OMe), 55.73 q (3-OMe), 74.51 s (C-7), 82.10 d (C-8), 101.40 t (–OCH_2O–), 108.51 d (C-11), 110.03 d (C-1),

111.46 d (C-4), 115.48 d (C-12), 124.92 s (C-8a), 125.93 s (C-7a), 128.62 s (C-4a), 134.98 s (C-12a), 144.16 s (C-9), 147.18 s (C-2), 147.31 s (C-10), 148.19 s (C-3).

Bicuculline. Substance identified by TLC, R_F 0.47 (S1); 0.63 (S2).

Densiflorine. Substance identified by TLC, R_F 0.69 (Sl); 0.86 (S2).

Fraction A_2

Parfumine. Obtained as a crystalline substance (52.1 mg), m.p. 110–112 °C (MeOH) (ref.[8]: 111–112 °C); the UV, ^{1}H NMR and ^{13}C NMR spectra are identical with those of the authentic sample.

Fumaritridine (**3**). Obtained as a crystalline substance (18.8 mg), m.p. 202–204 °C (EtOH) (ref.[8]: 198–200 °C), $[\alpha_D]$ +18° ($CHCl_3$). UV spectrum (MeOH), λ_{max}, nm (log ε): 208 (4.65), 230–232 (3.97), 290 (3.66). EI-MS, *m/z* (%): 369 (75, $C_{21}H_{23}NO_5$, M^+), 354 (100), 338 (63), 337 (48), 326 (27), 324 (28), 323 (24), 322 (51), 311 (51), 308 (11), 307 (11), 295 (10), 294 (10), 281 (14), 279 (15), 190 (10), 179 (64), 165 (16), 164 (22), 148 (12). ^{1}H NMR spectrum ($CDCl_3$): 2.26 s, 3 H (NMe); 2.55 ddd, 1 H, *J* = 14.3, 5.9, 3.8 (H-6a); 2.88 ddd, 1 H, *J* = 11.0, 11.0, 5.9 (H-5a); 2.97 ddd, 1 H, *J* = 11.0, 6.8, 6.8 (H-5b); 3.07 s, 3 H (OMe); 3.21 dddd, 1 H, *J* = 14.3, 11.0, 6.8, 0.7 (H-6b); 3.33 dd, 1 H, *J* = 16.4, 1.2 (H-13a); 3.46 d, 1 H, *J* = 16.4 (H-13b); 3.91 s, 3 H (OMe); 4.56 s, 1 H (H-8); 5.92 d, 1 H, *J* = 1.5 (–OCH_2O–); 6.02 d, 1 H, *J* = 1.5 (–OCH_2O–); 6.67 s, 1 H (H-4); 6.73 dd, 1 H, *J* = 7.7, 1.2 (H-12); 6.76 d, 1 H, *J* = 7.7 (H-11); 7.03 s, 1 H (H-1). ^{13}C NMR spectrum ($CDCl_3$): 33.19 t, 41.33 q, 42.54 t, 50.81 q, 55.80 t, 55.91 q, 70.72 d, 92.82 s, 100.83 t, 108.39 d, 112.07 d, 115.94 d, 116.85 d, 122.88 s, 130.25 s, 132.22 s, 136.40 s, 143.60 s, 144.63 s, 145.64 s, 146.31 s.

Scoulerine. Obtained as a crystalline substance (5.2 mg), m.p. 197 °C (MeOH) (ref.[8]: 198–200 °C); the UV, ^{1}H NMR and ^{13}C NMR spectra are identical with those of the authentic substance.

Fumarofine (**4**). Obtained as a crystalline substance (1.7 mg), m.p. 244–246 °C (MeOH) (ref.[8]: 240–245 °C). UV spectrum (MeOH), λ_{max}, nm (log ε): 212 (4.40). 236 (4.54), 245–258 sh (4.06–4.05), 266–275 sh (3.58–3.54), 339 (3.52). EI-MS, *m/z* (%): 369 (100, $C_{20}H_{19}NO_6$, M^+), 354 (55), 326 (21). 298 (11), 284 (28), 283 (13), 204 (13), 192 (24), 190 (12), 179 (17), 177 (13), 164 (14), 162 (15), 151 (13), 77 (10). ^{1}H NMR spectrum (CD_3SOCD_3): 1.97 m, 1 H; 2.28 m, 1 H; 2.36 m, 1 H; 2.47 m, 1 H; 2.49 s, 3 H (OMe); 3.79 s, 3 H (OMe); 4.22 d, 1 H, *J* = 1.0 (H-8); 6.21 d, 1 H, *J* = 1.0 (–CH_2O–); 6.22 d, 1 H, *J* = 1.0 (–OCH_2O–); 6.29 s, 1 H (OH); 6.62 s, 1 H (H-4); 7.13 dd, 1 H, *J* = 7.9, 1.0 (H-9); 7.16 s, 1 H (H-1); 7.31 d, 1 H, *J* = 7.9 (H-10); 8.68 s, 1 H (OH). ^{1}H NMR spectrum (CD_3OD): 2.25 m, 1 H; 2.43 m, 1 H; 2.54 m, 1 H; 2.58 s, 3 H (NMe); 2.66 m, 1 H; 3.86 s, 3 H (OMe); 4.61 s, 1 H; 6.71 s, 1 H; 6.92 s, 2 H (–OCH_2O–); 7.24 dd, 1 H, *J* = 7.9, 0.8; 7.26 d, 1 H, *J* = 7.9; 7.13 s, 1 H. ^{13}C NMR spectrum (CD_3SOCD_3): 31.92 t, 45.64 q, 48.94 t, 55.80 q, 72.36 d, 83.03 s, 103.13 t, 113.34 d, 114.66 d, 114.91 d, 119.04 s, 120.06 d, 126.18 s, 132.71 s, 141.61 s, 143.94 s, 144.27 s, 146.21 s, 148.24 s. The sample was too dilute for the C=O signal to be observable.

Fumaritine. Obtained as a crystalline substance (18.7 mg), m.p. 155–157 °C (Et_2O) (ref.[8]: 198–200 °C); the UV, ^{1}H NMR and ^{13}C NMR spectra were identical with those of the authentic sample.

Fumarophycine. Identified by TLC, R_F 0.25 (S1); 0.40 (S2).

Fraction B

Coptisine. Obtained in the chloride form (9.1 mg). The tetrahydro derivative, m.p. 204–205 °C, was obtained by reduction with zinc in hydrochloric acid; the UV, ^{1}H NMR, and ^{13}C NMR spectra were identical with those of stylopine.

Fumaflorine methyl ester (**5**). Obtained as a crystalline substance (10.6 mg), m.p. 184–186 °C (MeOH). UV spectrum (MeOH), λ_{max}, nm (log ε): 213 (4.62), 236 (4.54), 282–290 sh (4.03–4.01), 334 (4.07). EI-MS, *m/z* (%): 395 (1, $C_{21}H_{17}NO_7$, M^+), 364 (5, $C_{20}H_{14}NO_6$), 353 (8), 352 (37,

$C_{19}H_{14}NO_6$), 337 (22), 336 (100, $C_{19}H_{14}NO_5$), 320 (5), 292 (3), 207 (12), 188 (2), 164 (3), 45 (3). ^{1}H NMR spectrum ($CDCl_3$): 3.36 s, 3 H ($COOCH_3$); 4.07 s, 3 H (3-OMe); 3.08 s, 3 H (2-OMe); 6.19 s, 2 H (–OCH_2O–); 6.99 d, 1 H, *J* = 8.0 (H-12); 7.19 s, 1 H (H-1); 7.23 d, 1 H, *J* = 8.0 (H-11); 7.69 d, 1 H, *J* = 5.5 (H-5); 8.20 s, 1 H (H-4); 8.31 d, 1 H, *J* = 5.5 (H-6). ^{13}C NMR spectrum ($CDCl_3$): 52.65 q ($COOCH_3$), 56.51 q (2-OMe), 56.56 q (3-OMe), 103.34 t (–OCH_2O–), 104.93 d (C-4), 105.35 d (C-11), 110.38 d (C-12), 114.88 s, 123.19 d (C-5), 123.63 s, 126.45 d (C-1), 133.93 s, 134.91 s, 140.13 d (C-6), 147.84 s (C-8), 151.81 s, 151.94 s, 152.30 s, 153.72 s, 165.67 s, 196.05 s (C-13).

Fraction I

cis-N-Methylstylopinium iodide. Obtained as a crystalline substance (36.5 mg), m.p. 274 °C (MeOH) (ref.[9]: 275–280 °C); the UV, ^{1}H NMR, and ^{13}C NMR spectra were identical with those of the authentic sample.

Fumaflorine (**6**). Obtained as a crystalline substance (7.7 mg), m.p. 218–220 °C (MeOH). UV spectrum (MeOH), λ_{max}, nm (log ε): 236 (4.69), 330 (3.98). IR spectrum (KBr): 1 505, 1 590, 1 617, 1 662, 2 360, 2 923, 3 426 cm^{-1}. EI-MS, *m/z* (%): 381 (4, M^+), 365 (8, $C_{20}H_{15}NO_6$), 364 (11), 352 (21, $C_{19}H_{14}NO_6$), 337 (28), 336 (100, $C_{19}H_{14}NO_5$), 322 (13), 308 (11), 193 (8). ^{1}H NMR spectrum (CD_3OD): 4.04 s, 3 H (3-OMe); 4.07 s, 3 H (2-0Me); 6.19 s, 2 H (–OCH_2O–); 6.95 d, 1 H, *J* = 8.0 (H-12); 7.16 d, 1 H, *J* = 8.0 (H-11); 7.22 s, 1 H (H-1); 7.71 d, 1 H, *J* = 5.5 (H-5); 8.11 s, 1 H (H-4); 8.29 d, 1 H, *J* = 5.5 (H-6). ^{13}C NMR spectrum (CD_3OD): 56.65 q (2 C, 2 × OMe), 103.43 t (–OCH_2O–), 105.52 d (2 C, C-1, C-4), 110.02 d (C-12), 117.24 s, 123.23 d (C-5), 123.85 s, 126.40 d (C-11), 134.31 s, 134.19 s, 140.03 d (C-6), 147.77 s, 152.05 s, 152.28 s, 153.03 s, 154.01 s, 167.88 s, 195.93 s.

Corytuberine hydroiodide. Obtained as a crystalline substance (10.2 mg), m.p. 212–213 °C (MeOH) (ref.[10]: 215–217 °C); the UV, ^{1}H NMR, and ^{13}C NMR spectra are identical with those of the authentic sample.

Isolation of Hepatocytes, Preparation of Tissue Cultures, Hepatoprotective Effects

Hepatocytes were isolated by collagenase perfusion of rat liver[11]. Extracted from the abdomen, the liver was washed with Hank's buffer I containing a chelating agent (EDTA) for removal of Ca^{2+} for 4 min, and with Hank's buffer II containing 0.1% collagenase and Ca^{2+} for 6 min. Subsequently, the cells were extracted into the HEPES buffer, and the cell suspension was filtered through a sterile mull, centrifuged 3–5 times at 50 g for 1 min, and washed with HEPES buffer. The cell viability was determined using trypane blue stain; the yields were (3–5) . 10^8 cells per liver. The cell viability was higher than 90%.

The isolated hepatocytes suspended in a cultivating medium (Eagls' minimal essential medium) containing 5% inactivated bovine serum, penicillin (100 IU/l), streptomycin (100 μg/ml), and insulin (10^{-8} M) were placed in 96-well tissue culture plates (2.5 . 10^5 cells/ml) and preincubated for 1.5 h in a humidified incubator at 37 °C in an atmosphere containing 5% CO_2. The cells formed a monolayer on the bottom of the dishes[12,13] in 60 min.

After preincubation, the cells were exposed to the medium containing 10 mM CCl_4 and the extracts were added to produce a resulting concentration of 0, 0.25, 1 or 2 mg/ml, or the pure isolated alkaloids at a concentration of 0.1, 1 or 2 mM (all in DMSO, resulting concentration 0.5%). Control samples contained only DMSO. After 1 h, the activity of cytosolic lactate dehydrogenase (LDH) was determined in the medium. The results were expressed in terms of activity released into the medium by 10^6 cells, and as a per cent of damaged cells (LDH value for controls 0%, for damage with CCl_4 100%).

TABLE I

The cytoprotective effect of extracts of *Fumaria densiflora*, *F. officinalis*, Hepabene, and the alkaloids: protopine, cryptopine, and parfumine, on carbon tetrachloride-induced damage of primary cultures of rat hepatocytes. The values are given as averages of 5 measurements ± standard deviation

Sample	Concentration	LDH, ncat/10^6 cells
Carbon tetrachloride	10 mmol/l	27.08 ± 0.12 (100)
F. densiflora (crude extract)	0.25 mg/ml	26.12 ± 0.79 (91 ± 3)
	1.00 mg/ml	21.39 ± 0.77 (96 ± 3)
	2.00 mg/ml	17.16 ± 0.75[a] (65 ± 4)
F. densiflora (phenolic extract) (L)	0.25 mg/ml	24.10 ± 0.48 (89 ± 2)
	1.00 mg/ml	25.99 ± 0.42 (96 ± 2)
	2.00 mg/ml	22.74 ± 0.37[a] (84 ± 2)
F. officinalis (crude extract)	0.25 mg/ml	26.84 ± 0.80 (95 ± 2)
	1.00 mg/ml	22.21 ± 0.63 (80 ± 4)
	2.00 mg/ml	15.82 ± 0.44[a] (52 ± 5)
F. officinalis (phenolic extract) (L)	0.25 mg/ml	26.25 ± 0.52 (93 ± 1)
	1.00 mg/ml	25.43 ± 0.66 (90 ± 2)
	2.00 mg/ml	23.92 ± 0.41 (84 ± 2)
Hepabene® (total extract)	0.25 mg/ml	25.57 ± 0.70 (90 ± 3)
	1.00 mg/ml	23.14 ± 0.59 (82 ± 4)
	2.00 mg/ml	21.32 ± 0.41 (78 ± 1)

TABLE I
(*Continued*)

Sample	Concentration	LDH, ncat/10^6 cells
Hepabene®	0.25 mg/ml	24.72 ± 0.78
(alkaloid extract)		(89 ± 3)
	1.00 mg/ml	19.68 ± 0.33[a]
		(70 ± 2)
	2.00 mg/ml	12.37 ± 0.25[a]
		(43 ± 4)
Protopine	0.1 mmol/l	25.09 ± 0.42
		(90 ± 2)
	1.0 mmol/l	20.10 ± 0.49[a]
		(72 ± 3)
	2.0 mmol/l	11.10 ± 0.21[a]
		(41 ± 2)
Cryptopine	0.1 mmol/l	22.44 ± 0.91
		(83 ± 4)
	1.0 mmol/l	23.15 ± 0.94
		(85 ± 4)
	2.0 mmol/l	16.52 ± 0.83[a]
		(61 ± 5)
Parfumine	0.1 mmol/l	22.52 ± 1.15
		(83 ± 5)
	1.0 mmol/l	19.77 ± 0.82[a]
		(73 ± 4)
	2.0 mmol/l	18.41 ± 0.95[a]
		(68 ± 5)
Control		5.02 ± 0.05
		(0)

[a] $p < 0.05$ (Student's *t*-test), significantly different from cells damaged with CCl_4.

RESULTS AND DISCUSSION

The following acids were identified by GC-MS: azealic, ferulic, 3-hydrobenzoic, 5-hydroxyferulic, 3-hydroxy-4-methoxybenzoic, malic, caffeic, 4-methoxy-3-hydroxycinnamic, protocatechuic, sebacic, and cinnamic acids; also identified were methyl caffeate and methyl protocatechuate[14]. Crystallization of the ethereal extract L from methanol yielded fumaric acid and its monomethyl ester.

The alkaloid fraction A was separated into fraction A_1 containing non-phenolic bases and fraction A_2 containing phenolic bases. Fraction A_1 gave protopine and cryptopine

and, by column chromatographic separation, stylopine, sinactine, adlumine (**1**), fumariline, and fumaricine (**2**). Furthermore, bicuculline and densiflorine were identified in this fraction by TLC. Chromatography of fraction A_2 gave parfumine, fumaritridine (**3**), scoulerine, fumarofine (**4**), and fumaritine; fumarophycine was identified by TLC only. From fraction B, coptisine and fumaflorine methyl ester (**5**) were obtained by crystallization. Crystallization of fraction I from methanol provided *cis*-*N*-methylstylopinium iodide, and subsequent chromatography of the mother liquors gave the 1-benzylisoquinoline alkaloid fumaflorine (**6**), a substance which had not been found before and whose structure has been elucidated by spectral methods[15], and corytuberine hydroiodide. Fumaflorine methyl ester (**5**) seems to be an artifact, formed during the extraction procedure. Corytuberine, fumaricine (**2**), fumarophycine, stylopine, *cis*-*N*-methylstylopinium iodide, adlumine (**1**), fumaritridine (**3**), and fumaflorine (**2**) had not been identified in *F. densiflora* before. The differences in the proportions of the minority alkaloids in *F. densiflora*[3–5] must be attributed to varying biogeographic conditions.

The European pharmaceutical industry offers 20 phytopharmaceuticals based on *Fumaria* extracts[16]. Most of the preparations are indicated in diseases associated with malfunctions of the biliary tract. It is interesting that *Fumaria* extracts have never been evaluated with respect to their cytoprotective activity. Table I gives results obtained from testing of the cytoprotective effect of the crude extracts of *F. densiflora* and *F. officinalis* L. and of the major alkaloids: protopine, cryptopine, and parfumine, on primary cultures of rat hepatocytes intoxicated with CCl_4, a toxin which models radical hepatic damage seen in human disease. The activity of the leaked lactate dehydrogenase served as the evaluating index in this experiment. Hepabene, which contains the plant hepatoprotective silymarine in addition to the *F. officinalis* extract, was used as the positive control. A significant hepatocyte protection against CCl_4 was attained at a *F. densiflora* concentration of 2 mg/ml. The effect of the extract of *F. officinalis* was comparable. The phenolic fraction L exhibited no cytoprotective effect. The cytoprotective effect of the alkaloids was significant even at the lowest concentration of 1.0 mM. The total extract of Hepabene in the tablet form displayed no cytoprotective effect and the activity of the alkaloid extract of Hepabene was comparable to that of the crude extracts of the two *Fumariae* tested. It is thus evident that the alkaloids are primarily responsible for the hepatocyte protection from intoxication with CCl_4. None of the alkaloids can be regarded as antioxidant. Presumably, their protective effect is due to the inhibition of enzymes activating the radical cleavage of the carbon tetrachloride molecule to the trichloromethyl radical.

The authors wish to thank Dr M. E. Popova (Institute of Pharmacognosy, Varna University, Bulgaria) for plant material collection, Dr V. Havlicek (Institute of Microbiology, Academy of Sciences of the Czech Republic) for mass spectra measurements, and the Grant Agency of the Czech Republic for financial support.

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