Isoquinoline alkaloids from cell suspension cultures of *Fumaria capreolata*

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**ABSTRACT**

*Fumaria capreolata* was taken into cell suspension culture. The culture yielded a biomass of about 12 g dry weight per liter of medium; the dried cells contained ca. 0.1% of alkaloids. Besides choline, the following ten known isoquinoline alkaloids were isolated from the cell extract in crystalline form: coptisine, dehydrocheilanthifoline; (+)-isoboldine; magnoflorine; N-methylcoclaurine; (+)-reticuline; (-)-pallidine; protopine; sanguinarine; (-)-scoulerine. This is the most diverse isoquinoline alkaloid spectrum thus far published for a cell suspension culture.

**INTRODUCTION**

Plant cell cultures are an excellent source of enzymes to study the biosynthesis of alkaloids at the cell-free level (Stöckigt, 1980; Zenk, 1985). Using cell cultures mainly of the genus *Berberis* (Hinz and Zenk, 1981) it was recently shown in these laboratories that the entire sequence of enzymes leading from the biosynthetic precursors 3,4-dihydroxyphenylacetaldehyde and dopamine to berberine could be purified and characterized (Zenk, 1985). In order to extend this type of study to other alkaloid classes within the isoquinoline group and also to have access to material for physiological studies, we screened well over 200 tissue cultures from our collection of plant species known or presumed to synthesize this type of alkaloids. Among those species showing the most diverse alkaloid patterns was *Fumaria capreolata*. The chemistry of this plant, which occurs abundantly mainly in France and Syria has only been investigated superficially. Sanguinarine and coptisine are the only alkaloids known to occur in this species (Susplugas et al., 1974; 1976). In the present communication we report the occurrence and identity of the major alkaloids in cultivated suspension cells of this *Fumaria* species.

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**EXPERIMENTAL**

**Culture**

Callus cultures of *F. capreolata* were established from aseptically germinated seedlings using Linsmaier and Skoog (1965) medium. Flasks contained 250 ml of the same medium (shaken at 100 rev/min in continuous light) and were subcultured every 7 days. The cultures used in this study had been maintained as suspension for over 5 years.

**Extraction and Fractionation**

Frozen tissues (6.05 kg, dry weight 270 g) were percolated with MeOH. The filtered extract was concentrated *in vacuo*. The viscous mass was triturated repeatedly with 2% tartaric acid and the aqueous extracts were washed successively with benzene and CHCl₃. The acidic solution was made alkaline with NH₄OH and extracted with CHCl₃. The CHCl₃ extract was fractionated to afford non-phenolic bases (123 mg) and phenolic bases (180 mg) by treatment with 5% NaOH. The remaining aqueous layer was acidified with HCl and the quaternary alkaloids were precipitated by means of ammonium reineckate. The precipitate was dissolved in acetone-MeOH and passed through an ion exchange resin (Amberlite IRA-410, Cl-form). The eluate was concentrated under reduced pressure to afford the quaternary alkaloid chlorides (1.32 g).

**Isolation of alkaloids**

The non-phenolic base fraction was crystallized repeatedly from CHCl₃-MeOH to yield protopine (11) (56.1 mg). The mother liquors were concentrated and subjected to preparative TLC (benzene-Et₂O, 1:1), giving traces of sanguinarine (I). The phenolic base fraction was purified by repeated preparative TLC using the following solvent systems:
a) CHCl₃-MeOH-NH₄OH (95:5:0.5) (b) CHCl₃-MeOH-NH₄OH (92:8:0.5) (c) acetone-CHCl₃-MeOH-NH₄OH (35:15:3:0.5). Six alkaloids were isolated and identified as (-)-scoulerine (III) (9.6 mg), (+)-isoboldine (IV) (7.8 mg), (-)-pallidine (V) (2.9 mg), N-methylcoclaurine (VI) (6.2 mg), (+)-reticuline (VII) (47.3 mg), and a partially-characterized simple isoquinoline alkaloid (VIII) (2.4 mg). The mixture (1.09 g) of quaternary alkaloids was subjected to droplet countercurrent chromatography (DCCC). DCCC was carried out by the ascending method with solvent system CHCl₃-MeOH-H₂O (5:5:3) to yield coptisine (IX) (1.5 mg), dehydrocheilanthifoline (X) (9.9 mg), magnoflorine (XI) (24.9 mg), and choline (760 mg) as hydrochloride.

**Identification of the alkaloids**

**Sanguinarine**

(I) -- UV: λmax MeOH 226, 281, 322 nm. MS: m/z 332 (M⁺, 63), 317(100), 259(27), 201(46). This substance was observed as a characteristic orange band on TLC.

**Protopine**

(II) -- mp 208-210.5°. mmp 208-210.5°. MS: m/z 353 (M⁺, 4), 281(4), 267(5), 252(4), 251(3), 210(6), 163(17), 148(100), 134(9). This compound showed a purple colour upon treatment with AcOH-H₂SO₄.

**(-)-Scoulerine**

(III) -- [α]D12 256° (MeOH, c 0.14). UV: λmax MeOH 225(sh), 284; λmax EtOH+NaOH 245, 296 nm. MS: m/z 327 (M⁺, 100), 326(39), 178(74), 176(24), 150(38), 149(10), 135(25). 1H-NMR (CDCl₃): 3.86 (3H, s, OMe), 3.87(3H, s, OMe), 5.3-5.8 (2H, m, OH), 6.59(1H, s, ArH), 6.66 (1H, d, J=8.5 Hz, ArH), 6.73 (1H, d, J=8.5 Hz, ArH), 6.82 (1H, s, ArH).

**(+)-Isoboldine**

(IV) -- [α]D16 +650° (MeOH, c 0.11). UV: λmax MeOH 269(sh), 279, 303, 311 (sh); λmax MeOH+NaOH 322 nm. IR: ν CHCl₃ 3525 cm⁻¹. MS: m/z 327 (M⁺, 78), 326(100), 312(22), 295(14), 284(33), 269(10), 253(7); 1H-NMR (CDCl₃): 2.54 (3H, s, NMe), 3.93 (3H, s, OMe) 3.95 (3H, s, OMe), 6.10 (1H, br s, OH), 6.53 (1H, s, 3-H), 6.82 (1H, s, 8-H), 8.02 (1H, s, 11-H).

**N-methylcoclaurine**

(V) -- UV: λmax EtOH+NaOH 244, 300 nm. MS: m/z 299 (M⁺, 0.1), 193(24), 192(100), 178(6), 177(33), 149(6), 149(7), 107(5). 1H-NMR (CDCl₃): 2.46 (3H, s, NMe), 3.80 (3H, s, OMe), 6.36 (1H, s, 8-H), 6.54 (1H, s, 5-H), 6.79 (4H, A₂B₂ q, J=8.0 Hz, 2',3',5',6'-H).

**(+)-Reticuline**

(VII) -- [α]D16 +1070° (MeOH, c 0.16). UV: λmax EtOH 225(sh) 283.5; λmax EtOH+NaOH 253 nm. MS: m/z 192(100), 177(2), 149(5), 149(11). 1H-NMR (CDCl₃): 2.46 (3H, s, NMe), 3.83 (3H, s, OMe), 6.34 (3H, s, OMe), 5.12 (2H, br s, OH), 6.36 (1H, s, 8-H), 6.52 (1H, s, 5-H), 6.57 (1H, dd, J=3.0 and 9.0 Hz, 6'-H), 6.72 (1H, d, J=9.0 Hz, 5'-H), 6.76 (1H, d, J=3.0 Hz, 2'-H).

**Simple isoquinoline**

(VIII) -- UV λmax EtOH 226(sh), 285; λmax EtOH+NaOH 245, 301 nm. MS: m/z 207 (M⁺, 4), 206(4), 192(100), 177(32), 164(4), 149(11). 1H-NMR (CDCl₃): 1.46 (3H, d, J=6.8 Hz, CMe), 2.58 (3H, s, NMe), 2.73-3.27(4H, m, 3, 4-H), 3.76 (1H, q, J=6.8 Hz, 1-H), 3.85 (3H, s, OMe), 4.28 (1H, OH), 6.55 (1H, s, ArH), 6.65 (1H, s, ArH).

**Coptisine**

(IX) -- UV λmax EtOH 223, 240(sh), 265, 348, 358, 458 nm. 1H-NMR (CD₃COOD+D₂O): 3.23 (2H, t, J=6.0 Hz, 6-H), 4.83 (2H, t, J=6.0 Hz, 5-H), 6.07 (2H, s, OCH₂O-), 6.40 (2H, s, -OCH₃O-), 6.89 (1H, s, 4'-H), 7.45 (1H, s, 1'-H), 7.75 (1H, s, 11 or 12-H), 7.80 (1H, s, 12 or 11-H), 8.43 (1H, s, 13-H), 9.35 (1H, s, 3-H): Reduction of the compound with NaBH₄ gave (+)-stylopine. MS: m/z 323 (M⁺, 44), 322(39), 174(11), 148(100).