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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 continous 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 $CHCl_3$. The acidic solution was made alkaline with NH_4OH and extracted with $CHCl_3$. The $CHCl_3$ 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 presure to afford the quaternary alkaloid chlorides (1.32 g).

Isolation of alkaloids

The non-phenolic base fraction was crystallized repeatedly from $CHCl_3$-MeOH to yield protopine (II) (56.1 mg). The mother liquors were concentrated and subjected to preparative TLC (benzene-Et_2O, 1:1), giving traces of sanguinarine (I). The phenolic base fraction was purified by repeated preparative TLC using the following solvent systems:

a) $CHCl_3$-MeOH-NH_4OH (95:5:0.5) (b) $CHCl_3$--MeOH-NH_4OH (92:8:0.5) (c) acetone-$CHCl_3$--MeOH-NH_4OH (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 $CHCl_3$-MeOH-H_2O (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: λ_{max}^{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°. mmp 208-210.5°. MS: m/z 353 (M^+, 4), 281(4), 267(5), 252(4), 251(3), 190(6), 163(17), 148(100), 134(9). This compound showed a purple colour upon treatment with AcOH-H_2SO_4.

(-)-Scoulerine

(III)-$[\alpha]_D^{12}$ -256° (MeOH, c 0.14). UV: λ_{max}^{EtOH} 225(sh), 284; $\lambda_{max}^{EtOH+NaOH}$ 245, 296 nm. MS: m/z 327 (M^+, 100), 326(89), 178(74), 176(24), 150(38), 149(10), 135(25). ^{1}H-NMR ($CDCl_3$): 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)--$[\alpha]_D^{16}$ +65° (MeOH, c 0.11). UV: λ_{max}^{MeOH} 269(sh), 279, 303, 311 (sh); $\lambda_{max}^{MeOH+NaOH}$ 322 nm. IR: $\nu_{max}^{CHCl_3}$ 3525 cm^{-1}. MS: m/z 327 (M^+, 78), 326(100), 312(22), 295(14), 284(33), 269(10), 253(17); ^{1}H-NMR ($CDCl_3$): 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).

(-)-Pallidine

(V)--$[\alpha]_D^{16}$ -33° (MeOH, c 0.15). UV: λ_{max}^{EtOH} 239, 284.5 nm. IR: $\nu_{max}^{CHCl_3}$ 3535, 1670, 1645, 1625 cm^{-1}. MS: m/z 327 (M^+, 100), 312(36), 299(48), 284(75), 268(49), 256(31), 242(42). ^{1}H-NMR ($CDCl_3$): 2.46 (3H, s, NMe), 3.80 (3H, s, OMe), 3.89 (3H, s, OMe), 6.33 (1H, s, 8-H), 6.33 and 6.70 (2H, each s, 1 and 4-H), 6.77 (1H, s, 5-H).

N-methylcoclaurine

(VI)--UV: $\lambda_{max}^{EtOH+NaOH}$ 244, 300 nm. MS: m/z 299 (M^+, 0.1), 193(24), 192(100), 178(6), 177(33), 149(6), 148(7), 107(5). ^{1}H-NMR ($CDCl_3$): 2.49 (3H, s, NMe), 3.85 (3H, s, OMe), 6.36 (1H, s, 8-H), 6.54 (1H, s, 5-H), 6.79 (4H, A_2B_2 q, J=8.0 Hz, 2',3',5',6'-H).

(+)-Reticuline

(VII)--$[\alpha]_D^{16}$ +107° (MeOH c 0.16). UV: λ_{max}^{EtOH} 225(sh) 283.5; $\lambda_{max}^{EtOH+NaOH}$ 253 nm. MS: m/z 192(100), 177(21), 149(5), 137(3). ^{1}H-NMR ($CDCl_3$): 2.46 (3H, s, NMe), 3.68 (1H, m, 1-H), 3.83 (3H, s, OMe), 3.84 (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 λ_{max}^{EtOH} 226(sh), 285; $\lambda_{max}^{EtOH+NaOH}$ 245, 301 nm. MS: m/z 207 (M^+, 4), 206(4), 192(100), 177(32), 164(4), 149(11). ^{1}H-NMR ($CDCl_3$): 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 λ_{max}^{EtOH} 223, 240(sh), 265, 348, 358, 458 nm. ^{1}H-NMR ($CD_3COOD+D_2O$): 3.23 (2H, t, J=6.0 Hz, 6-H), 4.83 (2H, t, J=6.0 Hz, 5-H), 6.07 (2H, s, OCH_2O-), 6.40 (2H, s, -OCH_2O-), 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, 8-H): Reduction of the compound with $NaBH_4$ gave (±)-stylopine. MS: m/z 323 (M, 44), 322(39), 174(11), 148(100).

Dehydrocheilanthifoline

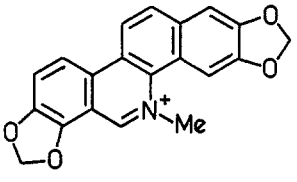
(X)--UV λ_{max}^{EtOH} 224(sh), 241(sh), 262, 347, 359, 463 nm. ^{1}H-NMR ($CF_3COOD+CD_3COOD$): 3.28 (2H, t, J=6.0 Hz, 6-H), 4.00 (3H, s, OMe), 4.37 (2H, t, J=6.0 Hz, 5-H), 6.38 (H, s, $-OCH_2O-$), 6.95 (1H, s, 4-H), 7.62 (1H, s, 1-H), 7.76 (2H, s, 11, 12-H), 8.44 (1H, s, 13-H), 9.32 (1H, s, 8-H). This substance was reduced to (±)-cheilanthifoline. MS: m/z 325 (M^+, 68), 324(56), 176(10), 148(100) (Tani et al., 1974).

Magnoflorine

(XI)--UV λ_{max}^{MeOH} 229, 270(sh), 278, 324 nm. MS: m/z 342 (M^+), 341, 327, 326, 312, 310, 297, 296, 283. ^{1}H-NMR (CD_3OD): 3.08 (3H, s, NMe), 3.43 (3H, s, NMe), 3.95 (3H, s, OMe), 3.98 (3H, s, OMe), 6.96 (1H, s, 3-H), 7.03 (2H, s, 8 and 9-H).

Choline

This compound was in all respects (NMR; IR; TLC) identical to authentic choline chloride (obtained from Sigma).



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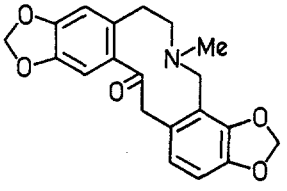

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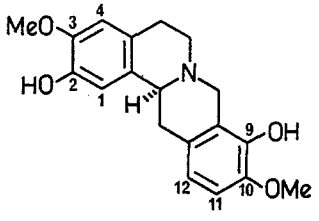

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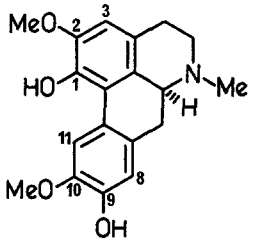

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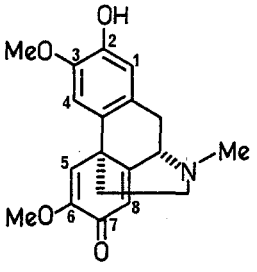

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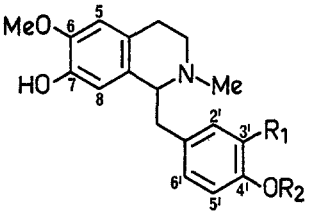
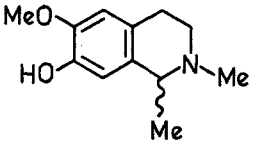


VI $R_1 = R_2 = H$

VII $R_1 = OH$, $R_2 = Me$



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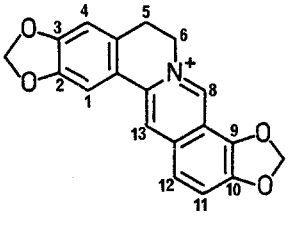

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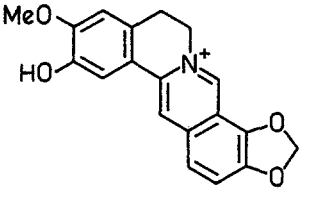

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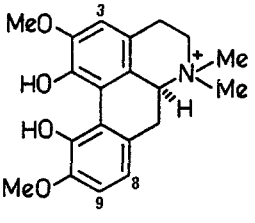

XI

RESULTS and DISCUSSION

F. capreolata cell suspension cultures proved to be an interesting source of structurally highly diverse isoquinoline alkaloids. A total of ten known alkaloids could be isolated and identified. The compounds ranged from a simple isoquinoline alkaloid (VIII) via benzylisoquinolines (VI, VII) to a tetrahydroprotoberberine (III), protoberberines (IX, X), a protopine (II), a benzophenanthridine (I), aporphines (IV, XI), and finally to a morphinanedienone-type alkaloid (V).
No plant cell culture has been shown previously to contain such a diversity of isoquinoline alkaloids. Optimization of this culture should give higher alkaloid yields or cellular selection for a given structural type by exploiting clonal variation (Zenk, 1978) will render this culture more suitable for biosynthetic studies. All of the alkaloids found in this cell suspension culture except VI and VIII are derived from (S)--reticuline, the compound which was also the most abundant in this culture. In a preliminary attempt to investigate the biosynthesis of VIII, no evidence was found that this simple alkaloid could be formed by degradation of any reticuline derived precursor. Labelling experiments using highly radioactive N-$^{14}CH_3$ -(S)-reticuline gave absolutely no incorporation into VIII. We hope that this culture will prove to be a tool for physiological and biosynthetic studies regarding the formation and storage of alkaloids.

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