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Studies on Orchidaceae Alkaloids. XXXIX.* Isolation of (−)-Cryptostyline I, II, III and two Quaternary Salts from *Cryptostylis erythroglossa* Hayata. Biosynthetic Studies of (−)-Cryptostyline I

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(−)-Cryptostyline I, II and III, together with 1-(3,4-methylenedioxyphenyl)-6,7-dimethoxy-2-methyl-3,4-dihydroisoquinolinium iodide and 1-(3,4-methylenedioxyphenyl)-6,7-dimethoxy-2-methylisoquinolinium chloride have been isolated from *Cryptostylis erythroglossa* Hayata.

The biosynthesis of (−)-cryptostyline I has been studied using radioactive precursors and the position of the radio-label determined by degradation. The biosynthetic results show that tyrosine and 3,4-dihydroxyphenylalanine as well as tyramine and dopamine are specifically incorporated. The finding that 3-hydroxy-4-methoxyphenethylamine is better incorporated than the isomeric 4-hydroxy-3-methoxyphenethylamine suggests that the ring closure to the tetrahydroisoquinoline skeleton is facilitated by a *para*-hydroxy group.

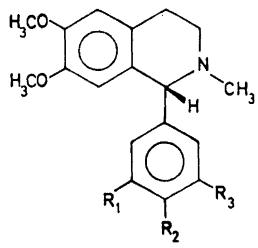
(+)-Cryptostyline I, II and III have been isolated by Leander *et al.*[2] from *Cryptostylis fulva* Schltr. The absolute configuration of these alkaloids has been established by two X-ray diffraction investigations.[3,4] In this paper we report the isolation of (−)-cryptostyline I, II and III from *C. erythroglossa* Hayata together with the immonium salt IV and the isoquinolinium salt V. Biosynthetic studies of (−)-cryptostyline I are also reported.

The structure of IV was established by comparing its iodide with an authentic sample of 1-(3,4-methylenedioxyphenyl)-6,7-dimethoxy-

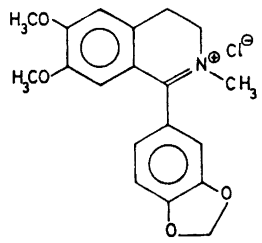
* For number XXXVIII, see Ref. 1.

Table 1.

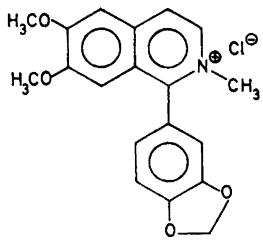
Precursor introduced	Amount fed mg	Amount fed μCi	Cryptostyline I Isolated mg	Cryptostyline I Dpm/mmol	Radioactivity % I	VIII	IX	X	XI
(±)-Tyrosine-α-^{14}C	0.18	50	100	68 300	100	84	7	88	
Tyramine-α-^{14}C	5.7	150	30	12 800	100	87	3	91	
(±)-3,4-Dihydroxyphenylalanine-α-^{14}C	0.12	50	30	13 500	100	94	5	91	
Dopamine-α-^{14}C	1.9	50	30	7 230	100	77	16	74	
3-Hydroxy-4-methoxyphenethylamine-α,β-^{3}H	3.9	70	25	6 870	100	69			25
4-Hydroxy-3-methoxyphenethylamine-α,β-^{3}	3.7	38	30	523					
4-Hydroxy-3-methoxyphenethylamine-5-^{3}H	0.003	250	30	1 470					



R(-)-Cryptostyline
I R_1=H, R_2R_3= O—CH_2—O
II R_1=H, R_2=R_3=OCH_3
III R_1=R_2=R_3=OCH_3



1-(3,4-Methylenedioxyphenyl)-
-6,7-dimethoxy-2-methyl-3,4-
-dihydroisoquinolinium chloride
(IV)



1-(3,4-Methylenedioxyphenyl)-
-6,7-dimethoxy-2-methyl-
-isoquinolinium chloride
(V)

2-methyl-3,4-dihydroisoquinolinium iodide.[2] Identification of V was accomplished by comparison with a synthetic sample, obtained by dehydrogenation of 1-(3,4-methylenedioxyphenyl)-6,7-dimethoxy-3,4-dihydroisoquinoline[2] with selenium, followed by methylation.

The cryptostyline alkaloids are interesting from a biogenetic point of view since they are the first 1-phenyl-tetrahydroisoquinolines isolated from Nature. For this reason the biosynthesis of (−)-cryptostyline I in *C. erythroglossa* has been studied using radioactive precursors.

The potential precursors shown in Table 1 were administered to the plant and (−)-cryptostyline I was isolated. The results show that tyrosine and 3,4-dihydroxyphenylalanine as well as the corresponding amines, tyramine and dopamine, were specifically incorporated, but in low yields. The results of feeding experiments with the two isomeric compounds 3-hydroxy-4-methoxyphenethylamine and 4-hydroxy-3-methoxyphenethylamine suggest that only the former compound (which occurs in, *e. g.*, *Pachycereus pecten-aboriginum* (Eng.) Br&R.[5]) is a precursor of (−)-cryptostyline I.

The early steps in the formation of 1-benzyltetrahydroisoquinoline alkaloids have so far received scant attention,[6] whereas the formation of the tetrahydroisoquinoline skeleton of cactus alkaloids has been extensively studied.[7–9] The present results indicating alternative paths (Fig. 2) to dopamine from tyrosine *via* tyramine or from 3,4-dihydroxyphenylalanine are analogous to previous results on the biosynthesis of anhalamine and anhalonidine in the cactus *Lophophora williamsii* (Lem.) Coult.[9]

In the biosynthesis of, *e.g.*, anhalamine, which is a 6,7,8-trisubstituted tetrahydroisoquinoline, 3-hydroxy-4,5-dimethoxyphenethylamine is the immediate progenitor of the tetrahydroisoquinoline skeleton, thus providing an *ortho*-activation suitable for ring-closure.[9] The present results with (−)-cryptostyline I suggest that the ring-closure is facilitated by a *para*-hydroxy group (Fig. 2). The origin of the remaining C_6-C_1 moiety of (−)-cryptostyline I remains to be elucidated. It may possibly be derived from protocatechualdehyde or partially *O*-methylated derivates thereof as has been shown for some Amaryllidaceae alkaloids.[6]

The specificity in the incorporation of the precursors into (−)-cryptostyline I was established by degradation. The ^{14}C-labelled precursors would, if incorporated without extensive break-down, label (−)-cryptostyline I at C-3. This carbon atom was isolated as the dimedone derivative of formaldehyde (X), obtained as shown in Fig. 1. The extensive presence of radioactivity in this position (Table

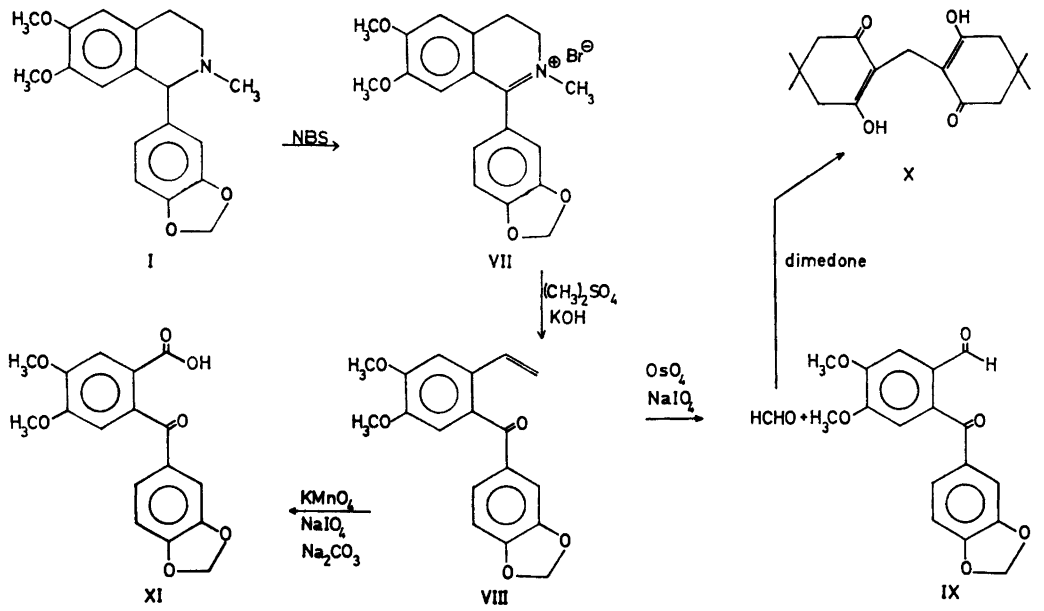


Fig. 1. Degradation of cryptostyline I.

1) indicates direct incorporation. The ^{3}H-labelled precursors would be expected to label the C_3 and C_4 positions in (−)-cryptostyline I. Degradation to compound XI showed predominant labelling in the expected positions (Table 1).

EXPERIMENTAL

Melting points are corrected. Mass spectra were measured on a Perkin-Elmer 270 instrument, the IR spectra on a Perkin-Elmer 257 instrument, the UV spectra on a Beckman DK 2 instrument and the NMR spectra on a Varian A-60A spectrometer. Elemental analyses were carried out at Alfred Bernhardt, Mikroanalytisches Laboratorium, Elbach über Engelskirchen, Germany, and Mikroanalyslaboratoriet, Lantbrukshögskolan, Uppsala, Sweden. Radioactivities were measured with a Packard Tri-Carb Model 3375 liquid scintillation spectrometer, in a solvent system consisting of 2 ml absolute ethanol and 10 ml Instagel® (Packard Instrument Corp.). External standardization was used for efficiency determination. Preparative thin-layer chromatography was carried out on alumina (1.5 mm) F-254 Type T (Merck) or silica gel (2 mm) 60F-254 (Merck).

(±)-Tyrosine-α-^{14}C, (±)-3,4-dihydroxyphenylalanine-α-^{14}C, tyramine-α-^{14}C, dopamine-α-^{14}C and 4-hydroxy-3-methoxy-5-^{3}H-phenethylamine were obtained from the Radiochemical Centre, Amersham, UK and New England Nuclear Corp., Boston, USA. The preparation of 4-hydroxy-3-methoxy-α,β-^{3}H-phenethylamine and 3-hydroxy-4-methoxy-α,β-^{3}H-phenethylamine has already been described.[10]

The plants were purchased from Chow Cheng Orchids, 194 Litoh-St. Taichung, Taiwan.

Feeding experiments. Each labelled precursor was dissolved in a minute quantity of water and injected into the stems of two plants of *C. erythroglossa*. After three weeks the alkaloid fraction was isolated as described below. Cryptostyline I ($R_F = 0.5$) was separated from the other alkaloids by preparative thin-layer chromatography on alumina plates with ether as eluent. The isolated alkaloid was diluted with 100 mg non-labelled (±)-cryptostyline I and recrystallized from ether to constant specific activity.

Isolation of the alkaloids. Fresh plants of *C. erythroglossa* (0.3 kg) were extracted with methanol (5 l). The extract was concentrated to 1 l, acidified (pH 3) with hydrochloric acid and washed with carbon tetrachloride (6 × 50 ml). The aqueous layer was made alkaline (pH 8) with sodium hydrogen carbonate and extracted with ether (3 × 50 ml). The combined ether solutions were treated as described earlier,[2] giving (−)-cryptostyline I (m.p. 101−102°; $[\alpha]_D^{22}$ −56°, *c* 0.4, chloroform), (−)-cryptostyline II (m.p. 116−117°; $[\alpha]_D^{22}$ −58°, *c* 0.4, chloroform) and (−)-cryptostyline III (m.p.

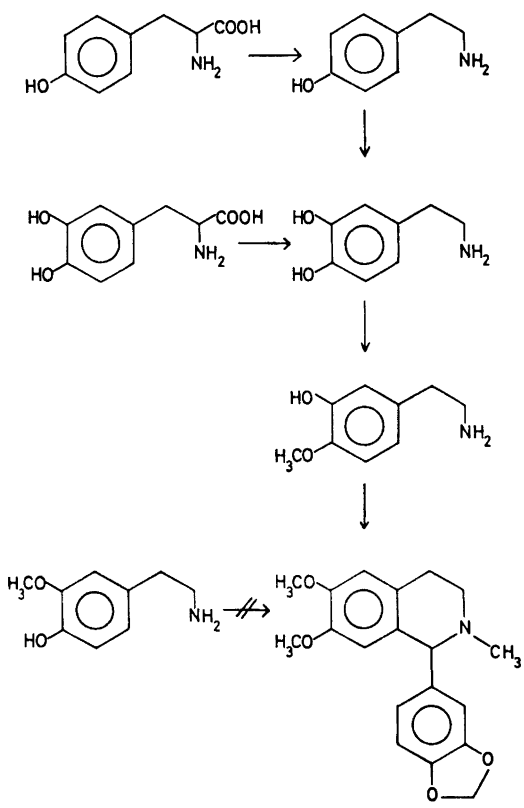


Fig. 2. Suggested pathway for the biosynthesis of (−)-cryptostyline I.

128 − 130°; $[\alpha]_D^{22}$ −52°, *c* 0.3, chloroform). The aqueous layer was then extracted with chloroform (8 × 50 ml). The chloroform extract was dried (Na_2SO_4) and evaporated to dryness. The residue was dissolved in water and filtered through a column of IRA-400 (Cl^-, 1 × 20 cm) irrigated with water, and the filtrate evaporated to dryness. This residue was separated into the crude chlorides IV ($R_F = 0.35$) and V ($R_F = 0.1$) by preparative thin-layer chromatography on alumina using chloroform/methanol (19:1) as eluent. The iodide corresponding to IV (5 mg), obtained by filtering the chloride through a column of IRA-400 (I^-, 1 × 15 cm) irrigated with water, was indistinguishable (m.p., TLC, IR) from an authentic sample of 1-(3,4-methylenedioxyphenyl-6,7-dimethoxy-2-methyl-3,4-dihydroisoquinolinium iodide.[2] Purification of V by preparative thin-layer chromatography on silica gel using chloroform/methanol (2:1) as eluent yielded V as an amorphous solid (25 mg) indistinguishable (TLC, UV, NMR, m.p. of the picrate) from an authentic sample of 1-(3,4-methylenedioxyphenyl)-6,7-dimethoxy-2-methylisoquinolinium chloride (*vide infra*).

1-(3,4-Methylenedioxyphenyl)-6,7-dimethoxyisoquinoline (VI). 1-(3,4-Methylenedioxyphenyl)- 6,7- dimethoxy- 3,4-dihydroisoquinoline [2] (0.40 g) and selenium (0.48 g) were heated under nitrogen at 220 − 230° for 15 min. The reaction mixture was dissolved in methanol and filtered through a column of silica gel. The eluate was chromatographed on neutral alumina using chloroform as eluent. The first fraction contained VI and unreacted imine, which were separated by preparative thin-layer chromatography on silica gel plates developed with ether. Recrystallization from methanol gave VI (0.10 g), m.p. 129 − 132°. (Found: C 69.8; H 5.1; N 4.4 Calc. for $C_{18}H_{15}NO_4$: C 69.9; H 4.9; N 4.5). UV spectrum, nm (ε): λ_{max} (ethanol) 329 (8000), 292 (8000), 238 (46 000); $\lambda_{shoulder}$ 245 (43 000). NMR spectrum ($CDCl_3$): τ 1.53 (d, 1 H, $J = 5$ Hz), 2.43 − 3.20 (m, 6 H); 3.95 (s, 2 H), 5.98 (s, 3 H), 6.12 (s, 3 H).

1-(3,4-Methylenedioxyphenyl)-6,7-dimethoxy-2-methylisoquinolinium chloride. An excess of methyl iodide was added to a solution of VI in acetone. The mixture was refluxed for 1.5 h, cooled and evaporated to dryness. The residue was filtered through a column of IRA-400 (Cl^-, 1 × 20 cm) irrigated with water. Evaporation of the eluate to dryness and purification of the residue by preparative thin-layer chromatography on silica gel using chloroform/methanol (2:1) as eluent gave the title compound as an amorphous solid. UV spectrum, nm (ε): λ_{max} (ethanol) 319 (4500), 290 (3500), 258 (25 100); $\lambda_{shoulder}$ 348 (2900). NMR spectrum ($CDCl_3$): τ 1.36 and 1.61 (AB spectrum, 2 H, $J = 6.5$ Hz), 2.22 (s, 1 H), 2.78 (s, 3 H), 3.00 (s, 1 H), 3.77 (s, 2 H), 5.77 (s, 3 H), 5.87 (s, 3 H), 6.17 (s, 3 H).

The *picrate*, m.p. 218 − 223°, was recrystallized from methanol-water. (Found: C 54.2; H 3.6; N 9.8. Calc. for $C_{25}H_{20}N_4O_{11}$: C 54.4; H 3.7; N 10.1).

1-(3,4-Methylenedioxyphenyl)-6,7- dimethoxy-2-methyl-3,4-dihydroisoquinolinium bromide (VII). *N*-Bromosuccinimide (75 mg) was added with stirring to a solution of cryptostyline I (100 mg) in absolute ether (20 ml). During the addition, a yellow precipitate appeared. After 1 h acetone (3 ml) was added and the reaction mixture was refluxed for 4 h to give the crude immonium salt (VII) as a light yellow crystalline precipitate. The precipitate was washed with a mixture of ether-acetone (7:1) to remove succinimide, giving VII (118 mg), m.p. 207 − 208°. (Found: C 56.0; H 4.8; Br 19.9; N 3.5; O 15.8. Calc. for $C_{19}H_{20}BrNO_4$: C 56.2; H 4.9; Br 19.7; N 3.5; O 15.8). IR spectrum: σ_{max} (KBr) 1640 (m) cm^{-1}. UV spectrum, nm (ε): λ_{max} (ethanol) 367 (13 400), 315 (11 500), 252 (22 000). NMR spectrum (CD_3OD): τ 2.78 − 2.95 (m, 4 H), 3.42 (s, 1 H), 3.85 (s, 2 H), 5.60 − 6.05 (m, 2 H), 6.50 − 6.85 (m, 2 H), 6.02 (s, 3 H), 6.38 (s, 6 H).

2-(3,4-Methylenedioxybenzoyl)-4,5-dimethoxystyrene (VIII). A mixture of VII (118 mg), dimethyl sulphate (0.06 ml), ethanol (0.12 ml) and aqueous potassium hydroxide (0.40 ml, 20 %) was refluxed for 3 h. Water (10 ml) was added and the cold solution was extracted with chloroform (5 × 10 ml). The combined chloroform solutions were dried (Na_2SO_4) and evaporated to dryness. The residue was purified by preparative thin-layer chromatography on silica gel using ethanol as eluent ($R_F = 0.9$). Recrystallization from ethanol gave VIII (77 mg), m.p. 98–99°. (Found: C 69.4; H 5.2; O 25.6 Calc. for $C_{18}H_{16}O_5$: C 69.2; H 5.1; O 25.6). IR spectrum: σ_{max} (KBr) 1620 (m), 1655 (s) cm^{-1}. UV spectrum, nm (ε): λ_{max} (ethanol) 314 (13 800), 258 (22 900), 237 (28 200). NMR spectrum ($CDCl_3$): τ 2.60–3.53 (m, 6 H), 3.95 (s, 2 H), 4,45 (q, 1 H, $J_1 = 1$ Hz, $J_2 = 17$ Hz), 4.85 (q, 1 H, $J_1 = 1$ Hz, $J_2 = 11$ Hz), 6.05 (s, 3 H), 6.15 (s, 3 H). MS: M^+ 312.

2-(3,4-Methylenedioxybenzoyl)-4,5-dimethoxybenzaldehyde (IX). A catalytic amount of osmium tetroxide (2 mg) was added to a solution of the alkene VIII (77 mg) in water-dioxane (1:4, 5 ml). After 0.5 h sodium periodate (145 mg) was added and the mixture was heated at 80° for 2 h. The solvent was evaporated and the residue was suspended in water and extracted with chloroform (5 × 25 ml). The combined chloroform solutions were dried (Na_2SO_4) and evaporated to dryness. The residue was purified by preparative thin-layer chromatography on silica gel using ethanol as eluent ($R_F = 0.7$). Crystallization from chloroform-ether gave IX (55 mg), m.p. 162–163°. (Found: C 64.8; H 4.5; O 30.4. Calc. for $C_{17}H_{14}O_6$: C 65.0; H 4.5; O 30.6). IR spectrum: σ_{max} (KBr) 1635 (m), 1685 (s) cm^{-1}. UV spectrum, nm (ε): λ_{max} (ethanol) 316 (12 600), 268 (10 800), 237 (21 700). NMR spectrum ($CDCl_3$): τ 1.23 (s, 1 H), 2.40–3.32 (m, 5 H), 3.92 (s, 2 H), 6.03 (s, 3 H), 6.06 (s, 3 H). MS: M^+ 314.

The formaldehyde formed in the reaction was trapped in a solution of dimedone (100 mg) in water (40 ml). The precipitate was recrystallized twice from ethanol giving methylenebisdimedone (X, 30 mg), m.p. 191° (Lit.[11] m.p. 191°).

2-(3,4-Methylenedioxybenzoyl)4,5-dimethoxybenzoic acid (XI). *t*-Butanol (4 ml) and 2-(3,4-methylenedioxybenzoyl)-4,5-dimethoxystyrene (VII, 55 mg) were added to a solution of sodium periodate (0.70 g) and potassium permanganate (0.35 g) in water (12 ml). The pH of the solution was adjusted to 8.5 by the addition of solid sodium carbonate. The mixture was stirred for 15 h at 25°, and then acidified (pH 4) with aqueous sulphuric acid. The excess of permanganate was destroyed with sodium sulphite and the solution extracted with chloroform (5 × 25 ml). The combined chloroform solutions were dried and evaporated to dryness. The residue was chromatographed on silica gel (3 × 10 cm, 70–230 mesh). Unreacted alkene (VII) was eluted with chloroform. Exchange of the solvent to ethanol eluted the acid (XI), which was further purified by preparative thin-layer chromatography on silica gel using ethanol as eluent ($R_F = 0.6$). Recrystallization from ethanol gave XI, m.p. 206–207°. (Found: C 61.8; H 4.4; O 33.8. Calc. for $C_{17}H_{14}O_7$: C 61.8; H 4.2; O 33.9). IR spectrum: σ_{max} (KBr) 1655 (m), 1665 (m), 3300–2500 (m) cm^{-1}. UV spectrum, nm (ε): λ_{max} (ethanol) 312 (9200), 275 (8400), 232 (24 300). NMR spectrum (CD_3OD): τ 2.16–3.27 (m, 5 H), 3.94 (s, 2 H), 6.02 (s, 3 H), 6.12 (s, 3 H).

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