Acta Chemica Scandinavica B 28 (1974) 1175—1179

Studies on Orchidaceae Alkaloids. XL.* Biosynthetic Studies of (−)-Cryptostyline I in *Cryptostylis erythroglossa* Hayata

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The biosynthesis of (−)-cryptostyline I (I) has been studied using radioactive precursors, the position of the radiolabel being determined by subsequent degradation.

The biosynthetic results show that 3,4-dimethoxyphenethylamine, *N*-(3-hydroxy-4-methoxybenzyl)-3-hydroxy-4-methoxyphenethylamine (XI) and 1-(3,4-methylenedioxyphenyl)-6,7-dimethoxy-2-methyl-3,4-dihydroisoquinolinium bromide (II) are specifically incorporated. Preliminary results indicate that dopamine is a precursor of the 1-phenyl group and the C-1 carbon atom. Vanillin is shown to be better incorporated than isovanillin.

* For number XXXIX, see Ref. 1.

In a previous report [1] we showed that tyrosine and 3,4-dihydroxyphenylalanine as well as tyramine and dopamine are specifically incorporated into the phenethylamine portion of (−)-cryptostyline I (I). It was also evident that 3-hydroxy-4-methoxyphenethylamine was incorporated to a greater degree than 4-hydroxy-3-methoxyphenethylamine. We now report further experiments on the biosynthesis of (−)-cryptostyline I (I). The specificity of the incorporation of the precursors into (−)-cryptostyline I (I) has been established by degradation studies (Table 1 and Fig. 1).

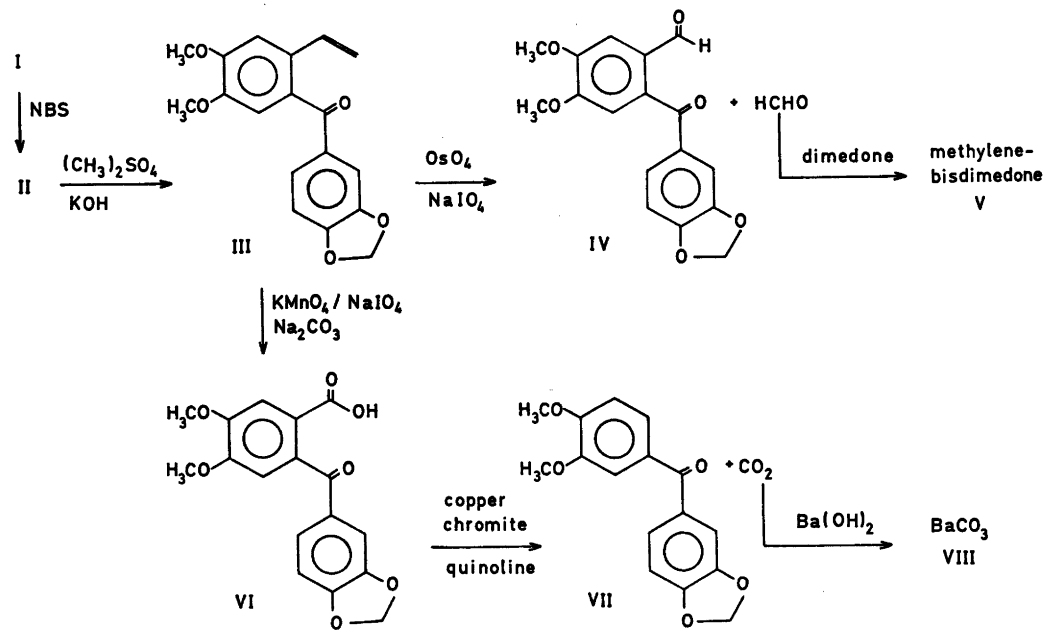

Fig. 1. Degradation of cryptostyline I.

Table 1.

Labelled compounds	Amount fed		Isolated (−)-cryptostyline I	Added carrier
	mg	μCi	mg	mg
Dopamine-β-^{14}C	0.26	100	60	240
3,4-Dimethoxyphenethylamine-α-^{14}C	2.4	100	30	60
N-(3-Hydroxy-4-methoxy-1′-^{3}H-benzyl)-3-hydroxy-4-methoxyphenethylamine-α-β-^{3}H (XI)[a]	5	325	55	40
1-(3,4-Methylenedioxyphenyl)-6,7-dimethoxy-2-methyl-3,4-dihydroisoquinolinium bromide-^{3}H	5	24	25	25
Vanillin-^{3}H	5	105	35	30
Isovanillin-^{3}H	5	150	40	35

Spec. act. in isol. (−)-cryptostyline I μCi/mmol × 10^{-3}	Recovered radioactivity % × 10^{-3}	Radioactivity % I	II	III	IV	V	VI	VII	VIII
77.0	14.0	100					96	57	35
1.7	0.16	100		98	12	91			
5.7	0.29	100[b]	81[b]				16[b]		
4700	1500	100[c]							
2.3	0.23	100[c]							
0.36	0.029	100[c]							

[a] The tritium-ratio between the C_1 and C_3-C_4 positions was 2.7 asuming non-exchange during debenzylation.

[b] The tritium-ratio between the C_1 and C_3-C_4 positions was 0.29. This figure was calculated by dividing the 19 % label shown by degradation to have occurred at the C_1 position by 65 % calculated by substracting the 16 % label in VI from the 81 % present in compound II. On the assumption that the losses of tritium and hydrogen at C-1 during the cyclization to (−)-cryptostyline I (I) are equal the ratio 0.29 in I should be doubled when I is compared with the precursor XI.

[c] Boiling with concentrated hydrochloric acid gave an unlabelled (−)-cryptostyline I (I).

As shown in Table 1, 3,4-dimethoxyphenethylamine is incorporated into (−)-cryptostyline I (I) but considerably less efficiently than dopamine. The latter was, however, administered in smaller amounts. It therefore appears probable that 3,4-dimethoxyphenethylamine is demethylated to the known precursor 3-hydroxy-4-methoxyphenethylamine before incorporation into I. An analogous reaction is known [2] for cactus species, where 3,4-dimethoxyphenethylamine was demethylated to 4-hydroxy-3-methoxyphenethylamine before further biosynthetic reactions.

Dopamine, labelled in the β-carbon, was administered to the plants to elucidate whether it is a progenitor of only one or of both the aromatic rings in I. The results suggest that dopamine is a precursor of both the aromatic parts of the molecule, since only about half of the radioactivity from dopamine-β-^{14}C was located at carbon C-4 of (−)-cryptostyline I (I). We have previously shown [1] that dopamine-α-^{14}C is incorporated into (−)-cryptostyline I (I) with only a slight scrambling of label and hence, it seems likely that the remaining label is present at C-1.

The origin of the 1-phenyl group was further investigated. Vanillin (Table 1) was decidedly better incorporated into I than was isovanillin. Incorporation rates were low but due to their minimal water solubilities both aldehydes had to be applied to the leaves as solutions in poly-

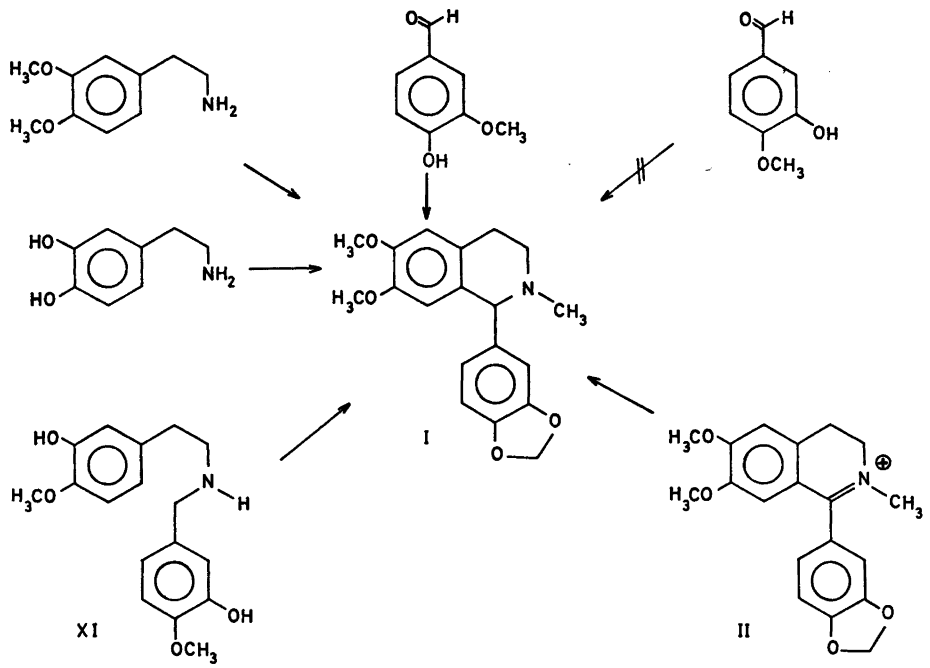

Fig. 2. Suggested precursors for the biosynthesis of (−)-cryptostyline I.

ethyleneglycol and thus may not be available for biosynthesis to the same extent as the amines. Barton *et al.*[3] have earlier found that isovanillin is poorly utilized in the biosynthesis of alkaloids owing to its poor absorption. We have not established which aromatic moiety became labelled from the aldehyde. The C_6-C_1 unit in some Amaryllidaceae alkaloids has previously been shown to be derived from protocatechualdehyde.[4]

N-(3-Hydroxy-4-methoxybenzyl)-3-hydroxy-4-methoxyphenethylamine (XI), labelled in both parts of the molecule, was incorporated in low yield. If we assume that XI, with the exception of the methyl groups, is incorporated intact into I it is clear from Table 1 that there has been a considerable loss of tritium from the position in XI which later appears at C_1 in (−)-cryptostyline I (I). The original tritium-ratio between the C_1 and the C_3-C_4 positions, which was 2.7 in XI, has decreased to 0.58 in I, as discussed in the footnotes to Table 1. This may be due to loss of tritium during passage through an intermediate such as the immonium salt of I (II). The indication that II is a main intermediate is supported by the efficient incorporation of II into (−)-cryptostyline I (I). Alternative explanations include metabolic degradation of XI to smaller fragments followed by re-incorporation. It should be noted that in XI the isovanillin substitution pattern is present in the 1-phenyl ring. In view of the results with vanillin and isovanillin it seems more probable that the isomer of XI, *N*-(4-hydroxy-3-methoxybenzyl)-3-hydroxy-4-methoxyphenethylamine, is the true precursor of II and (−)-cryptostyline I (I).

EXPERIMENTAL

The general methods described in Ref. 1 were used. 3,4-Dimethoxyphenethylamine-1-^{14}C and dopamine-2-^{14}C were obtained from the Radiochemical Centre, Amersham, UK and New England Nuclear Corp., Boston, USA, respectively.

Feeding experiments. The labelled amines were dissolved in small amounts of dilute acetic acid and the solutions were injected into the leaves of *C. erythroglossa.* The labelled vanillin and isovanillin were dissolved in small amounts of polyethyleneglycol and the solutions were applied to the leaves of the plants. (−)-Cryptostyline I (I) was isolated as described earlier.[1]

Unlabelled carrier was added to the isolated alkaloid in amounts specified in Table 1.

3,4-Dimethoxy-3',4'-methylenedioxybenzophenone (VII). A mixture of 2-(3,4-methylenedioxybenzoyl)-4,5-dimethoxybenzoic acid (VI, 70 mg), quinoline (1 ml) and copper chromite (20 mg) was refluxed for 10 min. Chloroform (10 ml) was added and the copper chromite filtered off. The chloroform phase was washed with hydrochloric acid (2 %) until all quinoline was removed, dried (Na_2SO_4) and concentrated. The residue was chromatographed on a silica gel column (2 × 5 cm) using chloroform as eluent. The fraction containing VII was further purified by preparative thin-layer chromatography on silica gel using chloroform as eluent ($R_F = 0.5$). Recrystallization from toluene gave VII (40 mg), m.p. 164–165 °C (Lit.[9] m.p. 164–165 °C). The carbon dioxide formed in the reaction was trapped in a saturated aqueous solution of barium hydroxide. The precipitate was filtered off and immediately dried under a high vacuum to give barium carbonate (VIII, 20 mg).

Vanillin labelled in the aromatic ring. Concentrated hydrochloric acid (0.03 ml) and tritiated water (0.04 ml, 0.2 Ci) were added to a solution of vanillin (46 mg) in dioxane (0.10 ml). The solution was heated in a sealed ampoule at 115 °C. After 3 h, water (4 ml) was added and the aqueous solution extracted with chloroform (4 × 5 ml). The combined chloroform solutions were dried (Na_2SO_4) and evaporated to dryness. The residue was dissolved in methanol and the solvent evaporated. This procedure was repeated five times. The residue was dissolved in chloroform and filtered through a short silica gel column. Evaporation of the eluate to dryness and recrystallization of the residue from toluene gave the tritiated vanillin, m.p. 80–81 °C (Lit.[5] m.p. 83–84 °C) with a specific activity of 3.2 mCi/mmol.

Tritiated isovanillin was prepared as described for vanillin, m.p. 116–117 °C (Lit.[6] m.p. 114–116 °C) with a specific activity of 4.6 mCi/mmol.

(±)-Cryptostyline I labelled with tritium in the aromatic rings was obtained by heating (±)-cryptostyline I (I, 34 mg) dissolved in dioxane (0.1 ml) with concentrated hydrochloric acid (0.03 ml) and tritiated water (0.05 ml, 0.25 Ci) in a sealed ampoule at 120 °C for 5 h. Water was added (2.5 ml) and the aqueous solution was washed with ether (5 ml), made alkaline (pH 13) with sodium hydroxide (1 M) and extracted with ether (2 × 5 ml). The combined ether solutions were dried (Na_2SO_4) and evaporated to dryness. Recrystallization of the residue from ether gave (±)-cryptostyline I (I, 21 mg), m.p. 117–118 °C (Lit.[7] m.p. 117–118 °C) with a specific activity of 2.1 mCi/mmol. Oxidation of this sample with *N*-bromosuccinimide [1] gave 1-(3,4-methylenedioxyphenyl)-6,7-dimethoxy-2-methyl-3,4-dihydroisoquinolinium bromide with a specific activity of 1.9 mCi/mmol.

N-(3-Benzyloxy-4-methoxy-1'-^{3}H-benzyl)-3-benzyloxy-4-methoxy-α,β-^{3}H-phenethylamine hydrochloride (X). 3-Benzyloxy-4-methoxybenzaldehyde (96 mg) and 3-benzyloxy-4-methoxy-α,β-^{3}H-phenethylamine [8] (103 mg, 5.38 mCi/mmol) were dissolved in ether (20 ml) and the solution was allowed to stand for 20 h at room temperature. Evaporation of the solvent gave the crude imine IX, which was dissolved in dioxane:methanol 3:1 (27 ml) and reduced with sodium borotriteride (11.8 mg, 62 mCi). After

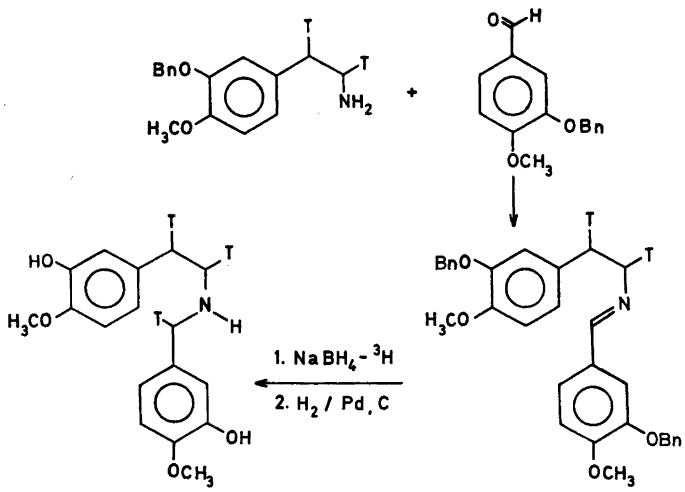

Fig. 3. Synthesis of the labelled compound XI.

stirring for 1.5 h an excess of sodium borohydride (120 mg) was added and the stirring continued for 2 h. The solution was diluted with water (25 ml) and extracted with ether (4 × 40 ml). The combined ether solutions were dried (Na_2SO_4) and evaporated to dryness leaving the crude phenethylamine derivative, which was converted to the corresponding hydrochloride. Recrystallization from ethanol gave X in the form of needles (150 mg), m.p. 148–150 °C. (Found: C 71.6; H 6.6; N 2.6. Calc. for $C_{31}H_{34}NO_4$: C 71.6; H 6.6; N 2.7.) UV spectrum, nm (ε): λ_{max} (ethanol) 279 (5600), 231 (16 900). NMR spectrum ($CDCl_3$): δ 2.98 (m, 4 H), 3.68 (s, 3 H), 3.78 (s, 3 H), 3.92 (s, 2 H), 5.05 (s, 2 H), 5.24 (s, 2 H), 6.65–7.65 (m, 16 H).

N-(3-Hydroxy-4-methoxy-1'-^{3}H-benzyl)-3-hydroxy-4-methoxy-α,β-^{3}H-phenethylamine (IX). A solution of X (150 mg) in acetic acid (15 ml) was hydrogenated over palladium on carbon (10 %, 10 mg) at room temperature and atmospheric pressure. When the theoretical amount of hydrogen had been consumed (40 min) the catalyst was filtered off and water (50 ml) was added. After washing with ether (2 × 25 ml) the pH of the solution was adjusted to 8.0–8.5 with sodium hydroxide (1 M) and extracted with ether (5 × 40 ml). The combined ether extracts were dried (Na_2SO_4) and the solvent evaporated. The residue was recrystallized from ethanol giving XI, m.p. 140–143 °C. (Found: C 66.8; H 6.5; N 4.4. Calc. for $C_{17}H_{21}NO_4$: C 67.3; H 7.0; N 4.6). NMR spectrum (pyridine-d_5): δ 2.92 (m, 4 H), 3.73 (s, 6 H), 3.84 (s, 2 H), 6.10–7.45 (m, 9 H). Specific activity: 19.7 mCi/mmol.

Acknowledgement. We thank Dr. Björn Lüning for his interest in this work and Mrs. Birgit Dittmer for measuring the radioactivities. This study was supported by the Swedish Natural Science Research Council.

REFERENCES

1. Agurell, S., Granelli, I., Leander, K., Lüning, B. and Rosenblom, J. *Acta Chem. Scand. B 28* (1974) 239.
2. Lundström, J. *Acta Pharm. Suecica 7* (1970) 651.
3. Barton, D. H. R., Kirby, G. W., Taylor, J. B. and Thomas, G. M. *J. Chem. Soc.* (1963) 4545.
4. Suhadolnic, R. J., Fischer, A. G. and Zulalian, J. *Proc. Chem. Soc. London* (1963) 132.
5. Wolynkin, N. I. *Ž. prikl. Chim. 11* (1938) 423.
6. Mac Donald, S. F. *J. Chem. Soc.* (1948) 376.
7. Leander, K., Lüning, B. and Ruusa, E. *Acta Chem. Scand. 23* (1969) 244.
8. Lundström, J. and Agurell, S. *Acta Pharm. Suecica 7* (1970) 247.
9. Inubushi, Y., Fales, H. M., Warnhoff, E. W. and Wildmann, W. C. *J. Org. Chem. 25* (1960) 2153.

Received May 24, 1974.