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BIOSYNTHESIS OF THE ALKALOIDS OF *CHELIDONIUM MAJUS*—II.

THE FORMATION OF CHELIDONINE FROM STYLOPINE

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Abstract—The tetrahydroprotoberberine alkaloid, stylopine, has been synthesized with carbon-14 label at C-6. The chelidonine isolated from *Chelidonium majus* L. plants which had been fed DL-stylopine-6-^{14}C was radioactive (0·7% incorporation), and systematic degradation established that all the activity was located at C-11. This result provides strong support for the hypothesis that the tetrahydroprotoberberines are precursors of the benzo[c]phenanthridine alkaloids.

WE HAVE previously established that chelidonine (III), one of the main alkaloids of the plant *Chelidonium majus* L., is derived from tyrosine[1] and dopamine.[2] The administration of tyrosine-2-^{14}C to the plant afforded radioactive chelidonine which was labelled at C-4b and C-11. The chelidonine derived from dopamine-α-^{14}C was labelled solely at C-11. These results strongly favored the suggestion of Bruchhausen and Bersch[3] that chelidonine is formed from a tetrahydroprotoberberine alkaloid such as stylopine (II) via an intermediate such as IV (Fig. 1).[4] This hypothesis is attractive since DL-stylopine is one of the minor alkaloids of *C. majus*.[5, 6] Strong support for this scheme has been obtained by Battersby and coworkers.[7] They fed to *C. majus* (+)reticuline (I), labelled with ^{14}C at C-3, the *N*-methyl group, and on the *O*-methyl group of the benzyl residue; a tritium label was also present at the assymmetric centre C-1. The resultant chelidonine was labelled with ^{14}C at C-11, C-6, and at the methylenedioxy group of ring D, the ratio of activity at these positions being consistent with the direct incorporation of the labelled reticuline via stylopine, as illustrated in Fig. 1. No tritium was found in the chelidonine, a result to be expected if a compound such as IV were a biosynthetic intermediate. Tritium-labelled stylopine was also fed to *C. majus*, and radioactive chelidonine (0·8% incorporation) was isolated from the plant.

We have carried out independent investigations with ^{14}C-labelled DL-stylopine which confirm Battersby's results. Haworth and Perkin[8] prepared DL-stylopine (II, 2,3,9,10-bismethylenedioxytetrahydroprotoberberine) in small yield and we have utilized their

[1] E. LEETE, *J. Am. Chem. Soc.* **85**, 473 (1963), Part I of this series.
[2] E. LEETE and J. B. MURRILL, *Tetrahedron Letters* 147 (1964).
[3] F. v. BRUCHHAUSEN and H. W. BERSCH, *Chem. Ber.* **63**, 2520 (1930).
[4] See also R. B. TURNER and R. B. WOODWARD, In *The Alkaloids* (Edited by R. H. F. MANSKE and H. L. HOLMES), Vol. III, p. 57. Academic Press, New York (1953).
[5] J. SLAVÍK, *Collection Czech. Chem. Commun.* **20**, 198 (1955).
[6] F. J. BANDELIN and W. MALESH, *J. Am. Pharm. Assoc.* **45**, 702 (1956).
[7] A. R. BATTERSBY, R. J. FRANCIS, E. A. RUVEDA and J. STAUTON, *Chem. Commun.* 89 (1965).
[8] R. D. HAWORTH and W. H. PERKIN, *J. Chem. Soc.* 1769 (1926).

general procedure with several variations, to increase yields, and make use of modern reagents (Fig. 2). Potassium cyanide-^{14}C was condensed with piperonal (V) affording the cyanhydrin VI which was acetylated yielding compound VII. Hydrogenation in the presence of palladium on charcoal afforded 3,4-methylenedioxyphenylethylamine (VIII) which was condensed with the anhydride IX yielding the amide XI. After esterification of the carboxyl group with diazomethane, cyclization to X was achieved with phosphorus oxychloride. The carbonyl group was reduced with lithium aluminum hydride, and the Δ^{13}-double bond hydrogenated in the presence of platinum to afford DL-stylopine-6-^{16}C in 8·8% yield from potassium cyanide.

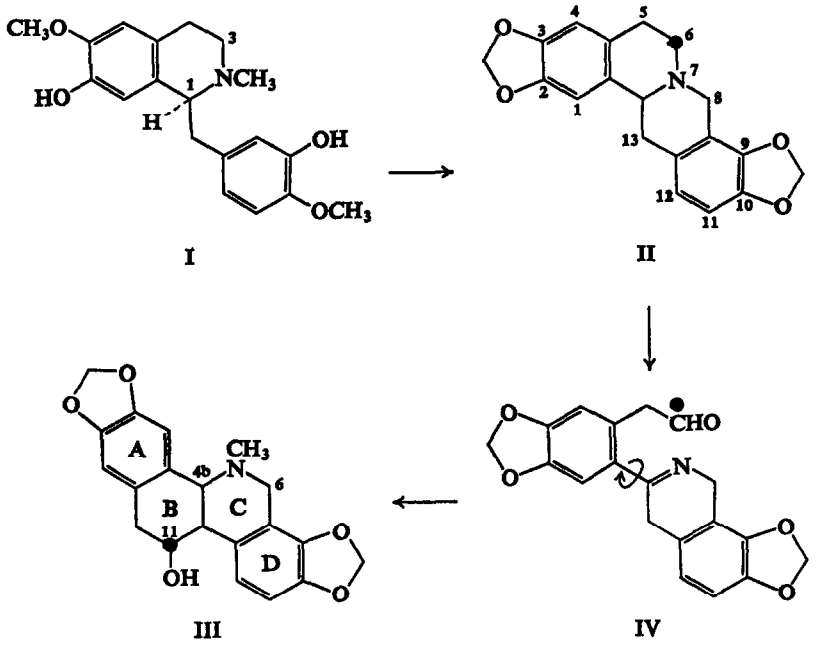

FIG. 1. BIOSYNTHESIS OF CHELIDONINE.

DL-Stylopine-6-^{14}C was fed to *C. majus* plants by means of cotton wicks inserted into the stems of the plant. After 8 days the plants were harvested and stylopine and chelidonine isolated and purified by chromatography. Details of the amount of tracer fed and the activity of the isolated alkaloids are recorded in Table 1. The recovery of unchanged DL-stylopine

TABLE 1

	Precursor fed DL-Stylopine-6-^{14}C	Isolated alkaloids	
		Chelidonine	DL-Stylopine
Wt. (g)	0·075	1·350	0·242
Total activity (dpm)	$2{\cdot}77\times10^{7}$	$1{\cdot}95\times10^{5}$	$2{\cdot}83\times10^{6}$
Specific activity (dpm/mM)	$1{\cdot}26\times10^{8}$	$5{\cdot}0\times10^{4}$	$4{\cdot}0\times10^{6}$
Incorporation (%)		0·7	10·2

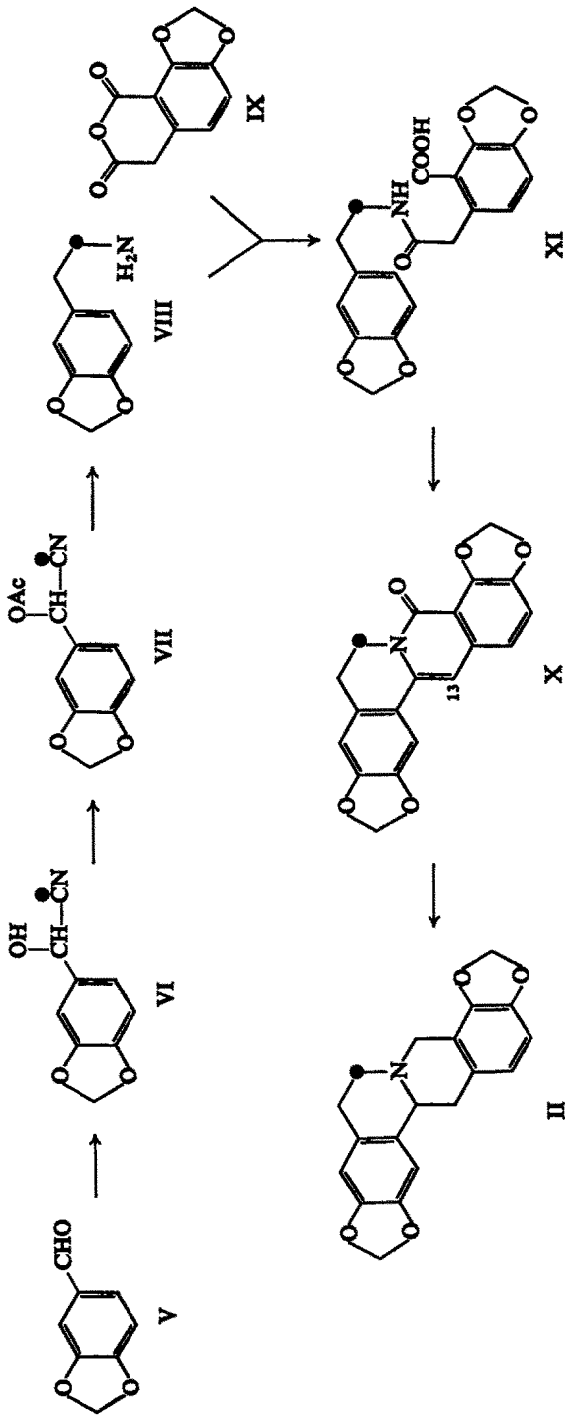

FIG. 2. SYNTHESIS OF DL-STYLOPINE-6-^{14}C.

was good (10·2%) and a moderate (0·7%) incorporation of tracer was found in the chelidonine. The radioactive chelidonine was degraded as previously described[1] (Table 2). Oxidation of the chelidonine with potassium permanganate yielded hydrastic acid and 3,4-methylenedioxyphthalic acid, which were isolated and separated as their *N*-ethyl-imides. Treatment of chelidonine with hydriodic acid afforded methyl iodide which was collected as triethylmethylammonium iodide. All these degradation products had negligible activity compared with the chelidonine. Since the only carbon atom which is not accounted for in these degradation products is C-11, all the radioactivity must be present at this position, in agreement with the biosynthesis scheme illustrated in Fig. 1.

TABLE 2. CHELIDONINE AND ITS DEGRADATION PRODUCTS

	Specific activity dpm/mM × 10^{-4}
Chelidonine	5·0
Chelidonine hydrochloride	4·9
O-Acetylchelidonine	4·9
Triethylmethylammonium iodide	0
N-Ethylhydrastimide	0·05
3,4-Methylenedioxy-*N*-ethylphthalimide	0·07

EXPERIMENTAL

DL-*Stylopine*-6-^{14}C

A mixture of piperonal (2·5 g, 0·0165 M), KCN-^{14}C[9] (1·0 g, 0·0154 M, 1·0 mc) and water (2 ml) was cooled in an ice bath and stirred vigorously with a magnetic stirrer while conc. HCl (1·3 ml) was slowly added. After stirring for 30 min the yellow oil which formed on the surface of reaction mixture was separated and added to acetic anhydride (4 ml) and anhydrous sodium acetate (2 g). After heating on a steam bath for 1 hr, the excess acetic anhydride was removed *in vacuo* leaving a solid residue which was crystallized from aqueous ethanol affording 3,4-methylenedioxy-*O*-acetylmandelonitrile (VII) (2·03 g, 60%), m.p. 71–72°, (lit.[10], m.p. 71°).

The nitrile VII (2·0 g) was dissolved in acetic acid (100 ml) containing conc. H_2SO_4 (2 ml) and hydrogenated at 3 atmos. pressure in the presence of 10% Pd on charcoal (1 g) for 1 hr. KOH (4·06 g) was added to the cooled filtered mixture, which was then evaporated to dryness. The residue was dissolved in water, made slightly acidic (H_2SO_4) and extracted with ether to remove non-basic materials. The aqueous solution was then made basic with KOH and extracted with ether. The dried (K_2CO_3) ether extract was evaporated and the residue distilled (136°, 4 mm) affording 3,4-methylenedioxyphenylethylamine (VIII) as a colorless oil (0·90 g, 60%).

A solution of 3,4-methylenedioxyphenylethylamine (0·90 g, 5·5 mM) in benzene (50 ml) was added slowly to a solution of 3,4-methylenedioxyhomophthalic anhydride (IX)[11] (1·39 g, 6·75 mM) in benzene (50 ml). The mixture was refluxed for 2 hr, then concentrated *in vacuo* to 50 ml. The reaction mixture was then extracted with 5% NaOH (3 × 10 ml). Addition of HCl to the alkaline extract yielded a white precipitate which was crystallized

[9] Purchased from Tracerlab, Waltham, Mass.
[10] A. ALBERT, *Chem. Ber.* **48**, 470 (1915).
[11] R. D. HAWORTH, W. H. PERKINS and T. S. STEVENS, *J. Chem. Soc.* 1764 (1926).

from acetic acid yielding compound XI (1·1 g, 55%), m.p. 194–195° (lit.[8] 194–195°). The acid XI (1·1 g) was esterified with an ethereal solution of diazomethane yielding the methyl ester of XI (1·12 g, 97%), m.p. 172–173°, after crystallization from methanol.

The methyl ester of XI (0·852 g) was refluxed with $POCl_3$ (3 ml) for 5 min. Excess $POCl_3$ was removed *in vacuo*, and water (5 ml) added to the residue. The grey solid obtained on addition of NaOH was crystallized from acetic acid affording colorless prisms of 2,3,9,10-bismethylenedioxyoxyprotoberberine (X) (530 mg, 71·5%), m.p. 291–292° (lit.[8] m.p. 292°).

Compound X (260 mg) was refluxed in tetrahydrofuran (50 ml) with $LiAlH_4$ (0·2 g) for 2 days. A small amount of water was then added and the granular precipitate filtered off and washed with acetone. The filtrate and washings were evaporated and the residue extracted with ether. Evaporation of the dried (K_2CO_3) ether extract afforded 2,3,9,10-bismethylenedioxydihydroprotoberberine, obtained as yellow prisms from acetone (190 mg, 78%), m.p. 193–195° (lit.[8] 194–196°).

The previously described compound (190 mg), dissolved in acetic acid (75 ml) was hydrogenated at 3 atmos. pressure in the presence of Pt_2O (0·2 g) for 3 hr. The residue obtained on evaporation of the filtered mixture was crystallized from a mixture of benzene and light petroleum (b.p. 60–70°) to afford colorless prisms of DL-stylopine-6-^{14}C (157 mg, 82%), m.p. 219–221° (lit.[8] m.p. 219°).

Administration of DL-*Stylopine-6-*^{14}*C to* Chelidonium majus *and Isolation of the Alkaloids*

DL-Stylopine-6-^{14}C (see Table 1 for the amount fed and activity) was dissolved in dil. acetic acid and fed to five *C. majus* L. plants (about 60 cm tall) growing in soil out of doors (July, 1965), by means of cotton wicks inserted in the stems near to ground level where the multiple stems come together into one stalk. The plants (wet wt. 1·5 kg) were harvested 8 days later and extracted with chloroform and ammonia.[1] Chromatography of the crude alkaloids on Woelm alumina (Activity II) as previously described[1] yielded DL-stylopine on elution with CH_2Cl_2, then chelidonine was eluted later with 1% ethanol in CH_2Cl_2.

The radioactive chelidonine was degraded as previously described[1] and the activity of its degradation products are recorded in Table 2. Radioactivity measurements were carried out in a Nuclear Chicago liquid scintillation spectrometer, Model 724, using as solvents either toluene, or dioxan-water, with the usual scintillators.[12]

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[12] A. R. FRIEDMAN and E. LEETE, *J. Am. Chem. Soc.* **85**, 2141 (1963).