[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY, UNIVERSITY OF MINNESOTA, MINNEAPOLIS, MINN.]

Biosynthesis of the Alkaloids of *Chelidonium majus*. I. The Incorporation of Tyrosine into Chelidonine[1]

BY EDWARD LEETE[2]

RECEIVED AUGUST 20, 1962

When DL-tyrosine-2-C^{14} was administered to *Chelidonium majus* plants the benzo[c]phenanthridine alkaloids, chelidonine and sanguinarine, became labeled. By systematic degradation of the radioactive chelidonine it was established that the label was located at specific positions, supporting the hypothesis that the biosynthesis of this class of alkaloid involves two molecules of tyrosine or closely related metabolites.

It has been suggested[3,4] that the benzo[c]phenanthridine alkaloids, of which chelidonine (III) and sanguinarine (VI) are members, are formed by a modification of a berberine skeleton (II) indicated schematically in Fig. 1. It is generally accepted that the berberine alkaloids are formed from two molecules of tyrosine or its hydroxylated derivative, 3,4-dihydroxyphenylalanine, and a one carbon fragment.[5] Quite recently this hypothesis has been strongly supported by tracer experiments. Spenser and Gear[6] fed tyrosine-2-C^{14} (I) to *Hydrastis canadensis* plants and obtained radioactive berberine which was labeled solely at the expected positions, C_1 and C_3. If chelidonine is indeed formed by a rearrangement of a berberine skeleton we would therefore expect chelidonine derived from tyrosine-2-C^{14} to be labeled at positions C_{4b} and C_{11} which

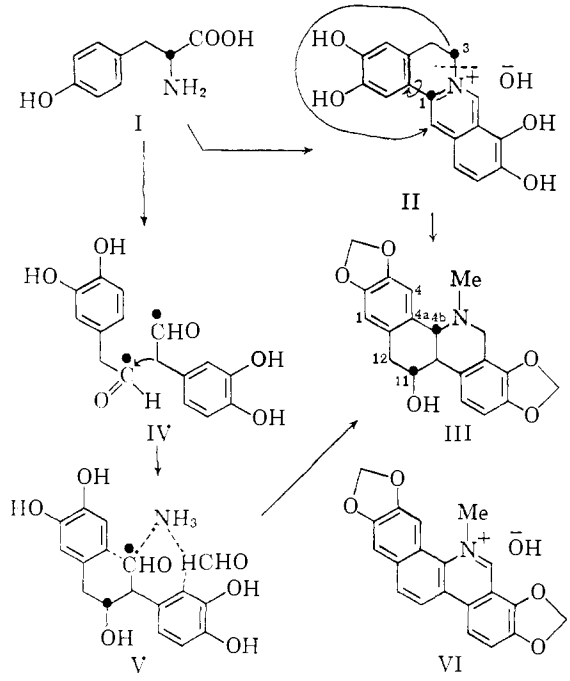


Fig. 1.—Biogenetic schemes for the benzo[c]phenanthridine alkaloids.

are indicated by heavy dots in formula III. It should be mentioned that the alternate scheme of Manske[7] would lead to the same pattern of labeling. He proposed that two molecules of 3,4-dihydroxyphenylacetaldehyde (IV), which is considered to arise by the oxidative decarboxylation of 3,4-dihydroxyphenylalanine, condense together to give the aldol (V), from which the nucleus of chelidonine and related alkaloids can be formed by two Mannich reactions involving ammonia and formaldehyde. Wenkert[8] has adumbrated a similar scheme involving two molecules of prephenic acid instead of the hydroxylated phenylacetaldehyde.

We have tested these hypotheses by feeding DL-tyrosine-2-C^{14} to five-month old *Chelidonium majus* plants growing in hydroponics. The tracer was absorbed rapidly by the roots and after eight days the plants were harvested yielding radioactive chelidonine and sanguinarine, which were purified by chromatography on alumina and Florisil. The radioactive chelidonine, which had a constant specific activity on repeated crystallization, was degraded according to the scheme illustrated in Fig. 2. Heating with hydriodic acid yielded methyl iodide which was absorbed in triethylamine to give triethylmethylammonium iodide which had negligible activity, implying that there was no activity on the N-methyl group. Oxidation with potassium permanganate according to the procedure of Späth and Kuffner[9] yielded a mixture of hydrastic (VII) and 3,4-methylenedioxyphthalic acid (VIII) which were converted to their N-ethylimides and separated by chromatography. Between them, these two compounds contain all the carbons of chelidonine except the N-methyl group and C_{11}. Since the N-methyl group was inactive, activity at C_{11} could be determined by difference and it was found that this position contained 61% of the total activity of the chelidonine. This method of determining the activity at C_{11} is dependent on reliable values for the specific activities of chelidonine and the N-ethylphthalimides. Subsequent degradation confirmed the activity of the latter compounds, and several derivatives of chelido-

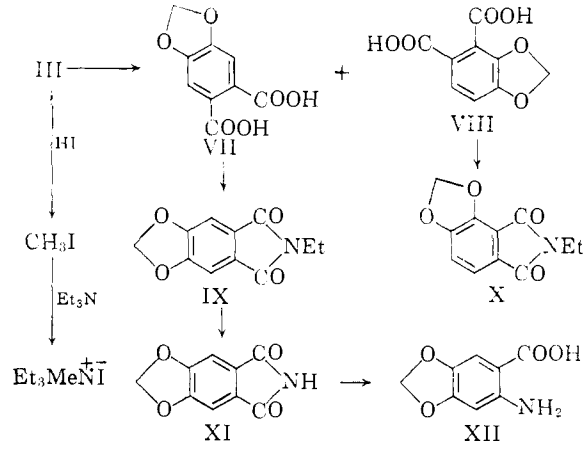


Fig. 2.—Degradative scheme for the chelidonine-C^{14}.

(1) An account of this work was presented at the Second International Symposium on the Chemistry of Natural Products, Prague, August 27 to September 2, 1962. This investigation was supported by a research grant, CY-5336, from the National Institutes of Health, U. S. Public Health Service.

(2) Alfred P. Sloan Research Fellow, 1962–1964.

(3) R. Robinson, "The Structural Relations of Natural Products," Clarendon Press, Oxford, 1955, p. 89.

(4) R. B. Turner and R. B. Woodward in "The Alkaloids," Vol. III, Ed., R. H. F. Manske and H. L. Holmes, Academic Press, New York, N. Y., 1953, p. 57.

(5) *Cf.* ref. 3, p. 86.

(6) I. D. Spenser and J. R. Gear, *Proc. Chem. Soc.*, 228 (1962).

(7) R. H. F. Manske in "The Alkaloids," Vol. IV, Ed. R. H. F. Manske and H. L. Holmes, Academic Press, New York, N. Y., 1954, p. 5.

(8) E. Wenkert, *Experientia*, **15**, 165 (1959).

(9) E. Späth and F. Kuffner, *Ber.*, **64**, 370 (1931).

nine were prepared, all of which had the same specific activity. Attempts were made to determine activity at C_{11} directly by oxidation to a C_{11} ketone which could then be phenylated and subsequently oxidized to benzoic acid. However several different methods of oxidation failed to yield the desired ketone.

The activity of the 3,4-methylenedioxy-N-ethylphthalimide (X) was negligible, a result which is consistent with the hypotheses illustrated in Fig. 1. The N-ethylhydrastimide (IX) contained 39% of the activity of the chelidonine. It was hydrolyzed to hydrastic acid, dehydrated to the anhydride, and then converted to hydrastimide (XI) by heating with urea. Treatment of this imide with sodium hypochlorite yielded 4,5-methylenedioxyanthranilic acid (XII) which had half the specific activity of the hydrastimide. This result indicates that all the activity was located on the carbonyl groups of the hydrastimide. Because of the symmetry of this compound the activity could be located on one or both of the carbonyl groups. We can thus state that the chelidonine was labeled at C_{4b} or C_{12} or both. However, it is impossible to conceive of a biogenetic scheme by which tyrosine-2-C^{14} could yield chelidonine labeled at C_{12} and we feel confident that the alkaloid was labeled only at C_{4b} and C_{11}. The activity was not equally divided between these two positions, and this result is another demonstration of the differential utilization of a single precursor in the biosynthesis of two segments of a "dimeric" alkaloid. Other examples of this phenomenon have been discovered by Gear and Spenser[10] in their study of the biosynthesis of hydrastine from tyrosine-2-C^{14}, and by Rapoport and co-workers[11] studying the incorporation of carbon dioxide-C^{14} into morphine.

Our results are consistent with the formation of chelidonine by the rearrangement of a berberine skeleton, but do not support Manske's hypothesis[7] which requires equal labeling at C_{4b} and C_{11}. The coexistence of the berberine and benzo[c]phenanthridine alkaloids in the *Papaveraceae* (*Chelidonium majus*, *Glaucium corniculatum*,[12] and several other species) and *Rutaceae* (*Toddalia aculeata*[13]) is strong circumstantial evidence in favor of their biosynthesis from common precursors.

Experimental[14]

Administration of the DL-Tyrosine-2-C^{14} to *Chelidonium majus* and Isolation of Chelidonine and Sanguniarine.—The *Chelidonium majus* plants were grown from seed in soil until they were about five months old. Four plants were then transferred to a hydroponics setup in which the roots were placed in aerated tap water. After several days new roots were produced and DL-tyrosine-2-C^{14} [15] (78.2 mg. 2.28×10^8 d.p.m.) was added to the aqueous solution in which the roots were growing. Uptake of the tracer was rapid and after three days less than 1% remained in the hydroponic solution. Eight days after administration of the tracer the plants were harvested (wet wt. 866 g.) and mascerated in a Waring blender with chloroform (3 l.) and 15 *N* ammonia (100 ml.). After standing for two days the mixture was filtered through cloth yielding an aqueous phase (530 ml., 2.0×10^7 d.p.m.[16]) and a chloroform layer which was taken to dryness in a rotary evaporator. The residue (6.5 g.) was extracted five times with 100 ml. portions of hot 10% acetic acid. The deep orange solution was made basic with concentrated ammonia (120 ml.) and extracted with chloroform. Evaporation of the dried chloroform extract yielded a mixture of crude alkaloids (2.2 g., 7.0×10^5 d.p.m.). These alkaloids were dissolved in benzene and chromatographed on a column of Woelm alumina (600 g.) (Activity II). Elution was carried out successively with benzene, methylene chloride, and finally with methylene chloride containing increasing amounts of ethanol (1–10%). The composition of the eluents was determined by thin layer chromatography, using alumina on glass plates, with methylene chloride as the developing solvent. Crude chelidonine (741 mg.) was eluted with 1% ethanol in methylene chloride, and was rechromatographed on a column of Florisil eluting with methylene chloride containing increasing amounts of ethanol (0–50%). Chelidonine was present in the fractions obtained by elution with 10% ethanol in methylene chloride, and was crystallized from aqueous methanol yielding colorless prisms of the monohydrate (297 mg., 1.13×10^5 d.p.m./m*M*) m.p. 135–136°, not depressed on admixture with an authentic specimen. Sanguinarine (95 mg., 1.1×10^5 d.p.m./m*M*) was obtained from the more polar fractions of the alumina and Florisil columns, and was crystallized from a mixture of ether and methanol. Work is proceeding on the isolation and characterisation of the minor alkaloids which have been obtained from the chromatographic columns.

Degradation of the Chelidonine-C^{14}. (a) **Demethylation.**—*Chelidonine (56 mg.)* was mixed with *ammonium iodide (50 mg.)*, gold chloride (1 mg.) and freshly distilled hydriodic acid (2 ml., d. 1.7) and heated to 360° in a current of nitrogen. The hydriodic acid which distilled was condensed in a water bath maintained at 70–80°. The nitrogen stream then was passed through a solution containing 2.5% cadmium sulfate and 2.5% sodium thiosulfate, and then into a 5% solution of triethylamine in ethanol (5 ml.) cooled to −80°. After standing overnight the triethylamine solution was evaporated yielding triethylmethylammonium iodide (32 mg.) which, after crystallization from a mixture of ethanol and ether, was identical (mixed m.p., infrared spectrum) with an authentic specimen.

(b) **Oxidation.**[9]—Chelidonine (250 mg.) was dissolved in 2 *N* sulfuric acid (5 ml.) and then diluted to 150 ml. with water. Sodium carbonate solution was added until the mixture was turbid and then potassium permanganate (1.5 g.) in water (150 ml.) was added with stirring during 3 hr. The mixture was warmed on a steam bath for 30 min., then cooled, decolorized with sulfur dioxide, and finally evaporated to small bulk (30 ml.). This solution was made strongly acidic with hydrochloric acid and extracted continuously for 2 days with ether. The residue obtained on evaporation of the ether extract was dissolved in dilute ammonia, and calcium chloride solution added when calcium oxalate precipitated. The filtered solution was acidified with hydrochloric acid and extracted again with ether. The residue from this second extraction was dissolved in 30% ethylamine solution (5 ml.), evaporated to dryness, heated at 180° and then sublimed (150°, 0.001 mm.). The sublimate (42.5 mg.) was dissolved in a 1:1 mixture of benzene and petroleum ether (b.p. 60–70°) and chromatographed on Woelm alumina (Activity III), eluting first with 1:1 benzene–pet. ether, and then with pure benzene. The N-ethylphthalimides readily were detected on the column by their strong fluorescence in ultraviolet light. N-Ethylhydrastic acid (green fluorescence) (16.9 mg.) m.p. 166–167°, was eluted first followed by 3,4-methylenedioxy-N-ethylphthalimide (blue fluorescence) (22.3 mg.) m.p. 124–125°. The identity of these imides was established by comparison with authentic specimens.[17,18]

(c) **Hydrastimide.**—The active N-ethylhydrastimide which had been diluted with inactive material to give a total wt. of 90 mg. was refluxed with 30% potassium hydroxide (5 ml.) for 30

TABLE I

ACTIVITY OF CHELIDONINE AND ITS DEGRADATION PRODUCTS

	Activity (10^{-5} × d.p.m./mM.)
Chelidonine (III)	1.13
Chelidonine hydrochloride	1.17
O-Acetylchelidonine[19]	1.12
Triethylmethylammonium iodide	<0.01
N-Ethylhydrastimide (IX)	.45
3,4-Methylenedioxy-N-ethylphthalimide (X)	< .01
Hydrastimide (XI)	.43
4,5-Methylenedioxyanthranilic acid (XII)	.22

(10) J. R. Gear and I. D. Spenser, *Nature*, **191**, 1393 (1961).

(11) H. Rapoport, N. Levy and F. R. Stermitz, *J. Am. Chem. Soc.*, **83**, 4298 (1961).

(12) J. Slavik and L. Slavikova, *Collection Czech. Chem. Commun;* **22**, 279 (1957).

(13) T. R. Govindachari and B. S. Thyagarajan, *J. Chem. Soc.*, 769 (1956).

(14) Melting points are corrected, and analyses were carried out by Mrs. Olga Hamerston and her assistants at the University of Minnesota.

(15) Purchased from Volk Radiochemical Co., Skokie, Illinois.

(16) Activities were determined in a Nuclear Chicago Model C-115 low background Q gas flow counter. Determinations were carried out on samples of finite thickness making corrections for efficiency and self absorption.

(17) We thank Professor W. Reeve of the University of Maryland for a generous sample of hydrastic acid, *cf.* W. Reeve and H. Myers, *J. Am. Chem. Soc.*, **73**, 1371 (1951).

(18) (a) W. H. Perkin and V. M. Trikojus, *J. Chem. Soc.*, 2925 (1926); (b) E. Späth and H. Holter, *Ber.*, **60**, 1891 (1927).

(19) Obtained by the procedure of J. Gadamer and K. Winterfeld, *Arch. Pharm.*, **262**, 452 (1924).

min. The aqueous solution was acidified with hydrochloric acid and extracted continuously with ether for 35 hr. The residue obtained by evaporation of the ether extract was heated to 190°, cooled, and mixed with powdered urea (60 mg.). On heating at 200°, this mixture first melted and then became solid. This solid was extracted with ethanol, filtered, and evaporated to small volume (1 ml.) when hydrastimide (66 mg.), m.p. 290–291°, separated.

(d) **4,5-Methylenedioxyanthranilic Acid.**—Hydrastimide (60 mg.) was dissolved in 10% sodium hydroxide solution (2 ml.) by warming to 45°. The solution was cooled to 0° and 0.76 *N* sodium hypochlorite solution (1 ml.) added. The mixture was stirred for 1 hr. at 0° and then warmed to 80° during one hr. After cooling the solution was almost neutralized with 4 *N* sulfuric acid and then brought to a pH of 5 with acetic acid. A buff colored precipitate separated (30 mg.) which was sublimed (200°, 0.001 mm.) to yield a pale yellow sublimate (11 mg.), m.p. 217–218° (dec.). Recrystallization from aqueous ethanol yielded colorless plates of 4,5-methylenedioxyanthranilic acid, m.p. 220–221° (reported,[20] 203°). The infrared spectrum in a KBr pellet had absorptions due to NH_2 at 3440 and 3340 cm.$^{-1}$, and a carbonyl absorption at 1658 cm.$^{-1}$.

Anal. Calcd. for $C_8H_7NO_4$: C, 53.04; H, 3.90; N, 7.73. Found: C, 53.43; H, 4.11; N, 7.91.

The activities recorded in Table I are calculated for non-diluted material.

(20) P. Friedlander and W. Schreiber, *Ber.*, **28**, 1382 (1905).

[CONTRIBUTION FROM THE RESEARCH DEPARTMENT, CIBA PHARMACEUTICAL CO., DIVISION OF CIBA CORPORATION, SUMMIT, N. J.]

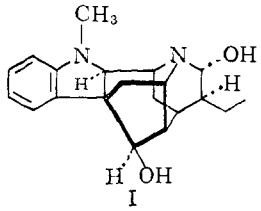
Rauwolfia Alkaloids. XLIII.[1] A Facile Ring Closure of Deoxyajmalal-A to Deoxyajmaline

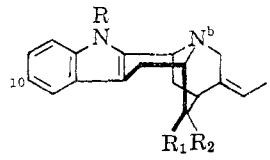
BY M. F. BARTLETT, B. F. LAMBERT, H. M. WERBLOOD AND W. I. TAYLOR

RECEIVED OCTOBER 18, 1962

Deoxyajmalal-A (IX) in strongly acidic solution is shown to exist in the ring closed form, the indoleninium salt X. Reduction of IX in such an acidic medium gives deoxyajmaline (VIII) along with 2-epideoxyajmaline (XIII).

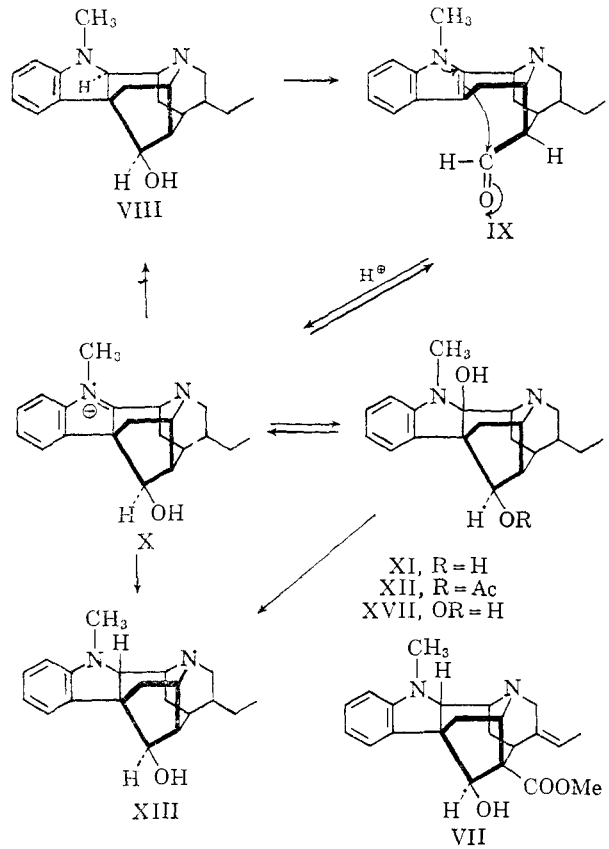
Ajmaline (I) has been degraded in a stepwise stereospecific manner which can be regarded as the reversal of its formal biogenesis.[3] The occurrence and established relationships[2,10] of indole alkaloids as sarpagine (II, $C_{10}OH$),[2,4] macusine-B (II, N_b-methiodide),[5] voachalotine (III),[6] akuammidine (IV),[7] polyneuridine (V)[8] and normacusine-A,[9] on the one hand, and the dihydroindoles, ajmaline[2,3] and its congeners, vincamajine (VII)[10] and its O-acetate, vincamedine, on the other, are in support of this belief. Since deoxyajmalal-A (IX)[2] was readily available from deoxyajmaline (VIII),[11] it was felt that this would be a suitable compound for testing the feasibility of this biogenetically possible ring closure reaction, IX → X → VIII. The





II, R = R_1 = H; R_2 = CH_2OH
III, R = Me; R_1 = CH_2OH; R_2 = COOMe
IV, R = H; R_1 = COOMe; R_2 = CH_2OH
V, R = H; R_1 = CH_2OH; R_2 = COOMe
VI, R = Me; R_1 = CHO; R_2 = COOMe

feeling was that experimental conditions could be found to take advantage of the vicinal location of the nucleophilic β-position of the indole nucleus[12] and the electrophilic aldehyde group.



As a model reaction deoxyajmalol-A (XIV)[2] was tosylated, thus setting up favorable conditions for an intramolecular substitution reaction, *viz.*, XV → XVI, which could result in 2-hydroxydideoxyajmaline (XVII). Indeed, the desired product was obtained, the cyclization occurring even at room temperature. Reduction of the hydroxy compound with lithium aluminum hydride gave 2-epidideoxyajmaline (XIII, OH = H) in good yield. When the reduction was carried out with zinc in hydrochloric acid, both of the epimeric dideoxyajmalines were produced as determined by paper and thin-layer chromatography.

Attention now focused on a study of deoxyajmalal-A (IX) itself. In dilute acid the indoleninium form X could not be detected spectroscopically nor could the

(1) Most recent paper, Part XLV, M. M. Robison, W. Pierson, R. A. Lucas, I. Hsu and R. Dziemian, *J. Org. Chem.*, in press.

(2) M. F. Bartlett, R. Sklar, W. I. Taylor, E. Schlittler, R. L. S. Amai, P. Beak, N. V. Bringi and E. Wenkert, *J. Am. Chem. Soc.*, **84**, 322 (1962).

(3) R. B. Woodward, *Angew. Chem.*, **68**, 13 (1956).

(4) D. Stauffacher, A. Hofmann and E. Seebeck, *Helv. Chim. Acta*, **40**, 508 (1957); S. K. Talapatra and A. Chatterjee, *Sci. and Culture*, **22**, 692 (1957).

(5) A. R. Battersby and D. A. Yeowell, *Proc. Chem. Soc.*, 17 (1961).

(6) N. Defay, M. Kaisin, J. Pecher and R. H. Martin, *Bull. soc. chim. Belges*, **70**, 475 (1961).

(7) J. Levy, J. Le Men and M.-M. Janot, *Compt. rend.*, **253**, 131 (1961).

(8) L. D. Antonaccio, N. A. Pereira, B. Gilbert, H. Vorbrueggen, H. Budzikiewicz, J. M. Wilson, L. J. Durham and C. Djerassi, *J. Am. Chem. Soc.*, **84**, 2161 (1962). The relationship of polyneuridine and macusine-C is discussed in footnote 40, ref. 8.

(9) A. T. McPhail, J. Monteath Robertson, G. A. Sim, A. R. Battersby, H. F. Hodson and D. A. Yeowell, *Proc. Chem. Soc.*, 223 (1961).

(10) M.-M. Janot, J. Le Men, J. Gosset and J. Levy, *Bull. soc. chim. France*, 1079 (1962).

(11) Woodward and Schenker (ref. 3), who first showed that the action of lead tetraacetate on deoxyajmaline gave an indole aldehyde, obtained only the diastereoisomer, deoxyajmalal-B.

(12) T. S. Stevens in "Chemistry of Carbon Compounds," E. H. Rodd, Editor, Vol. IVa, Elsevier Publishing Co., New York, N. Y., 1957, p. 78.