BIOSYNTHETIC INCORPORATION OF ONE-CARBON UNITS INTO BERBERINE AND HYDRASTINE[1]

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ABSTRACT

The utilization of one-carbon precursors in the biosynthesis of hydrastine and berberine was investigated by tracer experiments with plants of *Hydrastis canadensis* L. The lactone group of hydrastine and the bridge carbon of berberine are shown to be derived from the *S*-methyl group of methionine.

Recent tracer studies have established several intermediates of the pathway leading to the phthalideisoquinoline and the protoberberine alkaloids. By systematic carbon-by-carbon degradation of the labelled products which were isolated after administration of specifically tagged precursors to intact plants, it was shown that two tyrosine (I) units are incorporated into hydrastine (VIII) (2, 3), into narcotine (IX) (4), and into berberine (VII) (3, 5), that dopamine (III) is utilized in the biosynthesis of hydrastine (3, 6) and berberine (7), and that norlaudanosoline (V) serves as a precursor of narcotine (4). Incorporation of radioactivity from dopa (II) into berberine has also been reported (8). These findings, summarized in Fig. 1, are in remarkable agreement with the biogenetic speculations which were first put forward, mainly on the basis of structural considerations, almost half a century ago (9). They establish a source of all but one of the carbon atoms in the nucleus of the phthalideisoquinoline and protoberberine alkaloids. The remaining carbon atom in the skeletons of these alkaloids, the carbonyl carbon of hydrastine and narcotine (* in VIII and IX) and the so-called bridge carbon of berberine (* in VII), has long been postulated to arise from a one-carbon unit, which was presumed to enter at the oxidation level of formaldehyde (9). In agreement with this hypothesis, radioactivity from labelled formate has been found in the carbonyl group of narcotine (4).

We have tested the incorporation of ^{14}C-formate and of methyl-^{14}C-methionine into hydrastine and berberine in intact plants of *Hydrastis canadensis* L. (Golden Seal). The results indicate that the carbonyl group of hydrastine and the bridge carbon of berberine are indeed derived from one-carbon precursors. The methyl group of methionine is incorporated into these sites more efficiently than is formate. The findings lead to the inference that the one-carbon unit, from which the carbonyl carbon of hydrastine and the bridge carbon of berberine are ultimately derived, is introduced into a precursor molecule by a transmethylation step.

In a series of seven feeding experiments, sodium ^{14}C-formate (expts. 1–3) and L-methyl-^{14}C-methionine (expts. 4–7) were administered to plants of *Hydrastis canadensis* L., either via the roots or by infusion into the stem (3). In each experiment radioactive berberine and hydrastine was isolated from roots and rhizomes. Chemical and radiochemical yields are summarized in Table I. As observed previously with other precursors (3), berberine isolated from a particular experiment invariably had a higher specific activity than hydrastine (Table I). It is noteworthy that hydroponic administration of methionine led to more efficient incorporation of tracer than wick feeding, whereas wick

[1]*A preliminary account of parts of this work has been published (1).*

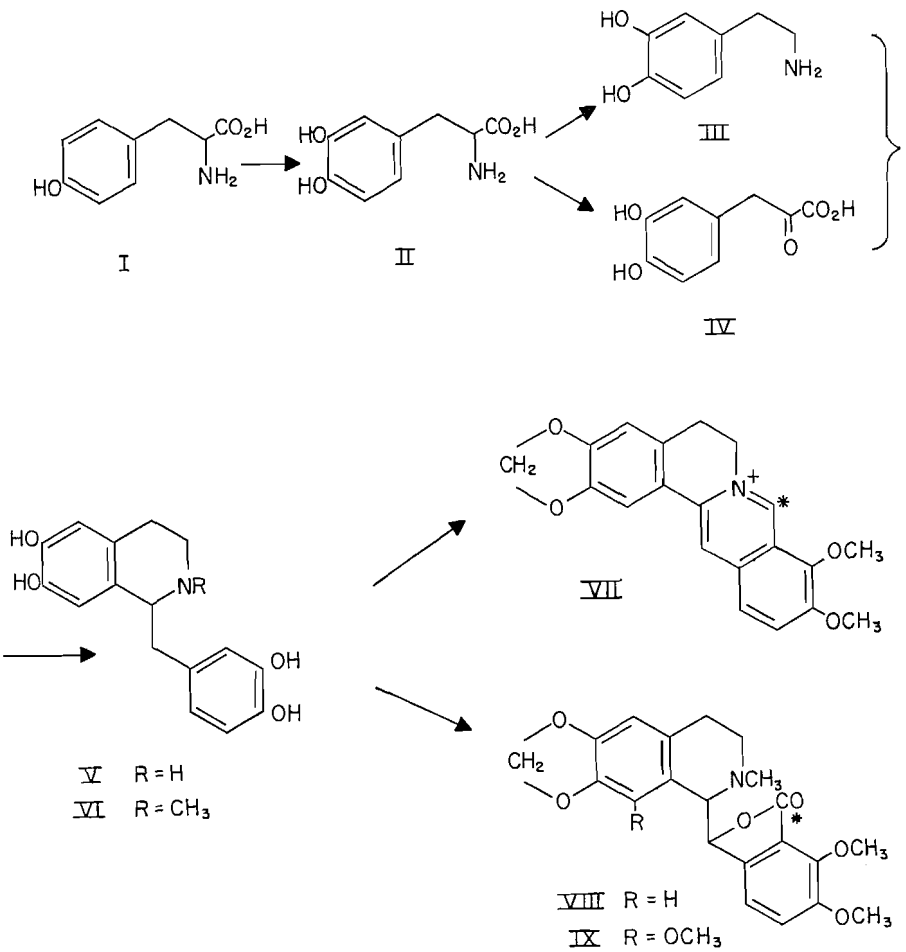

FIG. 1. Biosynthesis of berberine and hydrastine.

feeding was more efficient in the case of formate, in experiments conducted over the same time span. In terms of specific radiochemical yield, as well as in terms of recovery in the products of administered radioactivity (% incorporation), methionine was more efficiently incorporated than formate (Table II).

It was anticipated that incorporation of one-carbon precursors would yield hydrastine labelled at five centers (C=O, NCH_3, 2 × OCH_3, and CH_2O_2) and berberine labelled at four centers (bridge, 2 × OCH_3, and CH_2O_2). For an unequivocal interpretation of such multi-site incorporation of radioactivity, the labelled carbon atoms in each product had to be separated from one another, so that the extent of radioactivity at each labelled site could be determined. A method for degrading the alkaloids was worked out by pilot experiments with inactive material. The degradation sequences shown in Figs. 2 and 3 permitted determination of the radioactivity at the predicted one-carbon sites.

The labelled samples of hydrastine and berberine, obtained from six of the experiments, were degraded. The specific activities of the intermediates in the degradation sequences are shown in Tables III and IV. (Indicated limits are standard deviations of the mean.)

TABLE I

Chemical and radiochemical yields

Expt. No.	Precursor (total activity, counts $min^{-1} \times 10^{-7}$; specific activity, counts min^{-1} $mmole^{-1} \times 10^{-9}$)	Mode and duration of feeding (days)		Plants		Hydrastine			Berberine		
				No.	Dry weight (g)	Yield (mg)	Specific activity (counts min^{-1} $mmole^{-1} \times 10^{-4}$)	Total activity (counts min^{-1} $\times 10^{-4}$)	Yield (mg)	Specific activity (counts min^{-1} $mmole^{-1} \times 10^{-4}$)	Total activity (counts min^{-1} $\times 10^{-4}$)
1	$H^{14}COO^{-}Na^{+}$ (4.81; 2.55)	Root	3	8	10.0	184	0.36	0.17	220	0.71	0.38
2	"	Root	6	8	9.4	190	0.67	0.33	208	1.43	0.73
3	"	Wick	6	6	24.0	303	1.14	0.90	481	3.90	4.60
4	L-$^{14}CH_3$-methionine (2.51; 2.50)	Root	6	8	9.0	250	15.81	10.30	344	20.51	17.30
5		Wick	6	5	36.0	460	2.80	3.36	749	4.53	8.32
6	L-$^{14}CH_3$-methionine (1.92; 2.39)	Root	$1\frac{1}{4}$	9	16.0	244	4.47	2.84	268	16.0	10.91
7		Root	3	9	12.0	165	19.21	8.27	305	39.2	29.54

TABLE II

Efficiency of incorporation of radioactive precursors

Expt. No.	Precursor	Specific radiochemical yield* × 10^4		% incorporation of activity†		
		Hydrastine	Berberine	Hydrastine	Berberine	Total
1	^{14}C-Formate	1.4	2.8	0.003	0.008	0.011
2		2.6	5.6	0.007	0.015	0.022
3		4.5	15.3	0.019	0.096	0.12
4	L-Methyl-^{14}C-methionine	63.4	82.3	0.41	0.69	1.10
5		11.3	18.1	0.13	0.33	0.46
6		18.7	69.5	0.15	0.57	0.72
7		80.3	165.3	0.43	1.54	1.97

*Specific radiochemical yield $= \frac{\text{specific activity of product}}{\text{specific activity of precursor}} \times 100.$

†% incorporation $= \frac{\text{total activity of isolated product}}{\text{total activity of administered precursor}} \times 100.$

From these values the activity at the predicted one-carbon sites was deduced, in the manner indicated in the legend of Figs. 2 and 3. In each case these sites fully account for the activity of the original molecule. In Table V the contribution which each of these carbon atoms makes to the specific activity of the alkaloids obtained in individual experiments is expressed as a percentage of the specific activity of the intact alkaloids. The sum of the specific activities of the individual sites agrees, within experimental error, with the specific activity of the intact molecule, indicating the self-consistency of the results.

The results show that the lactone-carbonyl carbon of hydrastine and the bridge carbon of berberine, as well as the *O*- and *N*-attached one-carbon units of the two alkaloids, are specifically derived from one-carbon precursors. It is evident (Table V) that in every experiment the contribution which each of the one-carbon sites makes to the total specific activity of hydrastine and of berberine is of similar magnitude. This indicates that in each of the alkaloids all one-carbon sites are derived from the same immediate precursor. For if one of the one-carbon sites had been derived from some other immediate precursor than the other one-carbon sites in the same molecule, a different order of magnitude of the specific activity at that site, in either the formate or the methionine experiments, would have resulted. It follows that the lactone-carbonyl carbon of hydrastine and the bridge carbon of berberine are derived from the same immediate precursor which yields the extra-nuclear *O*- and *N*-attached one-carbon units.

There can be little doubt that transmethylation processes are responsible for the introduction of the one-carbon centers into the skeletons of hydrastine and berberine.

The evidence is strong that *O*- and *N*-methyl groups of alkaloids and other plant products originate in this manner (10). In a number of cases the transfer of methionine-methyl to such groups has been established by multiple labelling (11); and in others, transmethylase activity leading to the formation of *N*-methyl (12) and *O*-methyl groups (13, 14) by methyl transfer from *S*-adenosyl methionine has been demonstrated. In many further instances the methyl group of methionine proved to be more efficient than formate as a precursor of *O*-methyl (15) and *N*-methyl groups (15, 16) of plant alkaloids.

A transmethylation step is also required in the formation of the methylenedioxy group, (13, 17) which has been shown to arise from the methoxy group of an *o*-methoxyphenol (8, 18).

The origin of the bridge carbon of berberine and of the lactone group of hydrastine by a

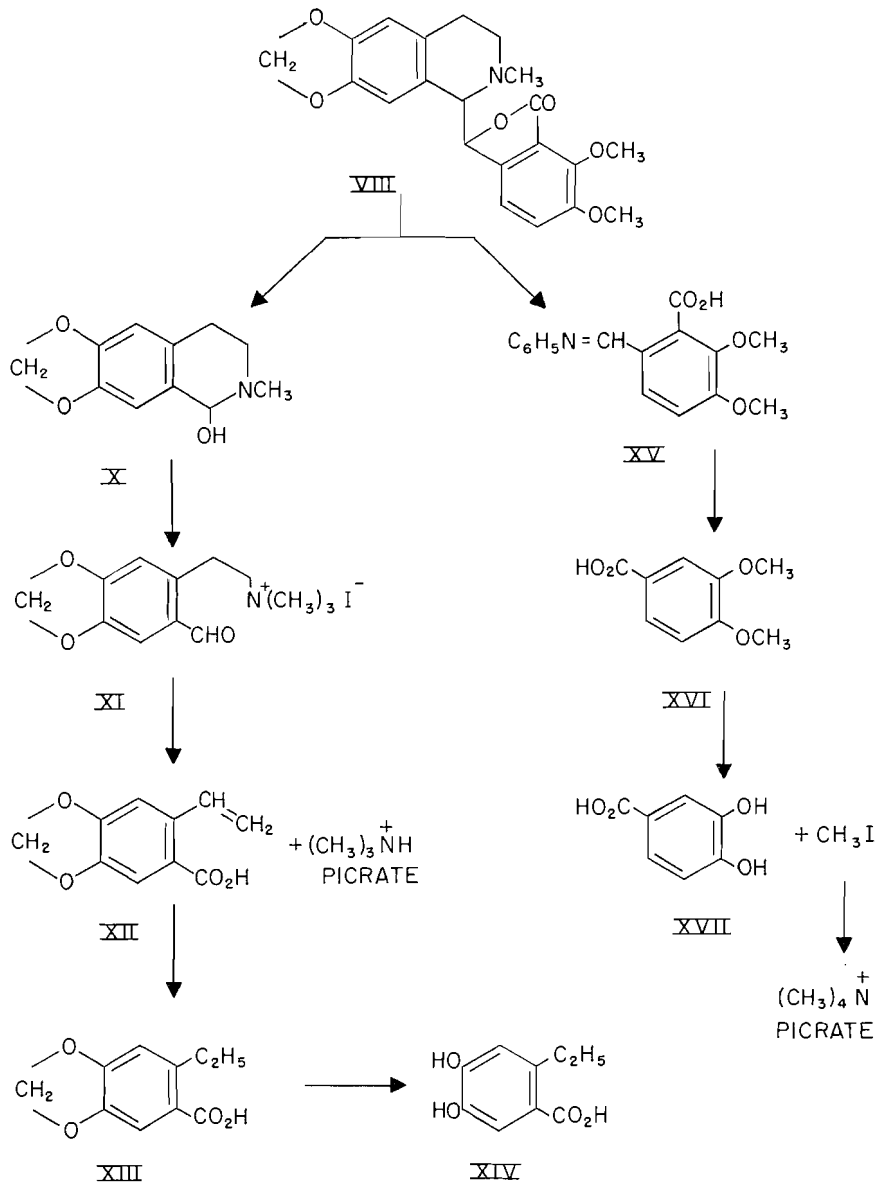

FIG. 2. Degradation of hydrastine, permitting isolation of one-carbon sites. NCH_3 = trimethylamine picrate; CH_2O_2 = 6-vinylpiperonylic acid (XII) minus 6-ethylprotocatechuic acid (XIV) (inactive) = 6-vinylpiperonylic acid (XII); $2 \times OCH_3$ = $2 \times$ tetramethylammonium picrate = veratric acid (XVI) minus protocatechuic acid (XVII) (inactive) = veratric acid (XVI); C=O = opianic acid anil (XV) minus veratric acid (XVI).

route involving transmethylation is less obvious. The early work of Sribney and Kirkwood (17) had indicated that radioactivity from methyl-labelled methionine entered the nucleus of protopine, an alkaloid structurally related to berberine. The site of activity was not determined but it was suggested that the label was located at a position corresponding to the berberine bridge. Recently it was shown that laudanosoline (VI) (19) and its di-*O*-methyl derivative, reticuline (8), are incorporated into berberine *in toto*, and that the

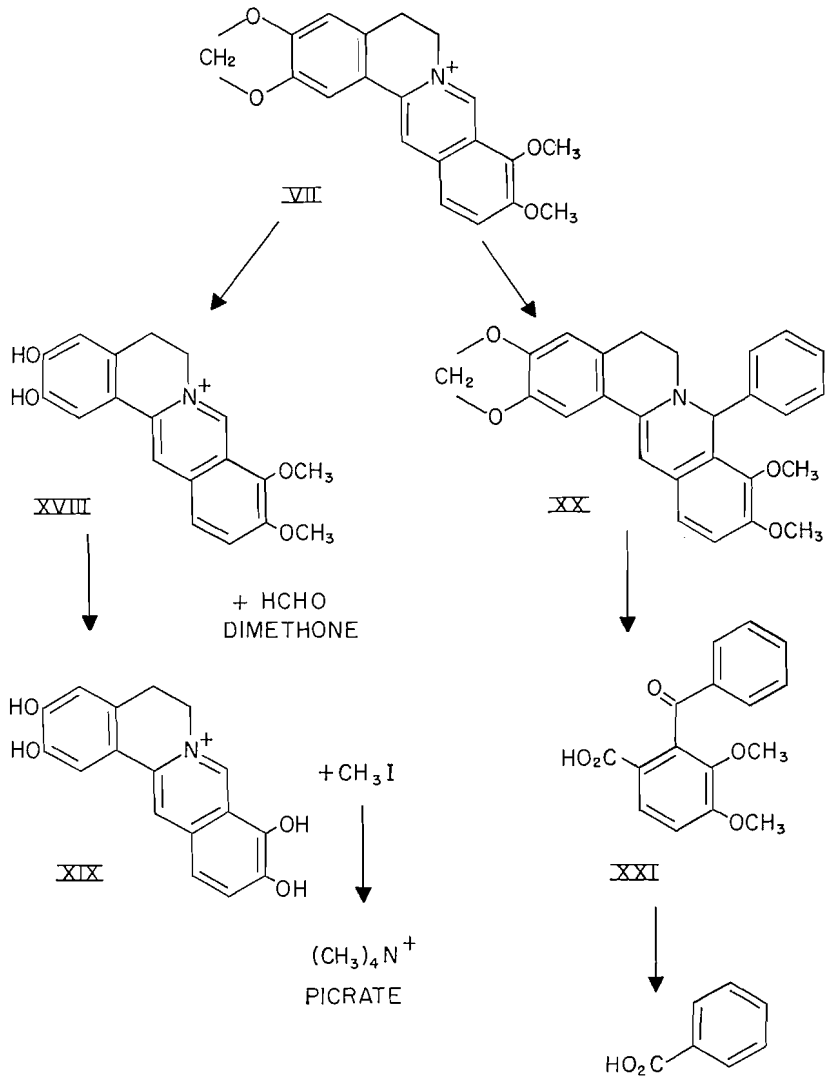

FIG. 3. Degradation of berberine, permitting isolation of one-carbon sites. CH_2O_2 = formaldehyde dimethone; 2 × OCH_3 = 2 × tetramethylammonium picrate; bridge = benzoic acid.

N-methyl group of these precursors is transformed into the bridge carbon of berberine. These results are consistent with the present findings, and with the view that the methyl group of methionine which gives rise to the bridge carbon of berberine enters the biosynthetic pathway by transmethylation onto the nitrogen atom of norlaudanosoline (V).

The present results indicate also that the lactone-carbonyl group of hydrastine, like the bridge carbon of berberine, is formed by modification of a methyl group, introduced into a precursor by transmethylation. Whether berberine and hydrastine arise from a common *N*-methylated precursor, laudanosoline (VI), by independent pathways, or whether hydrastine is formed by oxidative modification of berberine (20), or whether a *C*-methylated rather than an *N*-methylated derivative of norlaudanosoline is the intermediate of hydrastine biosynthesis, remains to be established.

TABLE III

Degradation of hydrastine

	Specific activity (counts min^{-1} $mmole^{-1}$ $\times 10^{-4}$)					
	Formate			Methionine		
	Expt. No. 1	Expt. No. 2	Expt. No. 3	Expt. No. 4	Expt. No. 5	Expt. No. 6
Hydrastine (VIII) (NCH_3, CH_2O_2, $2\times OCH_3$, C=O)	0.36±0.02	0.39±0.01*	0.66±0.02*	4.90±0.05*	2.80±0.04	1.56±0.01*
Hydrastinine (X)	0.11±0.01	0.13±0.01	0.25±0.01	2.14±0.02	1.28±0.04	0.74±0.01
Trimethylamine picrate (NCH_3)	0.064±0.007	0.081±0.007	0.10±0.01	1.21±0.02	0.86±0.02	0.33±0.01
6-Vinylpiperonylic acid (XII) (CH_2O_2)	0.070±0.004	0.084±0.008	0.16±0.01	0.98±0.01	0.48±0.01	0.32±0.01
6-Ethylprotocatechuic acid (XIV)	—	—	—	—	Inactive	—
Opianic acid anil (XV) ($2\times OCH_3$ + C=O)	0.21±0.01	0.24±0.01	0.40±0.04	2.88±0.06	1.52±0.02	0.98±0.02
Veratric acid (XVI) ($2\times OCH_3$)	0.14±0.01†	0.17±0.01	0.28±0.01	1.84±0.03	1.01±0.01	0.64±0.01
Tetramethylammonium picrate (OCH_3)	—	0.09±0.01	0.14±0.01	—	0.52±0.02	—
Protocatechuic acid (XVII)	Inactive	—	—	—	—	—

*Diluted with carrier.
†The sample of labelled opianic acid anil was diluted with carrier prior to decarboxylation. The value given for veratric acid so obtained takes into account the dilution factor.

TABLE IV

Degradation of berberine

	Specific activity (counts min^{-1} $mmole^{-1}$ $\times 10^{-4}$)					
	Formate			Methionine		
	Expt. No. 1	Expt. No. 2	Expt. No. 3	Expt. No. 4	Expt. No. 5	Expt. No. 6
Berberine picrate (CH_2O_2, 2×OCH_3, bridge)	0.71±0.03	1.43±0.02	1.90±0.02*	5.20±0.12*	4.53±0.11	8.20±0.19*
Formaldehyde dimethone (CH_2O_2)	0.15±0.01	0.33±0.01	0.43±0.02	1.27±0.04	1.13±0.03	2.07±0.10
Tetramethylammonium picrate (OCH_3)	0.17±0.01	0.35±0.01	0.43±0.02	1.36±0.04	1.17±0.04	1.95±0.09
Benzoic acid (bridge)	—	—	0.46±0.01	1.26±0.04	—	2.09±0.11

*Diluted with carrier.

TABLE V

Incorporation of one-carbon units into hydrastine and berberine

Expt. No.	Hydrastine					Berberine			
	Relative specific activity (%)*					Relative specific activity (%)			
	C=O	2 OCH_3	CH_2O_2	NCH_3	Total (%)†	Bridge	2 OCH_3	CH_2O_2	Total (%)‡
1	20±5	41±4	20±1	18±2	95±6		47±3‡	21±2‡	
2	18±4	41±3	21±2	20±2	103±5		49±2‡	23±1‡	
3	19±6	42±3	24±2	15±2	100±7	26±1*	49±1*	25±1*	92±2
4	21±1	36±1	19±1	24±1	103±3	24±1*	52±2*	24±1*	101±3
5	18±1	35±1	17±1	30±1	101±2		52±2‡	25±1‡	
6	21±1	39±1	20±1	20±1	104±3	26±1*	48±2*	26±1*	98±3

*Sum of specific activities of individual one-carbon sites = 100.
†Specific activity of intact hydrastine = 100.
‡Specific activity of intact berberine = 100.

EXPERIMENTAL

Degradation of Hydrastine

Conversion of Hydrastine (VIII) to Hydrastinine (X) and Opianic Acid Anil (XV) (3, 22)

Hydrastine (0.5 g) was suspended in 20% nitric acid (4 ml) and the mixture was kept at 40° for 16 h. The clear yellow solution was cooled, and cold 40% potassium hydroxide (3 ml) was added during stirring and cooling. The mixture was kept at 5° for 1 h, and the crystalline precipitate was filtered off and washed with a little cold water. Hydrastinine (227 mg, 84%), melting at 116–117° after recrystallization from ethyl acetate, was obtained; reported m.p. 116–117° (21).

Aniline (0.3 g) in ethanol (2 ml) was added to the filtrate and the solution was warmed for a few minutes at 60–70°. After the solution was cooled, cold 10% hydrochloric acid was added dropwise, with stirring, until the mixture was acidic to Congo red. The precipitate was filtered off, washed with water and with a little methanol, and crystallized from methanol. Opianic acid anil (170 mg, 45%), melting at 188–189°, was obtained; reported m.p. 191.5–192.5° (22).

Conversion of Hydrastinine to Hydrastinine Picrate (23)

Crude hydrastinine (10 mg) was dissolved in methanol (5 ml) and an excess of a hot saturated solution of picric acid in methanol was added. A red precipitate formed when the mixture was warmed and allowed to cool. This precipitate recrystallized from methanol as red crystals of hydrastinine picrate melting at 173° (reported m.p. 173° (23)).

Conversion of Hydrastinine (X) to β-[2-Formyl-4,5-methylenedioxyphenyl]-ethyltrimethylammonium Iodide (N-Methylhydrastinine Methiodide) (XI)

A one-step Hofmann degradation (3, 24) was not suitable for the present purpose, since, in the presence of excess methylating agent, methyl exchange on trimethylamine was likely to take place. Such an exchange would make the recovery of isotope in trimethylamine uncertain. *N*-Methylhydrastinine methiodide was therefore isolated.

Hydrastinine (105 mg) was suspended in water (2 ml), and potassium carbonate (100 mg) and methyl iodide (2 ml) were added. The mixture was shaken for 24 h and then kept at 40° for another 24 h. Methyl iodide was evaporated, the residue was cooled and filtered, and the product washed with a little cold water and recrystallized from aqueous methanol. *N*-Methylhydrastinine methiodide (135 mg, 75%), melting at 265–266°, was obtained; reported m.p. 267° (25) and 262–264° (26).

Hofmann Degradation of N-Methylhydrastinine Methiodide

A suspension of the above quaternary salt (180 mg) in 10% sodium hydroxide (5 ml) was kept on the steam bath for 1 h, during which time a current of nitrogen was passed through the suspension. The issuing gas was passed through an alcoholic solution of picric acid; from this solution, on concentration and recrystallization from methanol, trimethylamine picrate, melting at 216°, was isolated. Quantitative recovery of this picrate was not attempted.

The original reaction mixture was cooled, extracted with ether, and the ether extract was washed with water, dried, and evaporated. The residue was dissolved in a mixture of methanol (4 ml) and water (2 ml) and powdered silver nitrate (0.6 g) was added to the solution, followed by 20% sodium hydroxide (6 ml). The mixture was warmed on the steam bath, shaken for 2 h, filtered, and extracted with ether. The aqueous layer was warmed to remove ether and cooled and acidified with cold 50% (v/v) sulfuric acid. The precipitate, which was obtained on cooling, was washed with cold water and recrystallized from aqueous methanol. 6-Vinylpiperonylic acid (XII) (53 mg, 55%), melting at 167–168°, was obtained; reported m.p. 168° (3).

Reduction of 6-Vinylpiperonylic Acid (XII) to 6-Ethylpiperonylic Acid (XIII)

6-Vinylpiperonylic acid (100 mg) was dissolved in 10% sodium hydroxide (4 ml) and the solution maintained at 60–65°. Nickel–aluminium alloy (150 mg) was added in small portions with constant stirring. The addition of alloy was complete in 45 min. The temperature was raised to 75–80° and stirring was continued for 12 h. The catalyst was then filtered off and washed with water. The combined filtrate and washings were acidified with 50% hydrochloric acid and the product was filtered off, washed with a little cold water, and recrystallized from aqueous methanol. 6-Ethylpiperonylic acid (90 mg, 60%), melting at 125–126°, was obtained. (Found: C, 62.1; H, 5.2. $C_{10}H_{10}O_4$ requires C, 61.9; H, 5.2%.) Subsequent reductions were carried out at room temperature without affecting the yield or the nature of the reduction product. Cleavage of the methylenedioxy group, reported to take place in a number of other methylenedioxyphenyl derivatives (27), did not appear to occur with 6-vinylpiperonylic acid under these conditions.

Conversion of 6-Ethylpiperonylic Acid (XIII) to 6-Ethylprotocatechuic Acid (XIV)

6-Ethylpiperonylic acid (60 mg) and phosphorus pentachloride (180 mg) were sealed in a hard-glass test tube, which was then kept at 150–160° for 2 h. The tube was opened and the reaction mixture, after addition of water, was refluxed for 2 h in a current of nitrogen which had been purified by passing through potassium hydroxide solution. The exit gases were bubbled into *M* potassium hydroxide which was protected from atmospheric carbon dioxide by a trap of potassium hydroxide solution. The carbon dioxide

which had been swept out of the reaction mixture was converted to barium carbonate by addition of 17% barium chloride solution. The precipitate was washed with water and dried in air when barium carbonate (38 mg, 73%) was obtained.

The reaction mixture was cooled and extracted with ether. The ether extract was dried and evaporated and the residue sublimed at 130° and 1×10^{-3} mm. 6-Ethylprotocatechuic acid (24 mg, 40%), melting at 194–195°, was obtained; reported m.p. 164–165° (28). (Found: C, 59.2; H, 5.6. $C_9H_{10}O_4$ requires C, 59.3; H, 5.5%.)

Decarboxylation of Opianic Acid Anil (XV) (22)

A slow stream of nitrogen, which had been purified by passing through a saturated solution of potassium hydroxide and through sulfuric acid, was passed through the tube containing opianic acid anil (100 mg) and copper bronze (5 mg). The exit gases were bubbled through *M* potassium hydroxide, which was protected from the atmosphere by a trap of saturated potassium hydroxide solution. The reaction tube was placed in a bath of boiling ethyl benzoate (b.p. 212°) until the evolution of carbon dioxide ceased (10 min). Heating was discontinued, but passage of nitrogen was continued for another 0.5 h. Barium chloride (17%) was added to the potassium hydroxide trap which had absorbed the exit gases, to yield barium carbonate (60 mg, 90%).

The residue in the reaction tube, veratraldehyde anil, was hydrolyzed by refluxing with 5% acetic acid (1 ml) for 1 h (22). The mixture was cooled and extracted with ether. The ether extract was washed, in succession, with water, dilute potassium hydroxide solution, and water and was then dried and evaporated. The residue, veratraldehyde, was dissolved in a mixture of methanol (4 ml) and water (2 ml). Powdered silver nitrate (0.5 g) and 20% sodium hydroxide (5 ml) were added. The mixture was warmed on a steam bath for a few minutes, shaken for 2 h, and then filtered. Acidification of the filtrate with 50% (v/v) sulfuric acid yielded crude product, which was washed with water, dissolved in methanol, decolorized with charcoal, and recrystallized from aqueous methanol. Veratric acid (XVI) (34 mg, 50%), melting at 175–176°, was obtained; reported m.p. 179–181.5° (29).

Cleavage of the Methoxy Groups of Veratric Acid (cf. Ref. 30)

Veratric acid (200 mg) in freshly distilled hydriodic acid (density, 1.7, 10 ml) was gently refluxed in a current of dry nitrogen for 2 h. The exit gases were passed through a suspension of red phosphorus in 5% cadmium sulfate solution, passed over anhydrous potassium carbonate, and bubbled into a 5% methanolic solution of trimethylamine contained in a series of two tubes which were cooled in dry ice–acetone mixture. After 2 h passage of nitrogen was stopped, and the tubes containing trimethylamine solution were disconnected and allowed to stand at room temperature overnight. A crystalline precipitate of tetramethylammonium iodide was obtained. The solution was decanted and the precipitate repeatedly washed with dry methanol. The precipitate was dissolved in water, and a hot saturated aqueous solution of picric acid was added. The residue obtained on cooling was washed with ether and recrystallized twice from methanol, when pale red needles of tetramethylammonium picrate, melting at 312–313°, were obtained. No attempt was made to recover the picrate quantitatively.

The reaction mixture was diluted with water and extracted with ether. The extract was washed with cold dilute sodium thiosulfate solution to destroy iodine, and with water, dried, and evaporated. The residue sublimed at 125° and 3.5×10^{-3} mm when protocatechuic acid (XVII) (100 mg, 60%), melting at 200–202°, was obtained; reported m.p. 199°.

Degradation of Berberine

Conversion of Berberine Hydrochloride to Berberine Picrate

Berberine hydrochloride (10 mg) was dissolved in methanol and an excess of a hot saturated solution of picric acid in methanol was added. The mixture was heated and allowed to cool. An orange precipitate appeared which was filtered off, washed with methanol, and recrystallized from methanol–acetone, yielding orange needles of berberine picrate, melting at 238–239°; reported m.p. 239° (31).

Hydrolytic Cleavage of the Methylenedioxy Group of Berberine (cf. Ref. 17)

Berberine hydrochloride (100 mg) and dimedone (5,5-dimethyldihydroresorcinol) (300 mg) were suspended in dilute sulfuric acid (60 ml concentrated sulfuric acid in 100 ml water) (5ml) and the mixture was maintained at 98–100° for 18 h. The cooled reaction mixture was diluted with water (5 ml) and extracted with ether. The ether extract was washed with water, dried, and evaporated. The residue was crystallized from aqueous methanol when white silky needles of the dimedone derivative of formaldehyde (24 mg, 36%), melting at 190–191°, were obtained.

The aqueous layer, in which a precipitate had now appeared, was warmed to remove residual ether, and a saturated solution of potassium iodide (2 ml) was added. The mixture was cooled and filtered and the residue washed with a little sulfur dioxide water and dried. 2,3-Dihydroxy-9,10-dimethoxy-7,8,13,14-tetradehydroberbinium iodide (XVIII) (90 mg, 80%) was obtained (cf. ref. 32).

Cleavage of the Methoxy Groups

2,3-Dihydroxy-9,10-dimethoxy-7,8,13,14-tetradehydroberbinium iodide (XVIII) (90 mg) was refluxed with freshly distilled hydriodic acid (5 ml). Methyl iodide was isolated as tetramethylammonium picrate by the method described earlier for the cleavage of veratric acid.

The reaction mixture was diluted with water, decolorized with sulfur dioxide water, filtered, and dried. Berberoline iodide (XIX) (70 mg, 74%) was obtained. This compound could not be crystallized nor converted into a crystalline derivative (cf. ref. 33).

Isolation of the Bridge Carbon of Berberine as Benzoic Acid (cf. ref. 34)

Berberine hydrochloride (700 mg) was dried at 100° under vacuum for 2 h and then suspended in 10 ml ether. Excess of an ethereal solution of phenylmagnesium bromide, prepared from magnesium turnings (0.12 g) and bromobenzene (0.6 ml) in 5 ml anhydrous ether, was added. The mixture was stirred and refluxed for 2 h. Water (5 ml) and dilute hydrochloric acid (5 ml) was added and the resulting precipitate was washed with water and a little ether.

The crude phenyldihydroberberine hydrobromide (XX) was suspended in a solution of potassium carbonate (3 g) in water (150 ml). Potassium permanganate (3 g) was added in small portions over a period of 80 h with continuous stirring. Sulfur dioxide was passed through the solution until it was clear. The solution was then acidified and boiled to expel dissolved sulfur dioxide, cooled, and extracted with chloroform. The chloroform layer was washed with water and dilute acid and extracted with 10% sodium hydroxide. The basic extract was washed with ether, acidified with 50% hydrochloric acid, and extracted with ether. The ether extract was dried and evaporated. The residue was crystallized from 50% acetic acid. 2-Benzoyl-3,4-dimethoxybenzoic acid (XXI) (120 mg, 24%), melting at 193–194°, was obtained; reported m.p. 190–191° (34) and 192° (35).

2-Benzoyl-3,4-dimethoxybenzoic acid (XXI) (100 mg) was moistened with water (0.5 ml), potassium hydroxide (1 g) was added, and the mixture was fused for 2–3 min. The cooled melt was dissolved in water and the solution was acidified with 10% hydrochloric acid and extracted with chloroform. The chloroform layer was washed with water, dried, and evaporated. The residue was sublimed at 60–70° and 1×10^{-3} mm, yielding benzoic acid (35 mg, 83%) melting at 119–120°.

Administration of ^{14}C-labelled Compounds to Hydrastis canadensis L. and Extraction of Labelled Alkaloids

Methyl-^{14}C-methionine and sodium ^{14}C-formate were administered to 4-year-old plants of *Hydrastis canadensis* L., either hydroponically or by infusion into the stem, by reported methods (3). Hydrastine and berberine were extracted from roots and rhizomes by standard methods (3). Leaves and other aerial parts were rejected. Chemical and radioactive yields from all experiments are shown in Table I.

Degradation of Radioactive Alkaloids

The radioactive alkaloids were degraded by the reaction sequences described above. When the amount of material permitted, the alkaloid was degraded without dilution after crystallization to constant activity. In most cases, however, dilution with carrier was necessary to obtain sufficient material to carry out the complete degradation sequence. A minimum of 500 mg berberine and 350 mg hydrastine was desirable for this purpose.

The degradation products were solids, or were isolated as solid derivatives, and were crystallized to constant melting point. Their radioactivity was assayed on samples of finite thickness on aluminium planchettes, using a Model 1052 gas-flow counter with "micromil" window (Nuclear Chicago Corp.) in a low-background automatic sample changer (Nuclear Chicago Corp., Model C115). Counts were recorded on a printing timer (Nuclear Chicago Corp., C111B) in association with a scaler (Nuclear Chicago Corp., 181B).

The usual corrections for background and self-absorption were applied.

For plating, a 1% solution of collodion in dimethylformamide was used as the solvent. Only berberine hydrochloride and formaldehyde dimethone could not be plated in this way. Dissolution of these samples required prolonged contact with benzyl alcohol before application of the collodion dimethylformamide solution. In the case of benzoic acid, a drop of a 5% solution of sodium hydroxide in 50% aqueous methanol was required to avoid loss of benzoic acid by evaporation.

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