ONE-CARBON UNITS IN THE BIOSYNTHESIS OF HYDRASTINE AND BERBERINE*

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With the demonstration that two C_6-C_2 units, derived from tyrosine, are incorporated into hydrastine (Gear and Spenser, 1961, 1963), into berberine (Spenser and Gear, 1962a) and into narcotine (Battersby and McCaldin, 1962), and that dopamine (Spenser and Gear, 1962b) and norlaudanosoline (Battersby and McCaldin, 1962) are utilized in the biosynthesis of this group of compounds, a source of all but one of the carbon atoms in the nucleus of members of the pthalideisoquinoline and protoberberine alkaloids has been established.

The remaining carbon atom in the nucleus of these bases, i.e., the lactone-carbonyl carbon of hydrastine and of narcotine, and the so-called bridge-carbon of berberine, has long been postulated to arise from a one-carbon unit (Robinson, 1955) In agreement with this classical hypothesis it has been shown that radioactivity from labelled formate, administered to Papaver somniferum plants, was recovered from the lactone-carbonyl group of narcotine (Battersby and McCaldin, 1962).

In the present communication evidence is presented which shows that the lactone-carbonyl carbon of hydrastine, the bridge-carbon of berberine and the O- and N-attached extra-nuclear one-carbon substituents of both bases are derived from one-carbon precursors, and that the methyl group of methionine is incorporated into these sites more efficiently than formate.

In a series of experiments sodium formate-C^{14} and methionine methyl-C^{14} were administered to plants of Hydrastis canadensis L., either by way of the root or by infusion into the stem (c.f. Gear and Spenser, 1963). Labelled

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hydrastine and berberine were isolated from the roots and rhizomes. Chemical and radiochemical yields are recorded in Table 1. In terms of the specific activity of the products, as well as in terms of recovery in the products of administered tracer, methionine appears to be more efficiently incorporated than formate.

The labelled samples of hydrastine and berberine from each experiment were degraded (after dilution with carrier, when necessary) to isolate the predicted one-carbon sites. Oxidation of hydrastine (CH_2O_2, 2 x OCH_3, NCH_3, CO) gave hydrastinine (CH_2O_2, NCH_3) and opianic acid (2 x OCH_3, CO), which was isolated as the anil. Exhaustive methylation of hydrastinine gave trimethylamine (NCH_3), trapped as the picrate, and hydrastal, which was further converted to 6-vinyl-piperonylic acid (CH_2O_2). In one case the latter was reduced to 6-ethyl-piperonylic acid and then hydrolyzed to yield inactive 6-ethylprotocatechuic acid. Opianic acid anil (2 x OCH_3, CO) was decarboxylated to yield the anil of veratral, which was converted to veratric acid (2 x OCH_3). Treatment of veratric acid with hydrogen iodide gave inactive protocatechuic acid and methyl iodide which was isolated as tetramethylammonium picrate (2 x OCH_3). The specific activity of each of the different suspected one-carbon sites was thus determined separately. The sum of the specific activities of the individual sites agreed, within experimental error, with the specific activity of the original hydrastine, indicating the self-consistency of the results.

Hydrolysis of berberine (CH_2O_2, 2 x OCH_3, bridge) with sulphuric acid gave formaldehyde (CH_2O_2), isolated as the dimethone, and a phenolic product which on treatment with hydrogen iodide gave methyl iodide, recovered as tetramethyl-ammonium picrate (2 x OCH_3). Direct determination of the bridge-carbon atom is as yet incomplete, and its activity was therefore calculated by difference.

From the specific activities of the degradation products of hydrastine and berberine obtained in each of the five experiments, the contribution which each of the one-carbon sites makes to the specific activity of the original molecule was calculated (Table 2). The results clearly indicate that the lactone-carbonyl

TABLE 1

Chemical and Radiochemical Yields

	Precursor (total activity counts min^{-1} x 10^{-7})	Mode and duration of feeding (days)	Hydrastine			Berberine			% recovery of activity
			yield (mg)	specific activity counts min^{-1} mM^{-1} x 10^{-4}	total activity counts min^{-1} x 10^{-4}	yield (mg)	specific activity counts min^{-1} mM^{-1} x 10^{-4}	total activity counts min^{-1} x 10^{-4}	
1	$HC^{14}OO^{-}Na^{+}$ (4.81)	root 3	184	0.39	0.19	220	0.89	0.48	0.014
2	"	root 6	190	0.67	0.33	208	1.54	0.79	0.023
3	"	wick 6	303	1.14	0.90	481	3.90	4.60	0.12
4	$C^{14}H_3$-S-methionine (2.51)	root 6	250	15.83	10.3	344	20.50	17.3	1.10
5	"	wick 6	460	2.68	3.22	749	4.63	8.50	0.47

group, as well as the methylenedioxy, the N-methyl and the O-methyl groups of hydrastine are derived from one-carbon precursors. The results show further, that the methylenedioxy and the O-methyl groups of berberine are similarly derived. Although the one-carbon origin of the bridge-carbon of berberine is not demonstrated directly, it is shown that approximately one-quarter of the total activity of berberine derived from one-carbon precursors resides in the nucleus of the molecule. It has been shown earlier (Spenser and Gear, 1962a, Gear and Spenser, 1963) that two tyrosine units are specifically incorporated into the nucleus, accounting for all its carbon atoms, but one. The present activity of the nucleus must therefore be concentrated at the remaining carbon atom, i.e., at the bridge-carbon. Experiments designed to isolate the bridge-carbon for direct assay of radioactivity are in progress.

A number of further inferences may be drawn. It is evident (Table 2) that in every experiment the contribution each site makes to the total specific activity of hydrastine (and presumably also of berberine) is of similar magnitude. This indicates that all one-carbon sites in hydrastine (and berberine) are derived from the same immediate precursor. For if one of the one-carbon sites had been derived from some other immediate precursor than all other sites, a different order of magnitude of the specific activity at that site compared to the other sites, 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 precursor as the extra-nuclear one-carbon units.

Since the incorporation of methionine-methyl into both alkaloids was more efficient than the incorporation of formate, it follows either that methionine-methyl and formate give rise, by independent pathways, to one and the same ultimate one-carbon precursor, the former at a faster rate than the latter, or that formate and methionine each represent stages on a common route to the ultimate one-carbon precursor. On the basis of established pathways of one-carbon metabolism, the second alternative is the more attractive, and it is likely that the ultimate one-carbon precursor is S-adenosylmethionine, and that

TABLE 2

Incorporation of One-carbon Units into Hydrastine and Berberine

Exp.	Hydrastine					Berberine		
	Relative distribution of activity (%)[a]					Relative distribution of activity (%)[c]		
	C=O	2 OCH_3	CH_2O_2	NCH_3	Total (%)[b]	bridge[d]	2 OCH_3	CH_2O_2
1	17 ± 5	43 ± 5	21 ± 2	20 ± 3	92 ± 7	31 ± 3	47 ± 2	21 ± 2
2	18 ± 4	41 ± 3	21 ± 2	20 ± 2	103 ± 6	26 ± 3	50 ± 3	23 ± 1
3	18 ± 6	42 ± 5	24 ± 2	16 ± 2	102 ± 8	37 ± 3	41 ± 2	22 ± 1
4	21 ± 2	36 ± 2	19 ± 1	24 ± 1	103 ± 3	25 ± 2	52 ± 2	23 ± 2
5	18 ± 1	35 ± 1	17 ± 1	29 ± 1	101 ± 2	22 ± 2	53 ± 2	25 ± 1

a Sum of specific activities of individual one-carbon sites = 100

b Specific activity of intact hydrastine = 100

c Specific activity of intact berberine = 100

d by difference

all one-carbon sites originate by transmethylation processes. There is ample precedent in other systems for the introduction of O- and N-methyl groups in this manner.

That the formation of the methylenedioxy group requires such a step is very likely, since it was conclusively demonstrated (Barton, Kirby and Taylor, 1962) that such a group arises by oxidative cyclization of an o-methoxyphenol. The present results are consistent with such a process. They show (Table 2) that in every case the specific activity of the methylenedioxy group is identical, within experimental error, with the specific activity of each of the two methoxy groups, assuming that these are equally labelled, suggesting that both types of O-substituents arise from the same precursor and that they are introduced into the skeleton in close succession.

The origin of the lactone carbonyl group of hydrastine (and the bridge carbon of berberine) by a route involving a transmethylation step is at first glance more unusual. Such a methyl transfer onto, e.g., norlaudanosoline might take place either at the nitrogen atom or at the requisite carbon atom of the catechol nucleus, to yield an N- or a C-methylated intermediate, respectively.

Starting from either of these, hypothetical sequences can be written which lead to the endproducts in a plausible manner. Rather than speculate on the merits of these alternatives, we prefer to await the outcome of further experimen

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