second product (III) was isolated from the above reaction but could be obtained in better yield as described below.

Hydrogenation of vindoline (PtO_2/EtOH–HCl) afforded dihydrovindoline, $C_{25}H_{34}O_6N_2$, (m.p. 121–124° or 164–166°, pK_a' 5.9, no vinyl protons but four new protons at 1.5δ in the n.m.r., $[\alpha]^{25}D$ +52.4° [C = 1, $CHCl_3$]) which could be converted to an amorphous hygroscopic hydrochloride. Pyrolysis of this salt at 195–200° in vacuum gave a distillate from which the above second "soda lime" compound was obtained by direct crystallization from hexane in 15% over-all yield, $C_{21}H_{28}O_2N_2$, m.p. 130–132°, pK_a' 5.35, $[\alpha]^{25}D$ +12° (C = 1, $CHCl_3$), mol. wt. 340. The methoxydihydroindole portion of this compound was the same as in vindoline and the second oxygen was found to be present as a ketonic carbonyl ($\lambda_{max.}^{CHCl_3}$ 5.85 μ).

Careful comparison of the mass spectra of the pyrolysis ketone (III) and dihydrovindoline with that of N-methyl desacetyl aspidospermine[11] indicated the presence of the latter ring system in vindoline and its derivatives, since in all three compounds intense peaks were found at *m/e* 124, 174, 188 and 298.

The position of the carbonyl in III was assigned on the basis of the presence of the peak at *m/e* 298[11] (= M-42) which suggested that this group involves either C-3 or C-4. Position 4 was selected for two reasons: the typical ABX pattern at low field (3 to 3.5δ) in the n.m.r. spectrum (also consistent with structure III) indicated three protons between the carbonyl group and nitrogen; and equilibration with CH_3OD/methoxide resulted in the introduction of only two deuterium atoms per molecule (mol. wt., 342). The peak at *m/e* 124 comprises the atoms outlined by heavy lines in structure III while the 174 and 188 peaks contain the dihydroindole moiety (as indoles) with one or two carbons, respectively, of the "tryptophan" dimethylene bridge attached. The fragment of mass 298 involves the loss of the two-carbon bridge (C-3 and C-4) containing the oxygen functions (except the aromatic methoxyl) of each derivative. The only other major peak in the ketone occurs at *m/e* 166 which shifts to *m/e* 168 in the deuterio derivative. Structure IV is assigned to it since this peak corresponds to fragment V of mass 152[12] in the spectrum of aspidospermine.[13]

The mass spectral evidence, therefore, besides indicating the ring system of vindoline shows that the oxygen functions other than the aromatic methoxyl can only be present at positions 3 and 4, and therefore must be arranged as shown in structure I to be consistent with n.m.r. data. The formation of the ketone is also explained easily from this formulation (dehydration, hydrolysis, decarboxylation).

It is interesting to note that vindolinine,[8] another *Vinca* alkaloid with a slightly modified aspidospermine skeleton, contains the double bond at the same position[8] as vindoline.

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(11) K. Biemann, M. Friedmann-Spiteller and G. Spiteller, *Tetrahedron Letters*, No. 14, 485 (1961).

(12) K. Biemann, Symposium on Mass Spectrometry, Oxford, September, 1961.

(13) The fragmentation is more complex in dihydrovindoline, giving rise to a series of peaks at *m/e* 398, 311, 284 and 224 which are consistent with the proposed structure but will be discussed in the full paper.

THE BIOSYNTHETIC INCORPORATION OF DOPAMINE INTO HYDRASTINE[1]

Sir:

Recently we showed[2] that hydrastine (III) is derived from two tyrosine units, which, however, are not incorporated with equal efficiency. This was the first demonstration of differential utilization of a single precursor in the biosynthesis of the two segments of a "dimeric" alkaloid.[3]

This differential utilization of tyrosine implies that the "doubling" step of the anabolic sequence is preceded by one or more dissimilar structural modifications of either or both tyrosine units. It is likely that tyrosine gives rise by independent pathways to two different "monomeric" metabolic intermediates which join to form a "dimeric" precursor from which the alkaloid is then derived.

We have now found that 3,4-dihydroxyphenylethylamine (dopamine) can serve as a precursor of one, but not of the other, of these "monomeric" intermediates.

α-^{14}C-Dopamine hydrobromide (II) was infused by means of cotton wicks into the stems of six three-year old plants of *Hydrastis canadensis* L. In a simultaneous separate experiment, DL-β-^{14}C-tyrosine (I) was administered similarly to four three-year old plants. After nine days the plants were harvested and the alkaloids extracted from the roots. Chemical and radioactive yields are recorded in Table I.

Degradation of hydrastine was carried out as previously reported.[2] The activities of radiodopamine-derived hydrastine and its degradation products (Table II) clearly demonstrate that radiocarbon was confined to carbon atom 3, and show that dopamine serves as a specific precursor of hydrastine, *but that only one dopamine unit is utilized in the biosynthesis of the alkaloid.* Hydrastine isolated from plants fed with radiotyrosine, on the other hand, was derived from two tyrosine units.[4]

(1) Financial support by the National Research Council of Canada and by the Ontario Research Foundation is gratefully acknowledged.

(2) J. R. Gear and I. D. Spenser, 140th A.C.S. Meeting, Chicago, September, 1961, Abstracts; *Nature* (London) **191**, 1393 (1961).

(3) Similar results have since been obtained in a study of the biosynthesis of morphine from carbon-14-dioxide (H. Rapoport, N. Levy and F. R. Stermitz, *J. Am. Chem. Soc.*, **83**, 4298 (1961).

(4) Since specific incorporation of radioactivity from α-^{14}C-tyrosine into carbon atoms 1 and 3 of the hydrastine molecule already has been demonstrated,[3] it was sufficient in the present case to separate the two labelled carbon atoms (4 and 7'), derived from β-^{14}C-tyrosine, from one another, and unnecessary to isolate them completely.

TABLE I

Precursor: Activity	Products: Hydrastine: Weight, g.	Hydrastine: Specific activity counts/min./mM.	Hydrastine: Specific activity with respect to segment A., counts/min./mM.	Hydrastine: Specific radiochemical yield with respect to segment A[a]	Berberine: Weight, g.	Berberine: Specific activity, counts/min./mM.	Berberine: Specific radiochemical yield with respect to segment A, counts/min./mM.
DL-β-^{14}C-Tyrosine i. 13.9×10^8 ii. 2.15×10^7	0.424	5.76×10^4	3.28×10^4	2.36×10^{-3}	0.818	8.90×10^4	3.65×10^{-3}[b]
α-^{14}C-Dopamine i. 6.12×10^8 ii. 2.16×10^7	0.555	0.87×10^4	0.87×10^4	1.42×10^{-3}	0.825	4.50×10^4	7.35×10^{-3}[c]

[a] Specific radiochemical yield with respect to segment A = $\frac{\text{specific activity of product with respect to segment A}}{\text{specific activity of precursor}} \times 100$.

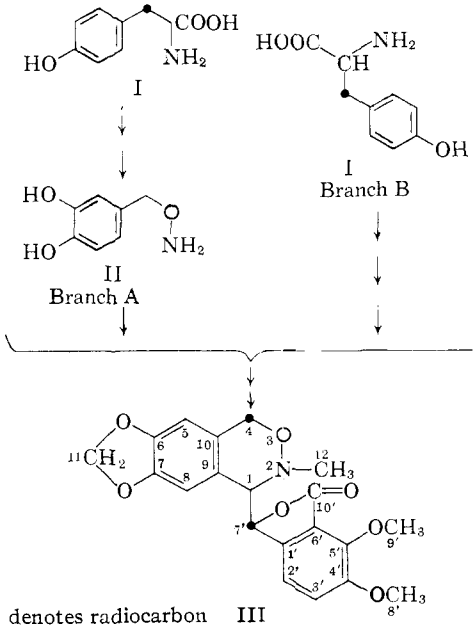
[b] Specific activity of berberine with respect to segment A was arbitrarily taken as 57% of total specific activity on the assumption that berberine is derived in a manner analogous to hydrastine. [c] Specific activity with respect to segment A was calculated on the assumption that, by analogy with hydrastine, radioactivity in berberine was confined to carbon atom 3.

TABLE II

Compound	Atoms of hydrastine	α-^{14}C-Dopamine experiment: Specific activity, counts/min./mM.	α-^{14}C-Dopamine experiment: Relative activity	β-^{14}C-Tyrosine experiment: Specific activity, counts/min./mM.	β-^{14}C-Tyrosine experiment: Relative activity
Hydrastine		0.87×10^4	100	5.76×10^4	100
Hydrastinine	1–12	0.87×10^4	100	3.28×10^4	57
Opianic acid	1′–10′	0.84×10^2	1	2.46×10^4	43
Hydrastal	1,3–11	0.83×10^4	96	3.39×10^4	59
6-Vinylpiperonylic acid	1,3–11	0.87×10^4	100		
6-Bromo-α-hydroxyhomopiperonyl bromide	3–11	0.89×10^4	102		
Benzoic acid	1	0	0		
6-Bromopiperonylideneacetone[a]	4–11	0.15×10^3	2		

[a] The homopiperonyl bromide derivative was oxidized to 6-bromopiperonal, which was converted to 6-bromopiperonylideneacetone.

Our earlier work[2] had indicated that hydrastine is synthesized in the plant by a branched metabolic route, one branch (A) giving rise to atoms 3–10, the other (B) to atoms 1, 1′–7′. The present results confirm that tyrosine serves as a precursor common to both branches[5] and indicate further that dopamine is associated with branch A only. Since no trace of activity was found in carbon atom 1 of the dopamine-derived hydrastine, branch A includes an irreversible step.



O,● denotes radiocarbon III

It is not possible to decide from the present experiments whether dopamine is an obligatory stage of branch A, whether it is the "monomeric" intermediate of this branch involved in the doubling step, or whether it merely serves as an alternative source of a true intermediate in this branch.

If dopamine were indeed on the direct route from tyrosine to hydrastine, the specific radiochemical yield with respect to segment A of hydrastine should be higher on feeding dopamine than on feeding tyrosine. Table I shows that this was not so. On the assumption, yet to be proven, that berberine is derived in a manner analogous to hydrastine, this yield calculated for berberine derived from dopamine is twice that calculated for berberine derived from tyrosine. Taken at face value these results would appear to indicate that dopamine lies on the route from tyrosine to berberine, but that it is not an obligatory intermediate in the biosynthesis of hydrastine. It would be premature, however, to draw such definitive conclusions

Clarification of this apparent anomaly must await the systematic degradation of the radio-berberine, and an investigation of the metabolic inter-relationship of the two alkaloids.

(5) It has yet to be shown whether tyrosine itself lies at the point of metabolic branching or whether it gives rise to another compound (*e.g.*, dopa) which disproportionates.

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