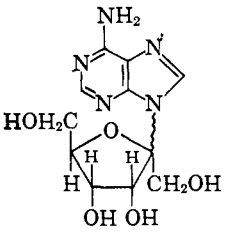
Table I. The Distribution of Carbon-14 in Psicofuranine

Expt.	Compd. added	% C^{14} in Adenine	% C^{14} in D-Psicose
1	Adenosine-U-C^{14}	98.0	2.0
2	Adenosine-U-C^{14}	86.0	14.0
3	Formate-C^{14}	99.5	0.5
4	Glucose-1-C^{14}	5.0	95.0
5	Glucose-6-C^{14}	25.0	75.0

Table II. Distribution of Carbon-14 in D-Psicose from Glucose-1-C^{14} and Glucose-6-C^{14}

Derivative	Carbon atoms	Glucose-1-C^{14}: C.p.m./mmole	Glucose-1-C^{14}: % C^{14}	Glucose-6-C^{14}: C.p.m./mmole	Glucose-6-C^{14}: % C^{14}
Psicosazone	1,2,3,4,5,6	52,200	100	4,580	100
Mesoxaldehyde-1,2-bisphenylhydrazone	1,2,3	42,000	81	0	0
Formaldimedone	6	5,000	10	4,020	88

communication reports the results on the incorporation of adenosine-U-C^{14}, formate-C^{14}, glucose-1-C^{14}, and glucose-6-C^{14} into the adenine and D-psicose of psicofuranine (6-amino-9-D-psicofuranosylpurine, I). The preparation of adenosine-U-C^{14} was reported earlier.[4] The labeled precursors were added to growing cultures



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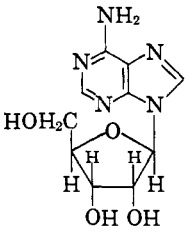

II

of *Streptomyces hygroscopicus*[8] two days after inoculation. Three days later, when the production of psicofuranine reached a maximum, the nucleoside was isolated.[9] Final purification of psicofuranine was achieved by paper chromatography. The chromatograms were developed in 1-butanol–3% ammonia (86:14). The psicofuranine was eluted and hydrolyzed.[10] After hydrolysis, the insoluble adenine sulfate was removed by filtration. Barium carbonate was added to the filtrate to remove the sulfuric acid. The mixture was filtered and the remaining adenine was removed by adsorption onto Dowex-50-H^+. The D-psicose which remained in solution was concentrated to a sirup at 60° with nitrogen. The distribution of carbon-14 in the psicofuranine is shown in Table I.

The distribution of radioactivity in the adenine-ribose of the adenosine-U-C^{14} used in these experiments was 40 and 60%, respectively. The per cent ratio of the radioactivity in the adenine-psicose moieties of psicofuranine was not the same (expt. 1 and 2, Table I). These data indicate that adenosine (II) does not serve as a direct precursor in the biosynthesis of psicofuranine. The lack of incorporation of formate-C^{14} into D-psicose supports the idea that psicofuranine is not derived directly from adenosine and a C_1 unit (as formate or formaldehyde). Although formate-C^{14} was incorporated into psicofuranine, 99.5% of the radioactivity resided in the adenine. This incorporation of formate is in agreement with the *de novo* synthesis of purine nucleotides. The fact that adenine-U-C^{14} was incorporated to a greater extent than was the D-ribose-U-C^{14} from the adenosine-U-C^{14} experiments (Table I) is taken as evidence that the purine moiety of psicofuranine arises from adenine. Both glucose-1-C^{14} and glucose-6-C^{14} were incorporated into psicofuranine. Most of the radioactivity resided in the D-psicose. To determine if the glucose were the direct precursor of D-psicose, this ketohexose from the glucose experiments was converted to the osazone[11] and degraded[12] to determine the distribution of radioactivity. The results of these studies are shown in Table II.

These findings indicate that the ketohexose D-psicose is arising from glucose or, more likely, a nucleotide–hexose intermediate.

Preliminary studies in our laboratory on the acid-soluble extracts from the mycelia from the glucose-1-C^{14} and glucose-6-C^{14} experiments show that a considerable amount of the radioactivity is retained on a Dowex-1-formate column. Studies are in progress to determine the nature of the radioactive intermediates in order to elucidate the mechanism by which glucose is converted to D-psicose by *S. hygroscopicus*.

(8) Kindly supplied by Dr. G. M. Savage, Microbiology Department, The Upjohn Company, Kalamazoo, Mich.
(9) H. Yüntsen, H. Yonehara, and H. Ui, *J. Antibiot.* (Tokyo), **7A**, 113 (1954).
(10) W. Schroeder and H. Hoeksema, *J. Am. Chem. Soc.*, **81**, 1767 (1959).
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(12) Y. J. Topper and A. B. Hastings, *J. Biol. Chem.*, **179**, 1255 (1949).
(13) Research Career Development Awardee of the United States Public Health Service (5-K3-GM-7100-04).

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Biosynthesis of Berberastine[1]

Sir:

A new alkaloid, berberastine (II), was recently isolated[2] in minute quantity from extracts of *Hydrastis canadensis* L. The structure assigned[2] to berberastine differs from that of berberine, the major alkaloid of *H. canadensis*, by an additional hydroxyl group located at a site which in the biosynthesis of berberine is specifically derived from the benzylic carbon atom of dihydroxyphenylethylamine (dopamine).[3]

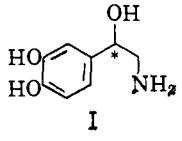
(1) Financial support by the National Institute of General Medical Sciences, U. S. Public Health Service (Grant No. GM-10043) and by the National Research Council of Canada is gratefully acknowledged.
(2) M. M. Nijland, *Pharm. Weekblad*, **98**, 301 (1963).
(3) I. Monković and I. D. Spenser, *Proc. Chem. Soc.*, 223 (1964).

Table I. Chemical and Radiochemical Yields

Isolated alkaloids	1-^{14}C-Dopamine hydrobromide[a] Yield, mg.	Sp. act., c.p.m./mmole × 10^{-4}	SRY[c] × 10^4	% incorpn.[d]	DL-2-^{14}C-Noradrenaline bitartrate[b] Yield, mg.	Sp. act., c.p.m./mmole × 10^{-4}	SRY[c] × 10^4	% incorpn.[d]
Berberastine	3	14.2 ± 0.34	65.2	0.0024	6	396 ± 6.7	894	0.10
Canadine	21	12.4 ± 0.14	56.7	0.021	24	0.22 ± 0.003	0.49	0.00032
Berberine	945	4.62 ± 0.03	21.2	0.29	895	0.073 ± 0.005	0.16	0.0033
Hydrastine	594	0.31 ± 0.02	1.42	0.013	600	0.028 ± 0.002	0.062	0.00088

[a] 3.90 mg., sp. act. 2.18 (± 0.03) × 10^9 c.p.m./mmole. [b] 3.94 mg., sp. act. 4.43 (± 0.06) × 10^9 c.p.m./mmole. [c] Specific radiochemical yield = (specific activity of product)/(specific activity of precursor) × 100. [d] % incorporation = (total activity of product)/(total activity of precursor) × 100. Limits are standard deviation of the mean.

We now present evidence which shows that berberastine is not derived from berberine, and which suggests that the benzylic hydroxyl group is generated at an early stage of biosynthesis, before formation of the benzylisoquinoline skeleton.



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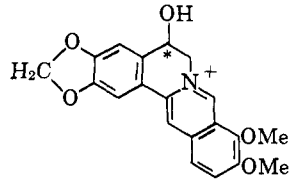

II

In separate feeding experiments 1-^{14}C-dopamine and DL-2-^{14}C-noradrenaline (I) were administered to plants of *H. canadensis* L. Berberine, hydrastine, canadine, and berberastine were isolated in each case and rigorously purified by column chromatography and crystallization. Chemical and radiochemical yields are given in Table I. Every one of the four alkaloids derived from the dopamine experiment was labeled. The noradrenaline experiment gave radioactive berberastine of high specific activity. Berberine, canadine, and hydrastine derived from noradrenaline-fed plants, on the other hand, were found to be almost devoid of radioactivity after they had been subjected to persistent chromatography which removed unweighable amounts of high-counting impurities, probably hydroxycanadine and hydroxyhydrastine.

The noradrenaline-derived berberastine was diluted with carrier and degraded. Radioactive carbon was shown to be confined to the predicted site. The thirteen-step degradation involved conversion of berberastine to berberine,[2] degradation of berberine to 6-bromopiperonylic acid[4] of a specific activity identical with that of the original alkaloid, and finally conversion of the acid to inactive 2-bromo-4,5-methylenedioxyaniline. Thus noradrenaline is incorporated into berberastine without randomization of activity.

We have previously shown that dopamine serves as a specific precursor of hydrastine[4] and berberine.[3] The present evidence indicates that it is incorporated also into canadine and berberastine. Since the specific activity of dopamine-derived berberastine was found to be substantially higher than that of the berberine isolated from the same experiment berberine cannot be a precursor of berberastine. Since noradrenaline is specifically incorporated into berberastine, but does not enter the other bases, canadine and berberine cannot be derived from berberastine, nor are they intermediates of the route from noradrenaline to berberastine. Also excluded as a stage between noradrenaline and berberastine is norlaudanosoline, whose O- and N-methyl derivatives have been shown to serve as specific precursors of berberine,[5] and which is generally regarded as the first "dimeric" intermediate[6] in the biosynthesis of berberine and all related alkaloids. If norlaudanosoline were an intermediate in the noradrenaline–berberastine conversion, labeled rather than unlabeled berberine would have been isolated from noradrenaline-fed plants. If noradrenaline is an obligatory stage of the pathway to berberastine, intermediacy of a hydroxynorlaudanosoline must be postulated.

The question arises whether the noradrenaline–berberastine conversion represents the normal pathway or an instance of aberrant biosynthesis. The natural occurrence of noradrenaline is not limited to mammalian tissues. The compound has been found in a number of plants[7] and a dopamine-β-hydroxylase preparation has been obtained from a plant source.[8] Noradrenaline has not hitherto been detected in *H. canadensis*, however, and we have not yet been able to demonstrate by isotope dilution the presence of noradrenaline in dopamine-fed *Hydrastis*. Even though it is tempting to regard the specific incorporation of noradrenaline into berberastine and the high specific radiochemical yield in this conversion as indicators of a normal biosynthetic pathway, judgment must be reserved until further evidence is available.

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Received December 21, 1964

Mechanistic and Exploratory Photochemistry. XII. Characterization of the Reactivity of the β-Carbon in the Triplet Excited State of α,β-Unsaturated Ketones[1]

Sir:

In previous publications[2a-d] we have discussed the mechanisms of ketone photochemical reactions. We

(1) Presented in part at the 10th Reaction Mechanisms Conference, Corvallis, Ore., June 1964.

(2) (a) Paper XI: H. E. Zimmerman and L. Craft, *Tetrahedron*