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BIOSYNTHESIS OF EPHEDRINE IN EPHEDRA

PARTICIPATION OF C_6-C_1 UNIT

Kazuo Yamasaki, Ushio Sankawa and Shoji Shibata

Faculty of Pharmaceutical Sciences, University of Tokyo, Japan

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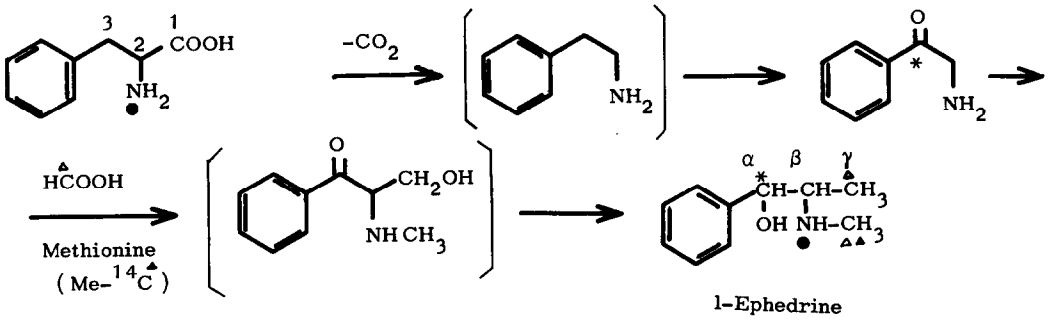
In the previous studies on the biosynthesis of ephedrine in Ephedra distachya L. (Ephedraceae) Shibata et al.[1-4] described that ^{15}N of labelled phenylalanine was incorporated into nitrogen of l-ephedrine and ^{14}C-methyl group of methionine into N-methyl group, while ^{14}C of formate was distributed 42% in N-methyl and 37% in C-methyl (at the γ-position of side chain), and carbonyl-^{14}C of ω-aminoacetophenone was highly incorporated into the α-position of ephedrine side chain.

On the basis of these results, the following scheme was proposed for the biosynthesis of l-ephedrine in Ephedra plant.



l-Ephedrine

However, our renewed experiments revealed that phenylalanine labelled at 2-^{14}C was not incorporated into l-ephedrine, whereas aromatic ^{3}H and 3-^{14}C were introduced in it.

The location of radioactivity was proved by the degradation of l-ephedrine by Kuhn-Roth oxidation into benzoic acid and acetic acid. The latter was characterized as the p-bromophenacyl ester.

Table I

Incorporation of DL-Phenylalanine into l-Ephedrine

Labelled position	Incorporation ratio	Specific activities of degradation products (100 = Specific activity of l-ephedrine)	
		Benzoic acid (ϕ-C_α)	Acetic acid (C_β-C_γ)
Aromatic-^{3}H	2.9×10^{-4}	--- *	---
2-^{14}C	nil	---	---
3-^{14}C	1.4×10^{-4}	---	---
Aromatic-^{3}H	5.3×10^{-4}	106	---
2-^{14}C	4.0×10^{-5}	---	---
Aromatic-^{3}H	8.5×10^{-4}	---	---
3-^{14}C	8.4×10^{-4}	100	nil
2-^{14}C, 3-^{14}C	8.2×10^{-5}	92	nil

* --- not measured

These results suggest that phenylalanine fed to the plant is cleaved between $C_{(2)}$ and $C_{(3)}$, and only C_6-C_1 portion is used for the biosynthesis of l-ephedrine. Then sodium benzoate (Carboxyl-^{14}C), benzaldehyde(Carbonyl-^{14}C) and sodium cinnamate-(3^{14}C) were administrated to Ephedra. All these labelled compounds were incorporated in higher ratios than phenylalanine into the corresponding positions of ephedrine molecule.

Table II

Incorporations of Benzoate, Benzaldehyde and Cinnamate into l-Ephedrine

Precursors	Incorporation ratio	Specific activities of degradation products (100 = Specific activity of l-ephedrine)	
		Benzoic acid (ϕ-C_α)	Acetic acid (C_β-C_γ)
Benzoate (Carboxyl-^{14}C)	1.3×10^{-1}	104	nil
Benzaldehyde(Carbonyl-^{14}C)	1.6×10^{-2}	96	nil
Cinnamate(3-^{14}C)	1.2×10^{-3}	100	nil

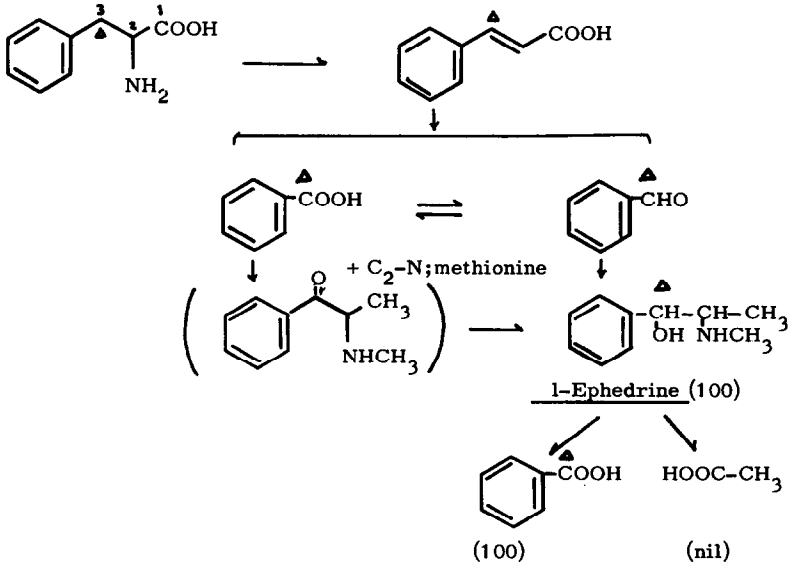
Mescaline in Cactus, hordenine in germinating barley and epinephrine in the medulla of the adrenals were experimentally proved to be biosynthesized from tyrosine via C_6-C_2-N intermediates. In contrast with them, ephedrine is biosynthesized from C_6-C_1 and C_2-N(+C_1) units.

The existence of benzylmethylamine was reported[5] in Ephedra, which would suggest the possibility of occurrence of C_6-C_1 intermediate.

The incorporation of phenylalanine-(3-^{14}C) into d-norpseudoephedrine in Catha edulis reported by Leete[6] is not incompatible with our new scheme of ephedrine biosynthesis.

Ephedrine and its homologues contained in Ephedra plants have S-configuration about the carbon atom at 3 -position of the side chain. Contrary to this, chloramphenicol has R-configuration in respect to the corresponding position and it is biosynthesized from L-p-aminophenylalanine without decarboxylation.[9] If L-serine or L-alanine are combined with benzoic acid or benzaldehyde, the configuration of the β-carbon atom must be S. A remarkable distribution (37 %) of the radioactivity of formate-^{14}C into the γ-carbon of ephedrine, which was shown in the previous experiment[3] could be explained by the contribution of formate via serine. The feeding experiment using L-serine(U-^{14}C) and L-alanine(U-^{14}C) showed poor incorporation into ephedrine (5.4×10^{-3}, and 7.4×10^{-4}%, respectively) and randomization of radioactivity giving no conclusive evidence for the origin of C_2-N unit. However, this cannot exclude the above possibility, since similar example of the formate incorporation was observed at the tryptophan moiety of evodiamine, a Rutaceous alkaloid[7].

The incorporation of ω-aminoacetophenone(Carbonyl-^{14}C) into ephedrine can be explained by its unstable nature decomposing into C_6-C_1 unit, and the incorporation of ^{15}N-labelled phenylalanine would be resulted by transamination. By the present experimental results the scheme of ephedrine biosynthesis proposed previously has to be amended, and the following pathway is now presented:



A biogenetical hypothesis involving the condensation of benzaldehyde and N-methylalanine was suggested by Akabori and Momotani[8] on the basis of their chemical synthesis of dl-pseudo-ephedrine.

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