the method reported in the preceding paper.*1 Yield, 56 mg. (45%). *Anal.* Calcd. for $C_{12}H_{10}O_4$ : C, 66.05; H, 4.62. Found : C, 66.03; H, 4.72.

**4,5-Dihydroxy-2-hydroxymethyl-1-methylnaphthalene (Terranaphthol) (Ib)**—Terranaphthol was obtained from 100 mg. of (XIVb) using the method described for (Ia), except that dehyd. $Et_2O$ was used as a solvent in place of tetrahydrofuran; m.p. 170~172°. This compound (Ib) was converted to its triacetate (Ic) by treating (Ib) with a mixture of 0.2 cc. of pyridine and 0.2 cc. of $Ac_2O$ at room temperature. The crude triacetate was recrystallized from cyclohexane, giving a slightly yellow powder of m.p. 142~143°. A sample for analysis was distilled at 0.001 mm. in an oil bath (150~200°), and recrystallized from cyclohexane to colorless crystals, m.p. 143.2~144°, showing no depression when mixed with a sample of terranaphthol triacetate, m. p. 145.5~146°, prepared from oxytetracycline (II). *Anal.* Calcd. for $C_{18}H_{18}O_6$ : C, 66.44, H, 5.49. Found : C, 65.85; H, 5.50.

## Summary

The structure of terranaphthol, a degradation product of oxytetracycline, was identified as 4,5-dihydroxy-2-hydroxymethyl-1-methylnaphthalene (Ib) by its synthesis through the Hauser rearrangement of 4,5-dimethoxy-1-dimethylaminomethylnaphthalene methiodide (VIIb).

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## 82. Shoji Shibata,*3 Izumi Imaseki,*4 and Mikio Yamazaki*3 : Phytochemical Investigation on Cultivation of Medicinal Plants. XV.*1 The Biogenesis of Ephedrine in Ephedra. (5).*2

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Previously, it was shown that *l*-ephedrine in *Ephedra distachya* L. is biosynthesized from phenylalanine using $^{15}N$-labeled tracer technique,[1] and that addition of one carbon fragment occurs during the process of biosynthesis after decarboxylation of the amino acid.[2] A biological methylation with methionine(*methyl*-$^{14}C$) was found to form N-$^{14}CH_3$ group in ephedrine molecule.[3]

Recently, after completion of the present work, it was learned that Leete[4] obtained an evidence for the incorporation of DL-phenylalanine(3-$^{14}C$) into *d*-norpseudoephedrine in the leaves of *Catha edulis* FORSK., which supports these previous results.

The present paper concerns chiefly with an investigation undertaken to elucidate in detail the intermediate process of ephedrine biosynthesis.

ω-Aminoacetophenone(*carbonyl*-$^{14}C$) was fed to the intact Ephedra plant and the labeled ephedrine isolated from the plant was degraded to determine the location of radioactivity.

ω-Aminoacetophenone was reported as being synthesized from acetophenone by bro-

*1 Part XIV : This Bulletin, **5**, 594(1957).
*2 (4). *Ibid.*, **5**, 594(1957). cf. Chem. & Ind. (London), **1958**, 1625.
*3 Hongo, Tokyo (柴田承二, 山崎幹夫).
*4 Kamimeguro 8-chome, Meguro-ku, Tokyo (今関和泉).
1) S. Shibata, I. Imaseki : This Bulletin, **4**, 277(1956).
2) S. Shibata, I. Imaseki, M. Yamazaki : *Ibid.*, **5**, 594(1957).
3) *Idem.* : *Ibid.*, **5**, 71(1957).
4) E. Leete : Chem. &. Ind. (London), **1958**, 1088.

mination followed by amination,[5] or via oxime-tosylate by rearrangement on detosylation.[6]

These methods, however, seemed not to be suitable for preparation of $^{14}C$-labeled $\omega$-aminoacetophenone. Because of the availability of $^{14}C$-labeled starting material and ease in treating radioactive intermediate products, $\omega$-aminoacetophenone(*carbonyl*-$^{14}C$) was prepared from glycine(*carboxyl*-$^{14}C$) by the reactions shown in Chart 1.

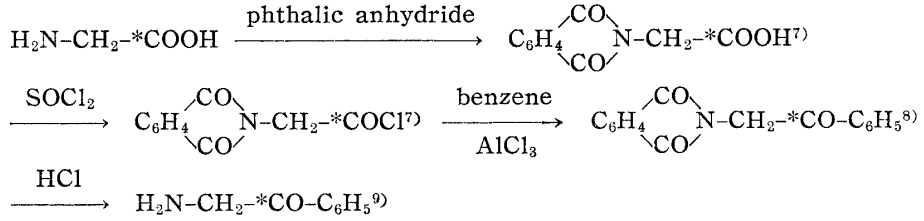


Chart 1.

## Experimental

**Plant Material**—*Ephedra distachya* L., cultivated in the Medicinal Plant Garden attached to the Tokyo University Forestry Experimental Station in Chiba Pref., was lifted from the ground on June 9, 1958, and the experiment was started from the next day.

The plant material (ca. 350 g. each) was dipped in the medium (500 cc.) by its roots, which were surrounded with glass wool, and 5 pots were used for the present experiment.

The cultivation medium employed was the Houghland solution added with $\omega$-aminoacetophenone-(*carbonyl*-$^{14}C$), non-labeled Na formate, and DL-methionine.

Table I. Components of Hydroponic Solution

| | | | |
|---|---|---|---|
| $KNO_3$ | $5\times10^{-3}M$ | $\omega$-Aminoacetophenone | $1\times10^{-3}M$ |
| $KH_2PO_4$ | $1\times10^{-3}$ | | $3.83\times10^5$ cpm/m$M$ (specif. activity)<br>$9.57\times10^5$ cpm (total activity) |
| $Ca(NO_3)_2\cdot4H_2O$ | $5\times10^{-3}$ | HCOONa | $3\times10^{-4}$ |
| $MgSO_4\cdot7H_2O$ | $2\times10^{-3}$ | DL-Methionine | $4.5\times10^{-4}$ |
| Minor elements | Trace | | |
| | | in 2.5 L. distilled water; pH 6.2 | |

The hydroponic cultivation lasted for 1 week under lightning in a draught chamber.

The grassy portion of the plant material (fresh wt., 830 g.; dry wt., 306 g.) was extracted by the procedure given in the previous paper to obtain *l*-ephedrine hydrochloride. Yield, 475 mg. or 0.13% of dry wt.

**Synthesis of $\omega$-Aminoacetophenone(*carbonyl*-$^{14}C$)—Phthalimidoacetic Acid(*carboxyl*-$^{14}C$)**—Radioactive glycine(1-$^{14}C$) (39.7 mg., 0.5 mc.), which was added with the carrier, non-labeled glycine (500 mg.), was mixed with phthalic anhydride (1 g.), and the mixture was heated in an oil bath at 160~170°, when it melted and then resolidified to form crude phthalimidoacetic acid. The product was recrystallized from water (70 cc.) to form colorless needles, m.p. 193°. Yield, 1.06 g. (67.9%).

**Phthalimidoacetyl Chloride(COCl-$^{14}C$)**—Dried and powdered phthalimidoacetic acid(*carboxyl*-$^{14}C$) was mixed with $SOCl_2$ (5 cc.) and heated in an oil bath at 60° for 3 hr. The excess of $SOCl_2$ was removed *in vacuo* from the reaction mixture. $CHCl_3$ was added and distilled off repeatedly to remove $SOCl_2$ completely. Purified phthalimidoacetyl chloride obtained by recrystallization from benzene and petr. ether was colorless needles, m.p. 84°.

**$\omega$-Phthalimidoacetophenone(*acetophenone-carbonyl*-$^{14}C$)**—Phthalimidoacetyl chloride was used without purification. It was dissolved in dehyd. benzene (5 cc.) and added with $AlCl_3$ (1.5 g.). The mixture was heated in an oil bath at 60° for 2 hr. when HCl gas evolved vigorously, which was trapped cautiously. The mixture was boiled for another 30 min. at 80°, when the reaction completed, and dil. HCl (5 cc.) and water (5 cc.) were cautiously added to the reaction mixture. The benzene layer was separated and the aqueous layer was extracted repeatedly with benzene. From the combined benzene solution, the solvent was distilled off *in vacuo* and the residue was recrystallized from glacial

5) C. Goedeckemeyer: Ber., **21**, 2685(1888).
6) S. Tatsuoka, K. Osugi, A. Miyake, M. Honjo, R. Nakamori: Yakugaku Zasshi, **71**, 778(1951).
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8) S. Gabriel: Ber., **40**, 2649(1907).
9) C. Mannich, F.L. Hahn: Ber., **44**, 1546(1911); cf. Footnote (5).

AcOH (ca. 6 cc.) to plates, m.p. 168°.

**ω-Aminoacetophenone(*carbonyl*-$^{14}$C)**—ω-Phthalimidoacetophenone labeled with $^{14}$C prepared as above was added with a mixture of conc. HCl (6 cc.), glacial AcOH (3 cc.), and water (3 cc.), and the mixture was refluxed in an oil bath for 5 hr. After the solid mass completely dissolved, the solvent was partly distilled off and the b.p. was constant at 104°. The concentrated solution was heated for another 10 hr. Phthalic acid which separated out after cooling was collected and washed with water. The filtrate combined with washings was evaporated *in vacuo* to leave a white residue, which was recrystallized from 98% EtOH to ω-aminoacetophenone(*carbonyl*-$^{14}$C), m.p. 185°. Yield, 430 mg. (37.3% of theor. amount calcd. from glycine).

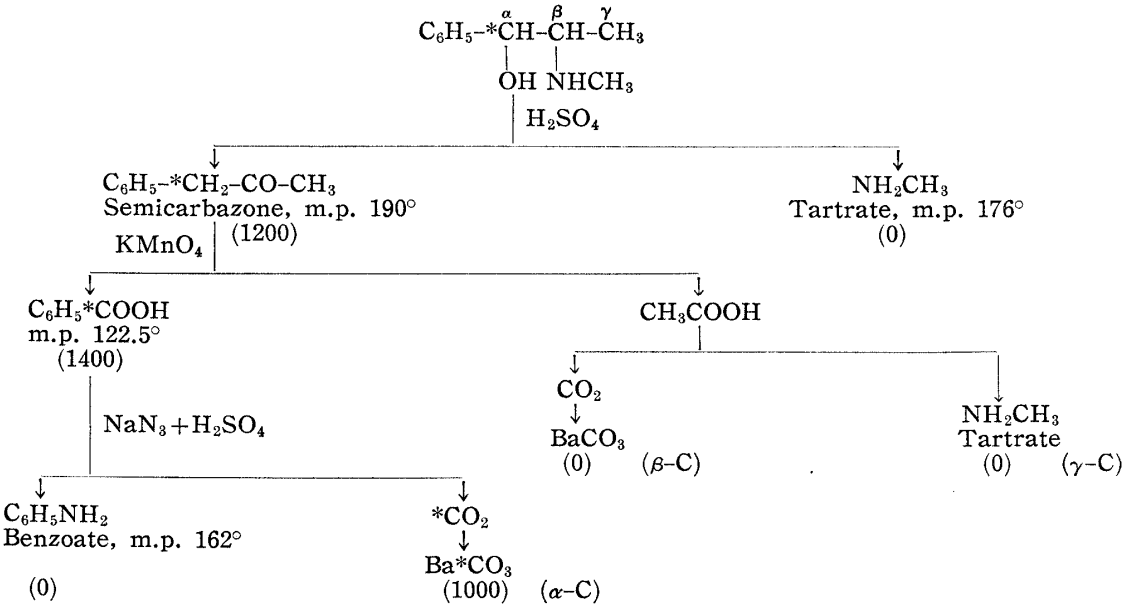
**Degradation of *l*-Ephedrine**—*l*-Ephedrine hydrochloride isolated from the plant was degraded as described in a previous paper.[2] *l*-Ephedrine hydrochloride (400 mg.) was heated with 83% $H_2SO_4$ (2.0 g.) for 8 hr. to obtain a methylamine which was purified as its tartrate, m.p. 176° (yield, 180 mg. or 47%), and a methyl benzyl ketone, which was concerted into its semicarbazone, m.p. 190°.

On oxidation with alkaline $KMnO_4$, methyl benzyl ketone yielded BzOH, m.p. 122.5° (yield, 100 mg. or 55%), and AcOH. BzOH was degraded by the Schmidt reaction to give aniline (characterized as benzoate, m.p. 162°; yield, 20%) and $CO_2$ (as $BaCO_3$; yield, 86%).

The AcOH portion was dissolved in a little amount of ice water and BzOH which separated out was removed. After repeated treatment as above, AcOH was cleaved by the Schmidt reaction. $CO_2$ liberated below 45° was removed and that obtained at 45~65° was absorbed in $Ba(OH)_2$ solution to form $BaCO_3$ (yield, 63 mg.). Methylamine which was formed by the same reaction was characterized as its tartrate (yield, 18 mg.).

## Results and Consideration

The determination of radioactivity of each fraction was made as described in the previous paper[2] using "Q" gas-flow counter. The labeling pattern thereby obtained is shown in Chart 2.




The figures in parentheses indicate specific activity (cpm/m*M*)

Chart 2.

Although previous observations indicated that phenylalanine is the original precursor of *l*-ephedrine and the methyl in γ-position of the side-chain is formed by one carbon fragment incorporated after decarboxylation of the amino acid, precise evidence for the oxidative stage in forming -CH(OH)- grouping of ephedrine molecule has not yet been obtained.

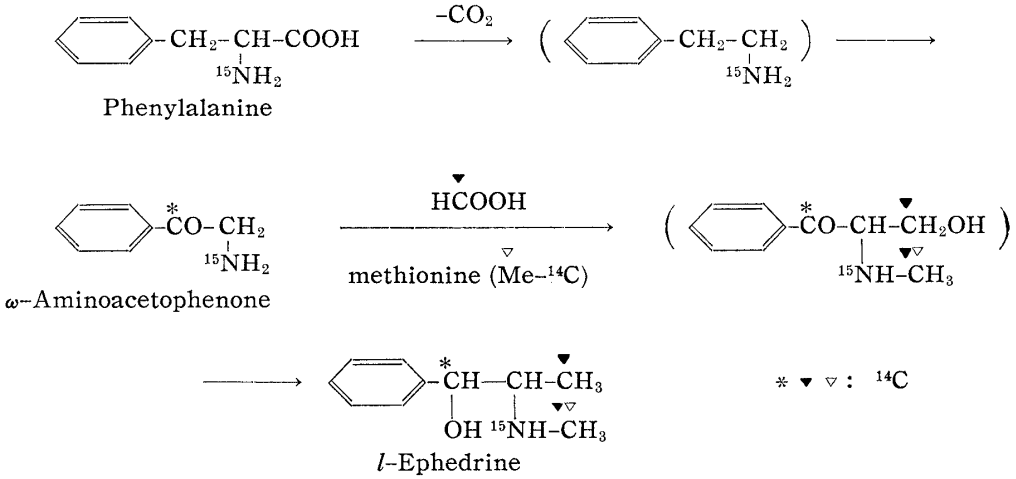
The present experimental result showing that the radioactivity of *l*-ephedrine localizes only in the α-carbon atom of the side-chain has indicated that ω-aminoacetophenone takes part as an intermediate compound in the process of ephedrine biosynthesis.

Similar biosynthetical schemes have been advanced for the formation of Adrenalin (epinephrine) in adrenal gland[10~12] and hordenine in sprouting barley[13~16] for which hydroxytyramine and tyramine, respectively, have been proved as the direct precursor.

Accordingly, the decarboxylation of corresponding amino acid occurs in the first step of biosynthesis. The oxidative stage forming noradrenalin from hydroxytyramine has not yet actually been proved.

It would be worthwhile to note that the incorporation of one carbon fragment by no means occurs in the biosyntheses of epinephrine and hordenine, doth of which possess hydroxyl in the benzene ring in the *para*-position of the side-chain, while it occurs in the case of *l*-ephedrine which possesses no substituent in the *para*-position, and it is quite possible, if it has not been actually confirmed, in the course of biogenesis of chloramphenicol which possesses nitro group in the corresponding position.

All the evidences which have been obtained in the course of these investigations has made it possible to propose the following scheme of biosynthesis for *l*-ephedrine in Ephedra plant.

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## Summary

It was proved that $^{14}C$ of ω-aminoacetophenone(*carbonyl*-$^{14}C$) is incorporated into the α-position of the side-chain of *l*-ephedrine molecule produced by *Ephedra distachya* L. during one week of hydroponic cultivation. The biosynthetic scheme of *l*-ephedrine starting from phenylalanine is discussed.

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16) E. Leete, L. Marion : *Ibid.*, **31**, 126(1953).