[Chem. Pharm. Bull. 14(10) 1108~1112(1966)]

UDC 581.13 : 582.751.9 : 547.94

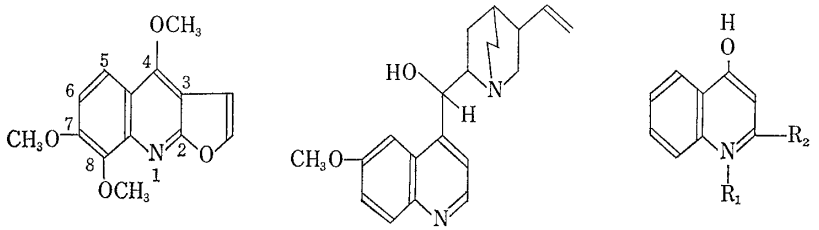
152. Mitsuyoshi Matsuo and Yosihiko Kasida : Biosynthesis of Skimmianine.*1

(*National Institute of Radiological Sciences**2)

Skimmianine (I), one of the furoquinoline alkaloids, was isolated from *Skimmia japonica* THUNB. by Honda in 1904[1] and its structure was elucidated by Asahina, *et al.*[2]

Only a few studies were reported on the biosynthesis of the quinoline alkaloids. Kowanko and Leete demonstrated that the quinoline moiety of quinine (II) was derived from tryptophan,[3] and Luckner and Ritter showed that the 2-*n*-alkyl-4-hydroxyquinolines (III) of *Pseudomonas aeruginosa* (SCHROET.) MIGULA were biosynthesized from anthranilic acid and acetate.[4]

A biogenetic hypothesis has been put forward for the furoquinoline alkaloids, in which dictamnine is expected to be derived from anthranilic acid, acetate and



I II III

mevalonate.[5,6] The present paper describes that the incorporation of anthranilic acid, acetate and tryptophan into skimmianine in *Skimmia japonica*.

TABLE I. Tracer Experiments on *Skimmia japonica*

Precursor	Fresh wt. of plants (g.)	Period (days)	Fed wt. (mg.)	Total act. (μCi)	Sp. act. (mCi/m*M*)	Skimmianine Yield (mg.)	Skimmianine Sp. act. (mμCi/m*M*)	Skimmianine Incorp. ratio[a] (%)	Skimmianine Dilution[b] (%)
Anthranilic acid(T)	235	10	20	39	0.27	89	622	0.54(1.1[c])	0.23(0.46[c])
Sodium acetate(1-^{14}C)	263	10	1.2	100	6.7	80	0.815	0.00025	0.000012
Sodium acetate(2-^{14}C)	284	10	2.7	100	3.0	115	17.0	0.0076	0.00057
DL-Tryptophan(3-^{14}C)	292	10	0.31	50	33.7	120	0.336	0.00031 (0.00062[d])	0.000010

a) Incorporation ratio = $\frac{\text{(Sp. act. of skimmianine, μCi/mg.)} \times \text{(Yield of skimmianine, mg.)} \times 100}{\text{(Total act. of precursor, μCi)}}$

b) Dilution = $\frac{\text{(Sp. act. of skimmianine, mCi/m}M\text{)} \times 100}{\text{(Sp. act. of precursor, mCi/m}M\text{)}}$

c) The value indicates the incorporation ratio calculated except the loss of tritium by substitution of methoxyl groups to the benzene ring of skimmianine.

d) The value considered for only L-form of precursor.

*1 Preliminary communication of this paper : Biochem. Biophys. Res. Commun., **23**, 679 (1966).

*2 9-1, Anagawa, 4-chome, Chiba (松尾光芳, 樫田義彦).

1) S. Honda : Arch. Exp. Pathol. Pharmakol., **52**, 68 (1905).
2) Y. Asahina, *et al.* : Chem. Ber., **63**, 2052, 2057 (1930); Yakugaku Zasshi, **50**, 1133, 1139 (1930).
3) N. Kowanko, E. Leete : J. Am. Chem. Soc., **84**, 4919 (1962).
4) M. Luckner, C. Ritter : Tetrahedron Letters, **1965**, 741.
5) R. Aneja, S.K. Mukerjee, T.R. Seshadri : Tetrahedron, **4**, 256 (1958).
6) T.A. Geissman : "Biogenesis of Natural Compounds," 607 (1963). Edited by P. Bernfeld, Pergamon Press Ltd., Oxford; E. Leete : *Ibid.*, 780 (1963).

Skimmia plants fed with anthranilic acid (T), acetate (1-^{14}C), acetate (2-^{14}C) and DL-tryptophan (3-^{14}C) was extracted to isolate radioactive skimmianine. The incorporation ratios and specific radioactivities of skimmianine were shown in Table I.

The incorporation of anthranilic acid into skimmianine was comparatively higher ratio than those of acetate and tryptophan. The difference of incorporation ratio as shown in the present experiment between acetate (1-^{14}C) and acetate (2-^{14}C) is seemed due to the difference of experimental condition.

If the foregoing hypothesis is correct, the activity of anthranilic acid (T) should be located in the benzene ring and that of acetate (2-^{14}C) at position 3 of quinoline nucleus of skimmianine. The tritium labeled skimmianine derived from anthranilic acid (T) was converted into skimmianal (Ⅳ) and skimmianic acid (Ⅴ) by potassium permanganate oxidation.[2] The methoxyl group of skimmianine was demethylated with hydrogen iodide to separate methyl iodide, which was converted into N-methylphthalimide (Ⅵ). The radioactivties of skimmianal, skimmianic acid and N-methylphthalimide were determined. All the radioactivity of skimmianine was found in skimmianic acid and no radioactivity was observed in N-methylphthalimide.

These results show that the radioactivity of anthranilic acid (T) was located in benzene ring of skimmianine.

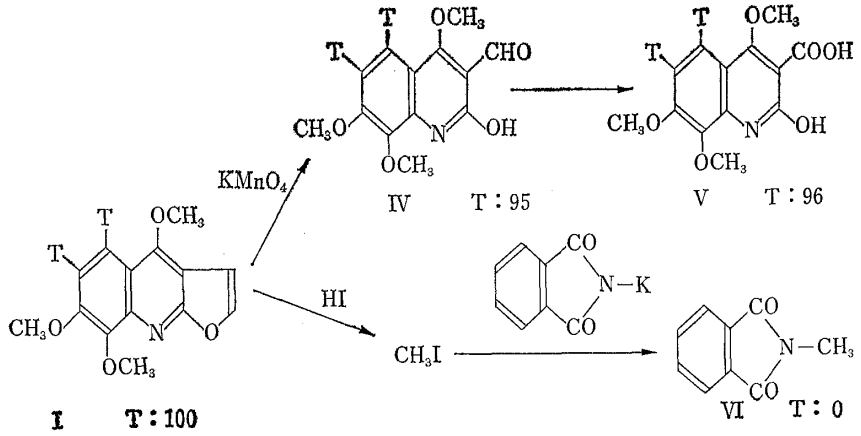
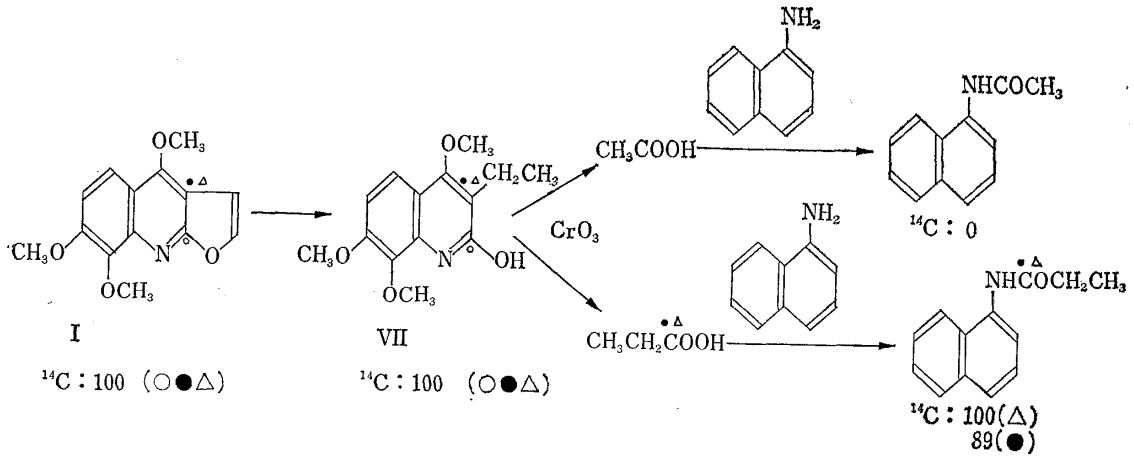


Chart 1. Degradation of Skimmianine derived from Anthranilic Acid (T)



(○) $CH_3{}^{14}COONa$ (•) $^{14}CH_3COONa$ (△) DL-Tryptophan(3-^{14}C)

Chart 2. Degradation of Skimmianine derived from Acetate(1-^{14}C), Acetate(2-^{14}C) and DL-Tryptophan(3-^{14}C)

The radioactive skimmianine obtained by the feeding experiments using acetate (1-^{14}C), acetate (2-^{14}C) and DL-tryptophan(3-^{14}C), were reduced catalytically with platinic oxide to 2-hydroxy-3-ethyl-4,7,8-trimethoxyquinoline (Ⅶ),[7] which was oxidized with chromium trioxide to afford acetic and propionic acid. These acids were separated on Hyflo Super-Cel column chromatography,[8] and radioassayed as 1-acetamido- (Ⅷ) and 1-propionamido-naphthalene (Ⅸ).[9] The radioactivity of the carbon atom at position 3 of skimmianine was calculated from the difference of activity between acetic and propionic acid. The results of degradation is shown in Table Ⅱ. About

Table Ⅱ. Degradation Products of Skimmianine

Degradation products	Sp. act. (d.p.m./mM)	%
1) From anthranilic acid(T)		
Skimmianine (I)	1.38×10^{6}	100
Skimmianal (Ⅳ)	1.31×10^{6}	95
Skimmianic acid (Ⅴ)	1.32×10^{6}	96
N-Methylphthalimide (Ⅵ)	1.12×10^{3}	<1
2) From sodium acetate(1-^{14}C)		
Skimmianine (I)	1.81×10^{3}	100
Reduced product (Ⅶ)	1.77×10^{3}	98
1-Propionamido naphthalene	1.78×10^{1}	1
3) From sodium acetate(2-^{14}C)		
Skimmianine (I)	3.78×10^{4}	100
Reduced product (Ⅶ)	3.74×10^{4}	99
1-Acetamidonaphthalene	3.34×10^{2}	<1
1-Propionamidonaphthalene	3.38×10^{4}	89
4) From DL-tryptophan(3-^{14}C)		
Skimmianine (I)	7.56×10^{2}	100
Reduced product (Ⅶ)	7.50×10^{2}	101
1-Acetamidonaphthalene	0.00	0
1-Propionamidonaphthalene	7.83×10^{2}	105

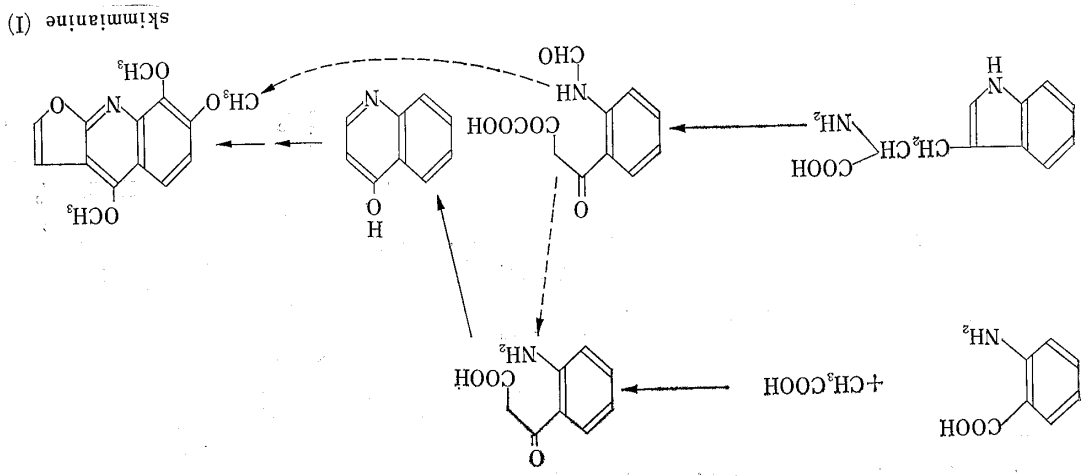


Chart 3. Biosynthetic Scheme of Skimmianine

7) T. Ohta, T. Miyazaki: This Bulletin, **1**, 184 (1953).
8) J. W. Cornforth, G. D. Hunter, G. Popjak: Biochem. J., **54**, 597 (1953).
9) E. Leete, H. Gregory, E. G. Gros: J. Am. Chem. Soc., **87**, 3475 (1965).

90% of the activity of skimmianine derived from acetate (2-^{14}C) was found in propionic acid, and no acitivity in acetic acid, on the other hand, no radioactivity was found in propionic acid obtained by the oxidation of skimmianine labeled by the acetate (1-^{14}C) feeding experiment. These results show that the carbon atoms at position 2 and 3 of skimmianine was derived from acetate. All the radioactivity of skimmianine derived from DL-tryptophan (3-^{14}C) was found also in propionic acid and no activity in acetic acid. It was shown that skimmianine was labeled at position 3 from DL-tryptophan (3-^{14}C) without randomization.

These results are interpreted as proof that the quinoline nucleus of skimmianine is formed by the condensation of anthranilic acid and acetate as shown in Chart 3. Moreover, another possibility is still remained since the radioactivity of DL-tryptophan (3-^{14}C) was found at position 3 of skimmianine without randomization though the incorporation ratio was very low. Therefore the biosynthetic scheme of skimmianine is shown in Chart 3.

Experimental

Radioactive Compound and Measurement of Radioactivity——Anthranilic acid(T) was synthesized by the hydrogen exchange method. Anthranilic acid (300 mg.), acetic acid (1 ml.) and THO (0.01 ml., 10 mCi.) was heated in a sealed tube in boilling water bath for 20 hr. Acetic acid was removed *in vacuo*. The residue was added to EtOH (20 ml.) and EtOH was removed *in vacuo*. The treatment was repeated 5 times. The residue was treated with Norit A and recrystallized from water to obtain a constant specific activity. The yield of anthranilic acid was 75 mg. (sp. activity : 0.27 mCi/mM).

The radioactivities were measured using Tri-Carb Liquid Scintillation Spectrometer, Series 314A (Packard Instrument Company, Inc.).*3

Administration of Precursors into Skimmia Plants and Isolation Skimmianine——The Skimmia plants, *Skimmia japonica* THUNB, were collected at Mt. Kiyosumi in Chiba Pref. The stems with leaves were cut off with a scissors, which were placed in small beakers containing the solution of radioactive precursor. The amounts of precursor used in each experiment is recorded in Table I. In all the experiments the plants were havested 10 days after the initial feeding of precursor, and skimmianine was isolated by the usual methods.[2,7] Skimmianine was chromatographed on silica gel (Kanto Chemicals Co., Inc. Tokyo; 100 mesh, diam., 4 cm., length, 20 cm.), and eluted with a mixture of benzene-methyl ethyl ketone (3:1). A pale blue fluorescent fraction was collected, and the solvent was evaporated *in vacuo* to obtain a residue which was recrystallized with EtOH until its specific activity became constant. The purity of skimmianine was examined by thin-layer chromatography (Silica gel G, solvent; benzenemethyl ethyl ketone, 3:1).

Degradation of Skimmianine derived from Anthranilic Acid(T)——Skimmianine (60 mg.) in acetone (4 ml.) was refluxed, and added with a solution of $KMnO_4$ (120 mg.) in acetone (10 ml.) dropwise for 1 hr. The mixture was filtered, the filtrate was concentrated *in vacuo* and the residue was recrystallized from EtOH to obtain skimmianal (5 mg.). The precipitate of the reaction mixture was extracted with 5% $NaCO_3$, and the extract was acidified with dil. HCl to separate precipitate which was recrystallized from acetic acid to obtain skimmianic acid (15 mg.).[2] Skimmianine (13 mg.) was added to HI (3 ml., d=1.7) and Ac_2O (2 drops), and the mixture was placed in a glycerol bath at 140° and CH_3I generated was trapped in cold EtOH (20 ml.) by N_2 stream. Potassium phthalimide (27.7 mg.) was added to the EtOH solution which was refluxed for 6 hr. and concentrated *in vacuo*. Water was added to the residue and the precipitate formed was washed several times with water and recrystallized with EtOH to afford the crystals of N-methylphthalimide (12 mg.), which was dissolved in toluene to use for liquid scintillation counting.

Degradation of Skimmianine derived from Acetate(1-^{14}C), Acetate(2-^{14}C) and DL-Tryptophan(3-^{14}C)——Skimmianine (50 mg.) in EtOH (25 ml.) was submitted to catalytic reduction with PtO_2 as a catalyst, at room temperature for 2 hr. under ordinary pressure. Almost 2 moles of hydrogen was absorbed smoothly. After removal of the catalyst by filtration, the solvent was evaporated to dryness. The residue was recrystllized from EtOH to obtain 2-hydroxy-3-ethyl-4,7,8-trimethoxyquinoline (Ⅶ) (47 mg.).[7] The compound (Ⅶ) was added to a mixture CrO_3 (8 g.) in 2N H_2SO_4 (30 ml.). Distillation was continued until about 100 ml. of distillate has been collected, water being added to maintain the volume in the distillation flask at 20~30 ml. The distillate was neutralized with 0.1N NaOH and evaporated to dryness. Paper chromatography indicated the presence of sodium acetate and propionate in the residue,[10] which were separated by Hyflo Super-Cel.[8]

*3 Sodium acetate(1-^{14}C), sodium acetate(2-^{14}C) and DL-tryptophan(3-^{14}C) was purchased from Daiich Pure Chemicals Co., Itd., Tokyo.

Both products were assayed by conversion to their 1-naphthylamine derivatives,[9] 1-acetamido- (4 mg.) and 1-propionamido- (6 mg.) naphthalene.

The authors are indebted to Prof. S. Shibata, University of Tokyo, for his encouragements through this work, to Prof. J. Haginiwa, University of Chiba, Dr. M. Yamazaki, and Dr. T. Hino, This Institute, for their kind advices and to the members of the Tokyo University Forestry Experimental Station in Chiba Pref., for the collection of plant materials.

Summary

Skimmianine isolated from *Skimmia* plants fed with anthranilic acid (T), acetate (1-^{14}C), acetate (2-^{14}C) and DL-tryptophan (3-^{14}C) was shown to be radioactive. Degradation of the labeled skimmianine showed that the radioactivity was located in the benzene ring when anthranilic acid (T) was administered, while it was located at the position 3 of quinoline nucleus when acetate (2-^{14}C) or DL-tryptophan (3-^{14}C) was used as the labeled precursors.

On the basis of these results, a scheme of the biosythesis of skimmianine was proposed in which skimmianine was shown to be derived from anthranilic acid and acetate.

(Received February 17, 1966)

10) E. P. Kennedy, H. A. Barker: Anal. Chem., **23**, 1033 (1951).

[Chem. Pharm. Bull. 14(10)1112~1121(1966)]

UDC 543.422.25 : 547.457

153. Shigeharu Inouye: Nuclear Magnetic Resonance Spectroscopy of Amino-sugars. I. Conformation of Methyl 3,6-Diamino-3,6-dideoxy-α-D-altropyranoside and Its Derivatives.

(*Central Research Laboratories, Meiji Seika Kaisha, Ltd.*[*1])

The synthesis of methyl 3,6-diamino-3,6-dideoxy-α-D-altropyranoside was recently reported by wolfrom, *et al.*[1] and the structure of this compound was confirmed by a sequence of degradation reactions to the known α-amino acids. The synthesis of this diamino-sugar *via* essentially the same route as that reported was independently carried out in this laboratory, as a modeled reaction relating to the synthesis of the aminated kanamycin derivatives.[2] The diamino-sugar was characterized by the crystalline free base, di-N-acetate, and disalicylidene derivative, and the structure was determined mainly on the basis of the nuclear magnetic resonance data.

Conformational analysis based on the instability factors presented by Reeves,[3] indicated that α-D-altropyranose existed in a C1⇆1C conformational equilibrium of about 1:1. It would be of interest, therefore, to see if this diamino-sugar and its derivatives are actually in an equal proportion of the C1 and 1C conformers.

*1 Morooka, Kohoku-ku, Yokohama-shi (井上重治).

1) M. L. Wolfrom, Yen-Lung Hung, D. Horton: J. Org. Chem., **30**, 3394 (1965).
2) S. Inouye: "Symposium Abstracts, 9th Symposium on the Chemistry of Natural Products," 7 (1965), Osaka.
3) E. L. Eliel, N. L. Allinger, S. J. Angyal, G. A. Morrison: "Conformational Analysis," 360 (1965), Interscience Publishers, New York.