The Biosynthesis of Sepedonin

Part I. Labeling Pattern of Anhydrosepedonin

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The origin of each carbon of anhydrosepedonin produced by *Sepedonium chrysospermum* has been elucidated by carbon-14 tracer method. At first, the degradation pathways adequate to such object was studied. The radioactivity incorporated in sepedonin and anhydrosepedonin was considerably high when acetate-1-¹⁴C, acetate-2-¹⁴C and formate-¹⁴C were used as the precursor. The feature of the labeling pattern of anhydrosepedonin was analogous to those of the other fungal tropolones. The carbon atom derived from formate was almost exclusively situated at C-7 position of the tropolone ring. As a whole, the labeling pattern demonstrated that anhydrosepedonin might be synthesized through the poly-ketomethylene pathway.

Mold tropolones, stipitatic, stipitatic, puberulic and puberulonic acids are known to be formed through the condensation of one acetate unit with three malonate units; the tropolone ring is completed by addition of a C₁ unit derived from formate or methionine.¹⁾ Recently Divekar *et al.* reported that the new tropolone derivatives, sepedonin I and anhydrosepedonin II were isolated from culture filtrates of *Sepedonium chrysospermum*.²⁾ The substituents in I and II are more complicated than those of the above mentioned mold tropolones. Therefore the authors were interested in the biosynthesis of I and II and carried out the studies on the labeling pattern of II, using carbon-14 precursors.

McInnes *et al.* have recently published an excellent report on the distribution of ¹³C in sepedonin, using the dexteros technique of

measurements of the ¹³C-H satellite signals.³⁾

1) a) S. W. Tanenbaum and E. W. Bassett, *Biochim. Biophys. Acta*, **59**, 524 (1962); b) R. Bentley, *J. Biol. Chem.*, **238**, 1895 (1963); c) I. G. Andrew and W. Segal, *J. Chem. Soc.*, **1964**, 607; d) L. D. Feretti and J. H. Richards, *Proc. Natl. Acad. Sci. U.S.A.*, **46**, 1438 (1960).

2) B. V. Divekar, H. Raistrick, T. A. Dobson and L. C. Vining, *Can. J. Chem.*, **43**, 1835 (1965).

3) A. G. McInnes, D. G. Smith, L. C. Vining and J. L. C. Wright, *Chem. Commun.*, **1968**, 1669.

SCHEME 2.

However, it is impossible by this method to determine the origin of the carbons which are not bound to any hydrogen atom. The method of the using of carbon-14 should be complementary to the above mentioned one, although it has need of troublesome degradations.

Degradation of anhydrosepedonin (II)

Degradation of anhydrosepedonin (II) was carried out as follows (Scheme 1).

In case of stipitatic and puberulonic acids, rearrangement to benzenoid compounds by fusion with alkali and oxidation with alkaline hydrogen peroxide to give malonic, oxalic and aconitic acids brought the favorable results.^{1b)} However, in case of II, the rearrangement to the benzenoid compound by fusion with potassium hydroxide gave only a small amount of the untractable oily substance under various conditions. Furthermore, oxidation with hydrogen peroxide or manganese dioxide was not suitable to determine the origin of each carbon atom, because it only gave fragments, such as oxalic, malonic and succinic acids. The reduction of II with hydrogen iodide in

acetic acid gave rise to fission of the vinyl ether bond in the side chain, affording VII, as colorless needles, mp 208~210°C, in high yield. Treatment of VII with sodium azide in concentrated sulfuric acid gave aminomethyl derivative VIII, showing that VII possessed an acetyl group. The analytical values of VII were in good agreement with $\text{C}_{11}\text{H}_{12}\text{O}_4$.

The compound VII gave the methylation products on treatment with diazomethane.⁴⁾ The IR spectrum of VII showed an intense band at 3225 cm^{-1} due to ν_{OH} , broad bands in the region between $3000\sim 2500\text{ cm}^{-1}$ due to the strongly associated intermolecular hydrogen bonding, and bands at 1713 cm^{-1} due to $\nu_{\text{C=O}}$, at 1614 and 1590 cm^{-1} due to $\nu_{\text{C=C}}$ of the tropolone ring, and at 903 cm^{-1} due to $\delta_{\text{C-H}}$ of the isolated ring hydrogens. The UV absorption maxima of VII at 260 and $330\text{ m}\mu$ were very similar to those of 4-hydroxy-tropolone derivatives. The NMR spectrum of VII in $\text{d}_6\text{-DMSO}$ showed the existence of

4) S. Takenaka and S. Seto, *Sci. Rep. Research Institutes Tohoku Univ.*, **A-21**, 106 (1969).

two ring protons (at 6.80 and 6.85 ppm), a methylene (at 3.92 ppm), a methyl in the side chain (at 2.16 ppm), and a methyl group attached to the ring (at 1.97 ppm). These data supported the structure of VII as 6-hydroxy-5-methyl-4-acetoniltropolone. The compound VII formed two kinds of crystals, VIIa, mp 208~210°C, and VIIb, mp 238~240°C. The NMR and UV spectra of VIIa in the solution were identical with those of VIIb. On fusion with potassium hydroxide, VII gave colorless needles (IX), mp 198.5~199.5°C, in high yield. Its analytical values corresponded to $C_8H_{10}O_3$, showing loss of C_2H_2O from the starting compound. The UV spectrum suggested that IX was a six-membered compound. The mass spectrum showed a parent peak at m/e 166, and the NMR spectrum showed signals for two methyls at 2.09 and 2.23 ppm (each 3H, s), and for two ring-protons overlapping around 7.25 ppm (2H, broad). From these data and the consideration of the course of formation of IX, its structure was elucidated to be 3-hydroxy-4,5-dimethylbenzoic acid, although the melting point of our specimen considerably lower than that of the literature.⁵⁾

Decarboxylation of IX in quinoline in the presence of $CuCrO_2$ gave a colorless volatile oil (X), which without further purification was nitrated with fuming nitric acid in concentrated sulfuric acid. Then the nitration product (XI) was obtained as yellow needles, mp 79~80°C. The analytical values agreed with $C_8H_8O_5N_2$. The UV spectrum showed intense maxima at 270 and 354 $m\mu$, and the NMR spectrum showed signals at 2.37 (3H, s), 2.52 (3H, s) and 8.47 ppm (1H, s). Therefore, XI must be 2,4-dinitro-5,6-dimethylphenol.

The exhaustive catalytic hydrogenation of II in the presence of PtO_2 gave a colorless viscous oil (XII). The IR spectrum of XII showed the absence of any double bond and

carbonyl group. Only terminal absorption was observed in its UV spectrum. The thin layer chromatogram of XII on Silica gel G (Merck) showed several spots, so that XII was believed to be a mixture of several stereoisomers. Treatment of XII with Kiriani reagent resulted in an evolution of one molar CO_2 to afford an oil (XIII). Reaction of XIII with iodine in alkaline solution gave iodoform and an oil (XIV). The structure of XIV was not investigated in detail, but XIV should have the assigned structure shown in Scheme 2.

The combination of the above mentioned reactions made it possible to identify almost all carbons of II. The carbon atoms of II were numbered as follows:

SCHEME 3.

Alkaline fusion of VII gave IX and acetic acid which originated from the C-9 and C-10 carbons. Then, by the Schmidt reaction, the C-9 carbon was trapped as $BaCO_3$, and the C-10 carbon was trapped as methylamine which in turn was converted to $BaCO_3$ with a combustion apparatus.

The rearrangement mechanism being taken in consideration, the carbon atom of the carboxyl group of IX should be derived from the C-1 carbon. This carbon atom could be trapped as $BaCO_3$ on decarboxylation of IX to X. Treatment of XI with Kuhn-Roth oxidizing reagent gave acetic acid which consisted of methyl group derived from the C-8 and C-11 carbons, and of carboxyl group derived from the C-4 and C-5 carbons. Treatment of XI with bromine in aqueous solution of barium hydroxide gave bromopicrin, whose carbon atom originated from the C-3 and C-7 carbons. Bromopicrin was converted to $BaCO_3$ with a combustion apparatus.

5) P. V. Divekar and L. C. Vining, *Can. J. Chem.*, **42**, 63 (1964).

TABLE I. DISTRIBUTION OF RADIOACTIVITY IN ANHYDROSEPEDONIN (II)

C-Position	Precursors		
	Acetate-1- ¹⁴ C × 10 ⁻⁴ dpm/mmmole	Acetate-2- ¹⁴ C × 10 ⁻⁴ dpm/mmmole	Formate- ¹⁴ C × 10 ⁻⁴ dpm/mmmole
Anhydrosepedonin	22.5	78.1	69.6
1	0.71 (2.9%)	13.8 (16.0%)	
2+6	8.07 (33.2)	1.38 (1.6)	
3 ^{a)}	0.54 (2.2)	16.2 (18.8)	
4+5	3.40 (14.0)	13.8 (16.1)	
7	0.80 (3.3)	3.95 (4.6)	45.5 ^{b)} (79.3%)
8+11	4.52 (18.6)	18.30 (21.3)	
9	5.69 (23.4)	1.72 (2.0)	
10	0.56 (2.3)	17.18 (20.0)	

^{a)} The activity of C-3 was estimated by subtraction of the activity of C-7 from sum of the activities of C-3 and C-7.

^{b)} The activities of other carbons were not measured.

Moreover, the reaction of XII to XIII evolved one molar CO₂ derived from the C-1 carbon, and iodoform obtained from XIII originated from the C-7 carbon. The decarboxylation of XIV gave two molar CO₂ derived from the C-6 and C-2 carbons.

RESULTS

¹⁴C-Labeled anhydrosepedonin was isolated from the cultures of *Sepedonium chrysospermum* in Czapeck-Dox medium containing acetate-1-¹⁴C, acetate-2-¹⁴C and formate-¹⁴C. The labeled precursors were added below the mycelial mat on a day of 10~16th day of growth. The labeled anhydrosepedonin was degraded as mentioned above. The results are summarized in Table I.

DISCUSSION

The previous studies¹⁾ with ¹⁴C-labeled precursors on the biosynthesis of simpler fungal tropolones revealed that the molecules were assembled via polyketide pathway and the labeling patterns were as shown in Fig. 1.

As to the labeling pattern of sepedonin

FIG. 1.

skeleton, McInnes *et al.*³⁾ established that the C-3, C-8 and C-10 carbons of sepedonin were derived from the methyl carbon of acetic acid, the C-11 carbon from the carboxyl carbon of acetic acid, and the C-7 carbon from formate.

Although, in our experiments, it was impossible to discriminate between the C-4 and C-5 carbons, and between the C-8 and C-11 carbons, the mutual complement of the studies of McInnes group and the present authors, elucidated unequivocally the labeling pattern of sepedonin skeleton as shown in Fig. 2.

The labeling pattern of anhydrosepedonin is similar to those of simpler fungal tropolones, and this suggests that both biosyntheses involve

FIG. 2.

the initial formation of a polyketide chain. Many biosynthetic pathway of mold tropolone have been postulated,^{1b, 6a-c)} but they lack evidences that the precursors postulated are directly incorporated into the tropolone. The fact that, in addition to stipitatic acid, α -pyrone derivatives were isolated from the culture of *P. stipitatum* grown in the presence of ethionine implies the formation of the tropolone via acetate-polymalonate pathway,⁷⁾ but these α -pyrone derivatives were not directly incorporated into the tropolone.^{6a, 8a, b)} *S. chrysospermum* behaved toward ethionine like *P. stipitatum* and formed a small amount of 6-acetonyl- α -pyrone derivative (XV).⁹⁾ However, this compound does not seem to be incorporated into sepedonin directly. In case of 6-methyl salicylic acid, 6-methyl- α -pyrone derivative (XVI) could not be an intermediate.¹⁰⁾ Moreover, although fatty acid synthetase gives 6-methyl- α -pyrone derivative in case of defect of TPNH, this derivative is not incorporated into long chain fatty acids directly.¹¹⁾

6) a) T. E. Acker, P. E. Brenneisen and S. W. Tanenbaum, *J. Am. Chem. Soc.*, **88**, 834 (1966); b) G. S. Marx and S. W. Tanenbaum, *ibid.*, **90**, 5302 (1968); c) R. Bentley and P. M. Zwitkowitz, *ibid.*, **89**, 681 (1967).

7) A. J. Birch, *Science*, **156**, 202 (1967).

8) a) R. Bentley, J. A. Ghaphery and J. G. Kell, *Arch. Biochem. Biophys.*, **111**, 80 (1965); b) R. Bentley and P. M. Zwitkowitz, *J. Am. Chem. Soc.*, **89**, 676 (1967).

9) Unpublished results in our laboratory.

10) R. J. Light, T. M. Harris and C. M. Harris, *Biochem.*, **5**, 4037 (1966).

11) a) D. J. H. Brock and K. Bloch, *Biochem. Biophys. Res. Commun.*, **23**, 775 (1966); b) J. E. Nixon, G. R. Putz and J. W. Porter, *J. Biol. Chem.*, **243**, 5471 (1968).

XV: R = CH_2COCH_3

XVI: R = CH_3

On the other hand, Gatenbeck *et al.*¹²⁾ reported that protein bounded polyketo-methylene is isolated from the cell free extract of *Aspergillus flaviceps* and lead to triacetic lactone by alkaline hydrolysis. Moreover, Gaucher postulated that orsellinic acid synthetase contained acyl carrier protein.¹³⁾ In case of biosyntheses of the tropolones, the protein such as acyl carrier protein bound polyketomethylene may also be involved. Therefore, in order to elucidate the biosynthetic pathway of the tropolone in detail, the studies with cell free system should be carried out.

EXPERIMENTAL

All melting points are uncorrected. The measurements of the UV and IR spectra were made with a Cary recording spectrophotometer model 14 and with a Hitachi EPI-G2 spectrophotometer, respectively. The NMR spectra were measured in a deuterio-dimethylsulfoxide or deuteriochloroform solution, using tetramethylsilane as an internal standard, with a Varian A-60, T-60 and Hitachi H-60 spectrometers. The microanalyses were carried out by Misses Noriko Matsukawa, Emiko Yoshida and Noriko Sato of this institute, to whom the authors are indebted. Sodium acetate-1-¹⁴C, acetate-2-¹⁴C and formate-¹⁴C were purchased from Daiichi Chemical Co. Ltd. Cab-O-sil was obtained from Beckman Instruments, Inc. Measurement of radioactivity was made in dioxane solution containing 7 g of 2,5-diphenyloxazole, 300 mg of 1,4-bis (5-phenyloxazolyl) benzene and 100 g of naphthalene in 1000 ml of dioxane or 2 g of 2,5-diphenyloxazole and 50 mg of 1,4-bis (phenyloxazolyl) benzene in 500 ml of toluene with a Ten Model GSL-111 liquid scintillation counter.

12) S. Gatenbeck, P. O. Erikson and Y. Hansson, *Acta Chem. Scand.*, **23**, 699 (1969).

13) G. M. Gaucher and M. G. Shepherd, *Biochem. Biophys. Res. Commun.*, **32**, 664 (1968).

Anhydrosepedonin. The culture condition was the same as the literature.²⁾ A mixture of sepedonin and anhydrosepedonin (1.0 g) was heated in glacial acetic acid (100 ml) under the nitrogen atmosphere, by which sepedonin was converted to anhydrosepedonin. The resulting solution was concentrated to a half volume and the crystals separated out on cooling were collected by filtration. Recrystallization from methanol gave yellow needles, mp 205°C.

¹⁴C-Labeled anhydrosepedonin. Sodium acetate-2-¹⁴C (100 μ Ci) in 1 ml of sterilized water was added to the cultures of *Sepedonium chrysospermum* below the surface of the mycelium on 14 days after inoculation. After 25 days growth, ¹⁴C-labelled anhydrosepedonin was isolated from the culture fluid (1000 ml). The fourth recrystallization from a mixture of methanol and acetic acid gave the crystals (IIa) of constant specific activity. Yield, 1.06 g, 3.79×10^8 dpm/mmole.

The samples (IIb and IIc) of anhydrosepedonin labelled with acetate-1-¹⁴C and formate-¹⁴C were obtained by the same way; IIb, 0.515 g, 1.61×10^9 dpm/mmole; IIc, 1.206 g, 6.40×10^8 dpm/mmole, respectively.

6-Hydroxy-5-methyl-4-acetonyltropolone (VII). A mixture of anhydrosepedonin (II, 200 mg), iodine (800 mg) and red phosphorus (800 mg) in 15 ml of acetic acid and 2 ml of water was refluxed for 7 hr. The resulting solution was concentrated to dryness and the residue was adjusted to pH 3 with 2N sodium hydroxide and a large excess of sodium thiosulfate was added to the acidic solution. This solution was continuously extracted with ether for 48 hr. The colorless crystals obtained from the ether layer were recrystallized from a mixture of methanol and benzene to give VII, as colorless needles, mp 208~210°C. Found: C, 63.18; H, 6.22. Calcd. for C₁₁H₁₂O₄: C, 63.45; H, 5.81%. UV $\lambda_{\text{max}}^{\text{MeOH}}$ m μ (log ϵ): 254 (4.52), 330 (3.81), 350 (3.71).

VIIa (VII labeled with acetate-2-¹⁴C). VIIa was obtained by the same manner as mentioned above from 1.0 g of labeled anhydrosepedonin (7.81×10^8 dpm/mmole), 890 mg (89% of theoretical).

VIIb (VII labeled with acetate-1-¹⁴C). VIIb was also obtained by the same operation from 1.06 g of labeled anhydrosepedonin (2.25×10^8 dpm/mmole), 911 mg (85.9% of theoretical).

3-Hydroxy-4,5-dimethylbenzoic acid (IX). A mixture

of VII (350 mg), potassium hydroxide (5 g) and water (1 ml) was heated at 320~330°C for 10 min under the nitrogen atmosphere. The brownish solid was dissolved in 2 ml of water and the solution was acidified to pH 1 with 6N hydrochloric acid. The resulting solution was extracted continuously with ether. The yellowish solid obtained by removal of ether was chromatographed on a silica gel column. The solid obtained from the ether-effluent was recrystallized from a mixture of ether and pet-ether to give IX, mp 198.5~199.5°C, yield, 218 mg. Found: C, 64.74; H, 6.08. Calcd. for C₉H₁₀O₃: C, 65.05; H, 6.07%. UV $\lambda_{\text{max}}^{\text{MeOH}}$ m μ (log ϵ): 246 (4.03), 297 (3.54).

IXa (IX labeled with acetate-2-¹⁴C). IXa (5.94×10^5 dpm/mmole, 511 mg, 72% of theoretical) was obtained by the same way as above from VIIa (890 mg). Thereby, acetic acid cleaved from the side chain of VII was trapped as sodium salt by distillation from the ether extract, yield 239 mg (68% of theoretical). This salt (239 mg) and sodium azide (200 mg) were dissolved in 1 ml of conc. sulfuric acid (1 ml) and chloroform (1 ml), and then this solution was stirred for 3 hr under the nitrogen atmosphere. Thereby, evolved carbon dioxide was trapped as barium carbonate (1.72×10^4 dpm/mmole, 546 mg, 95% of theoretical). Monomethylamine hydrochloride (83 mg, 42% of theoretical) was obtained from the inorganic layer, and converted to barium carbonate by a combustion apparatus (1.718×10^5 dpm/mmole, 218 mg, 90% of theoretical).

IXb (IX labeled with acetate-1-¹⁴C). IXb (1.81×10^5 dpm/mmole, 538 mg, 74% of theoretical) was obtained by the same way as above from VIIb (911 mg). Sodium acetate (258 mg, 72% of theoretical) thereby obtained was converted to barium carbonate (5.69×10^4 dpm/mmole, 609 mg, 98% of theoretical) and monomethylamine hydrochloride (76 mg, 36% of theoretical) which was also converted to barium carbonate (0.56×10^4 dpm/mmole, 213 mg, 96% of theoretical).

2,3-Dimethylphenol (X). A suspension of CuCrO₂ (1 g), and IX (314 mg) in 15 ml of quinoline was heated at 230°C for 2 hr under the nitrogen atmosphere, and then quinoline was distilled at 30 mmHg. Quinoline was acidified by the addition of a large excess of 6N hydrochloric acid, and was extracted with ether. Gradual evaporation of the ether extract left the residual oil (X) which was nitrated im-

mediately. In the course of decarboxylation, carbon dioxide was trapped as barium carbonate.

Xa (X labeled with acetate-2-¹⁴C). Xa was obtained by the same operation as above from IXa (5.94×10^5 dpm/mmmole, 505 mg). Carbon dioxide thereby evolved was trapped as barium carbonate (1.38×10^5 dpm/mmmole, 596 mg, 100% of theoretical).

Xb (X labeled with acetate-1-¹⁴C). Xb was obtained by the same manner as above from IXb (1.81×10^5 dpm/mmmole, 530 mg). Carbon dioxide was trapped as barium carbonate (0.71×10^4 dpm/mmmole, 611 mg, 97% of theoretical).

2,4-Dinitro-5,6-dimethylphenol (XI). To a solution of 2,3-dimethylphenol (X) which was obtained from the ether extract as mentioned above in 0.5 ml of conc. sulfuric acid, a mixture of 0.5 ml of sulfuric acid and 0.3 ml of conc. nitric acid was added dropwise under ice cooling. After a few minutes, 0.6 ml of nitric acid was added to the solution. Then the solution was adjusted to pH 3 with 6N sodium hydroxide and extracted with ether. The ether extract was evaporated and the residue was recrystallized from pet-ether to give XI as orange plates, mp 79~80°C, (184 mg, 46% of theoretical). Found: C, 45.48; H, 3.79; N, 11.9. Calcd. for $C_8H_8N_2O_5$: C, 45.29; H, 3.80; N, 13.21%. UV $\lambda_{\max}^{MeOH}$ m μ (log ϵ): 270 (4.02), 354 (3.75).

XIa (XI labeled with acetate-2-¹⁴C). XIa (5.36×10^5 dpm/mmmole, 342 mg, 53% of theoretical) was obtained from IXa by the same operation as above.

XIb (XI labeled with acetate-1-¹⁴C). XIb (1.73×10^5 dpm/mmmole, 325 mg, 48% of theoretical) was obtained from IXb similarly.

Kuhn-Roth oxidation of XIa and XIb. XIa (150 mg) in 50 ml of $CrO_3-H_2SO_4$ was heated for 2 hr at 130°C. Then the solution, after addition of anhydrous magnesium sulfate (7 g) was distilled by steam. Acetic acid in the distillate was collected as sodium salt (70 mg, 60% of theoretical). By Schmidt reaction, sodium acetate (70 mg) was converted to barium carbonate (1.38×10^5 dpm/mmmole, 128 mg, 76% of theoretical) and monomethylamine hydrochloride (31 mg, 54% of theoretical), which was led to barium carbonate (1.83×10^5 dpm/mmmole, 85.1 mg, 94% of theoretical) with a combustion apparatus.

By the similar operation, XIb (150 mg) gave barium

carbonate (3.40×10^4 dpm/mmmole, 136 mg, 77% of theoretical) and monomethylamine hydrochloride (31 mg, 56% of theoretical), which was led to barium carbonate (4.52×10^4 dpm/mmmole, 81 mg, 82% of theoretical).

Bromopicrin reaction of XIa and XIb. XIa (150 mg), barium hydroxide (15 mg) and bromine (4 g) were dissolved in 150 ml of water, and the solution was heated gradually on a water bath under the nitrogen atmosphere for 1 hr. After the addition of 30 ml of a saturated aqueous solution of sodium hydrogen carbonate, the resulting mixture was distilled. The distillate (80 ml) was extracted with ether continuously. Evaporation of the extract gave bromopicrin (2.02×10^5 dpm/mmmole, 200 mg, 71% of theoretical).

XIb (150 mg) was treated similarly to give bromopicrin (1.34×10^4 dpm/mmmole, 180 mg, 64% of theoretical).

Hydrogenation of II. II (1.2 g) in 100 ml of acetic acid was hydrogenated in the presence of 50 mg of PtO_2 at room temperature. After exhaustive hydrogenation the solvent was removed under the reduced pressure, and the residue was passed through a silica gel column, affording a colorless oil (XII, 1.558 g). This substance showed several spots on the thin layer chromatography of silica gel.

Hydrogenation of IIa, IIb and IIc (II labeled with acetate-2-¹⁴C, acetate-1-¹⁴C and formate-¹⁴C, respectively). IIa (7.85×10^5 dpm/mmmole, 1.0 g), IIb (2.25×10^5 dpm/mmmole, 780 mg) and IIc (6.96×10^5 dpm/mmmole, 1.206 g) gave XIIa (1.049 g), XIIb (811 mg) and XIIc (1.260 g), respectively, in hydrogenation as above.

Oxidation of XIIa, XIIb and XIIc (XII labeled with acetate-2-¹⁴C, acetate-1-¹⁴C and formate-¹⁴C, respectively). To a solution of XIIa (950 mg) in 10 ml of acetic acid, 16 g of Kiliani reagent was added slowly under cooling, and then the solution was stirred for 4 hr at room temperature. Extraction with ether gave XIIIa (503 mg, 57% of theoretical).

Similarly, oxidation of XIIb (811 mg) gave XIIIb as a colorless oil (501 mg, 67% of theoretical).

Oxidation of XIIc (960 mg) as above gave XIIIc as a colorless oil (521 mg, 59% of theoretical).

Iodoform reaction of XIIIa, XIIIb and XIIIc (XIII labeled with acetate-2-¹⁴C, acetate-1-¹⁴C and formate-¹⁴C, respectively). To a solution of XIIIa (6.56×10^5 dpm/mmmole, 500 mg) in 3 ml of 6N sodium hydroxide,

I₂-KI solution (I₂, 10 g; KI, 20 g in 100 ml of water) was added and the crystals precipitated were collected by filtration (3.95×10^4 dpm/mmole, 84% of theoretical). The filtrate was acidified to pH 2 with 6N hydrochloric acid and continuously extracted with ether. The oily substance obtained from the ether extract was purified through a silica gel column to give XIVa (6.20×10^5 dpm/mmole, 343 mg, 68% of theoretical).

Iodoform reaction of XIIIb (2.18×10^5 dpm/mmole, 485 mg) as above iodoform (0.80×10^4 dpm/mmole, 725 mg, 76% of theoretical) and XIVb (2.10×10^5 dpm/mmole, 314 mg, 64% of theoretical).

Iodoform reaction of XIIIc (6.96×10^5 dpm/mmole, 521 mg) as above gave iodoform (4.55×10^5 dpm/mmole, 817 mg, 81% of theoretical) and XIVc.

Decarboxylation of XIVa and XIVb (XIV labeled with acetate-2-¹⁴C and acetate-1-¹⁴C). A mixture of XIVa (6.20×10^5 dpm/mmole, 325 mg) and CuCrO₂ (200 mg)

in quinoline (5 ml) was heated at 230°C for 4 hr under the nitrogen atmosphere. Carbon dioxide thereby evolved was trapped as barium carbonate (1.38×10^4 dpm/mmole, 577 mg, 91% of theoretical).

Decarboxylation of XIVb (2.10×10^5 dpm/mmole, 300 mg) as above gave barium carbonate (8.07×10^4 dpm/mmole, 510 mg, 87% of theoretical).

Schmidt reaction of VII. Sodium azide (130 mg) was added to a stirred mixture of VII (144 mg), conc. sulfuric acid (0.7 ml) and chloroform (5 ml) at room temperature. The initial red color of the acidic layer changed gradually to yellow with progress of the reaction. The precipitate separated out on dilution of the aqueous layer with 20 ml of water, was collected. Recrystallization from acetic acid gave VIII (139 mg) as colorless needles, mp 230~231°C. Found: C, 60.39; H, 6.90. Calcd. for C₉H₁₁O₃N: C, 59.66; H, 6.12%. UV $\lambda_{\text{max}}^{\text{MeOH}}$ m μ (log ϵ): 253 (4.43), 329 (3.73), 352 (3.66).