Synthesis of Phthalthrin- ^{14}C (Neo-Pynamin®- ^{14}C)*

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Phthalthrin labelled with ^{14}C in the alcoholic moiety was synthesized *via* an intermediate Δ^1 -tetrahydrophthalic anhydride- ^{14}C . A preparative pathway leading to the intermediate was investigated in order to shorten the synthetic steps. Thus the intermediate was prepared in two steps starting with potassium cyanide- ^{14}C . Position of double bond introduced into the molecule was confirmed from IR spectrum and melting point. Chemical and radiochemical purities of intermediates and the final product were ascertained on scanning the radio-thin-layer chromatograms. A brief description on the self-decomposition of phthalthrin- ^{14}C and an intermediate is also given.

Phthalthrin labelled with isotopic carbon in the alcoholic moiety was required for its metabolic study.¹⁾ This compound can be prepared from Δ^1 -tetrahydrophthalic anhydride (Δ^1 -TPA- ^{14}C , IV) through the corresponding N-methylol imide (Δ^1 -MTI- ^{14}C , VI).^{2,3)}

A synthetic route to reach Δ^1 -tetrahydrophthalic anhydride- ^{14}C , which is readily converted into Δ^1 -TPA- ^{14}C ⁴⁾ was reported by Nystrom and his coworkers⁵⁾: according to their procedure, the isotopic carbon was introduced into the molecule at the first step of the reaction sequence and thereafter seven steps were required to reach the desired compound.

Since such a long procedure had not proved consistently successful in obtaining high radiochemical yield, the present investigation was undertaken in order to find such a precursor that could be transformed, after introducing ^{14}C label into the molecule, into Δ^1 -TPA- ^{14}C in one or two steps.

Thus a number of routes leading to Δ^1 -TPA were compared and following synthetic pathway starting with ethyl cyclohexanone-2-carboxylate (I) was elaborated in a semimicro scale.

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* A part of this study was conducted while the authors were in Takarazuka Radiation Laboratory, Division of Sumitomo Atomic Energy Industries Ltd., Takarazuka.

1) J. Miyamoto, Y. Sato, K. Yamamoto, M. Endo and S. Suzuki, *Agr. Biol. Chem.*, **32**, 628 (1968).

2) T. Kato, K. Ueda, S. Horie, T. Mizutani, K. Fujimoto and Y. Okuno, (to Sumitomo Chemical Co., Ltd.) U. S. Patent 3268398 (Aug. 23, 1966).

3) *idem.*, (to Sumitomo Chemical Co., Ltd.) Japan Patent 462108 (Dec. 22, 1965) and 468732 (Mar. 15, 1966).

4) M. E. Bailey and E. D. Amstutz, *J. Am. Chem. Soc.*, **78**, 3832 (1956).

5) R. F. Nystrom, Y. H. Loo and J. C. Leak, *ibid.*, **74**, 3434 (1952).

Then, phthalthrin- ^{14}C was derived through the following route:

The synthetic route to Δ^1 -TPA described above was reported by Hückel and Lampert.⁶⁾ On applying their procedure, several modifications were necessary in order to introduce ^{14}C -label efficiently and to carry out the processes in a small scale.

Potassium cyanide- ^{14}C , prepared from barium carbonate- ^{14}C by Hasegawa and Shimamura's method,⁷⁾ was added into ethyl cyclohexanone-2-carboxylate to obtain ethyl 1-cyanohexanol-2-carboxylate (II). To make good use of isotopic material, potassium cyanide- ^{14}C just equimolar to (I) was used. Provided a large excess of potassium cyanide was used as Hückel and Lampert reported, expensive isotopic starting material was wasted. Whereas the use of less amount of that than equivalent to (I) brought about difficulty in eliminating a resultant by-product, cyclohexanone-2-carboxylic acid, after hydrolysis.

Cyano-ester (II) in the reaction mixture was hydrolyzed without separation to hydroxydicarboxylic acid (III) which was dehydrated in nitrogen atmosphere to Δ^1 -TPA- ^{14}C in one step. To remove undehydrated acidic impurities which were detected on infrared spectrum and thin-layer chromatogram even after recrystallization from methyl cyclohexane, the

product was repeatedly washed with warm water.

The infrared absorption bands between 6μ and 12μ , which are characteristic of each isomeric anhydride, and melting point of the purified compound come to good agreement with that of non-radioactive authentic sample and also the results reported by Bailey and Amstutz about Δ^1 -isomer,⁴⁾ showing the position of the double bond introduced in the compound was Δ^1 -positional.

Regarding the procedure leading to phthalthrin- ^{14}C starting with this Δ^1 -TPA- ^{14}C , general processes reported by Kato and his coworkers,^{2,3)} were employed after some amendment come from a small scale and radioactive synthesis. To determine the radiochemical and chemical purity of intermediates and the final product, radio-thin-layer chromatographic technique was used.

EXPERIMENTAL

Melting point was measured with "Yanagimoto Micro Melting Point Apparatus" and the corrected values are described. For measurement of radioactivity of products, a liquid scintillation counting system (Kobe Kogyo, model GSL-163) was used, dissolving an aliquot of products into a liquid scintillator of the usual toluene-POPOP-*p*-terphenyl system. Radio-thin-layer chromatogram was scanned by "Aloka Thin-Layer Chromatography Scanner, model TLC-28" at the scanning speed of 12.5 mm/min with the slit width of 1 mm.

Cyclohexanol-1, 2-dicarboxylic acid- ^{14}C (III). To 1.7 g of cooled ($0\sim 5^\circ\text{C}$) ethyl cyclohexanone-2-carboxylate, 0.65 g of potassium cyanide- ^{14}C (43.3 mCi) dissolved in 2 ml of water was added under stirring in 10 min. After additional stirring for 3 hr at the same temperature, the reaction mixture was acidified with 1.3 ml of concentrated hydrochloric acid keeping the temperature not to exceed 10°C . The acidified reaction mixture was stirred for further 30 min, stoppered, then kept stand for 20 hr at $0\sim 5^\circ\text{C}$ to complete the reaction. After addition of 2 ml of concentrated hydrochloric acid, cyano-ester (II) was hydrolyzed without separation by heating for 6 hr under reflux. Resulting hydroxy-dicarboxylic acid (III) was ex-

6) W. Hückel and V. Lampert, *Chem. Ber.*, **67**, 1911 (1934).

7) M. Hasegawa and O. Shimamura, *Bull. Chem. Soc. Japan*, **29**, 445 (1955).

tracted with diethyl ether by a Soxhlet-type liquid extractor, then the solvent was evaporated to dryness. The crystalline product was washed with small portions of dry benzene to remove impurities and dried *in vacuo*. Yield of cyclohexanol-1, 2-dicarboxylic acid- ^{14}C was 1.71 g, 91% based on potassium cyanide- ^{14}C , mp 148~149°C.

Δ^1 -TPA- ^{14}C (IV). Under a stream of nitrogen gas, 1.71 g of hydroxy-dicarboxylic acid- ^{14}C (III) in a modified Auschütz's distillation flask was heated to 210~230°C. When the content reached to melt, water from dehydration reaction began to condense at the wall of the flask. After heating for 30 min when the evolution of water ceased, the flask was cooled to room temperature and water condensed in the saber-shaped receiver was evaporated by heating under reduced pressure. Then, the brownish residue in the flask was distilled *in vacuo* (2~3 mmHg) at 200°C into the receiver. The distillate, solidified in the receiver, was recrystallized from methyl cyclohexane. In order to remove water-soluble impurities, the product was repeatedly washed with warm water. After drying *in vacuo*, 0.838 g of Δ^1 -TPA- ^{14}C was obtained. Yield 57.4%, mp 70.5~72°C. *Anal.* Found: C, 63.15; H, 5.41. Calcd. for $\text{C}_8\text{H}_6\text{O}_3$: C, 63.15; H, 5.30%.

The radioactivity of the product was 22.6 mCi and the radiochemical yield estimated from potassium cyanide- ^{14}C was 52.3%. Radio-thin-layer chromatography of the product on Silica gel G with benzene-methanol (1:2) showed that most of radioactivity was

FIG. 1. Radio-thin-layer Chromatogram of Δ^1 -TPA- ^{14}C .

Adsorbent; Silica gel G, solvent; benzene-methanol (1:2). Full scale; 3×10^5 cpm, scanning speed; 25 mm/min.

FIG. 2. Infrared Spectrum of Δ^1 -TPA- ^{14}C .

"Hitachi Infrared Spectrophotometer, EPI-500" in paraffin oil.

co-chromatographed to Δ^1 -TPA- ^{14}C (Fig. 1). Only small amount of radioactivity, probably caused by undehydrated impurities, was found on the origin. No non-radioactive impurities were able to be detected with iodine visualization. Infrared spectrum of purified product, shown in Fig. 2, agreed with that of an authentic sample and with the result reported by Bailey and Amstutz.⁴⁾

Δ^1 -TPI- ^{14}C (V). A mixture of 1.185 g of Δ^1 -TPA- ^{14}C and 7.4 ml of aq. ammonia (20% conc.) was heated to 60°C with stirring to make clean solution. After additional stirring for 30 min, the temperature was elevated to 70~80°C and excess aq. ammonia was evaporated by blowing nitrogen gas to dryness. Resulting white crystals ammonium salt of Δ^1 -tetrahydrophthalic acid- ^{14}C , were heated to 110~120°C for 30 min so as to convert it into the imide-form by thermal elimination of ammonia accompanied with dehydration from the salt, and the reaction was accomplished by further heating at 170~180°C and keeping at this temperature with moderate stirring for 2 hr. After cooling to room temperature, the

FIG. 3. Radio-thin-layer Chromatogram of Δ^1 -TPI- ^{14}C .

Adsorbent; Silica gel G, solvent; benzene-ethyl acetate. (4:1). Full scale 3×10^5 cpm, scanning speed; 12.5 mm/min.

reaction mixture was washed out with water, then crystalline Δ^1 -TPI- ^{14}C was collected on glass filter and dried. The crude product was dissolved in toluene, treated with a small amount of active-charcoal. Δ^1 -TPI- ^{14}C , crystallized from the solution by adding *n*-heptane, was collected and finally dried under reduced pressure. Yield of pure Δ^1 -TPI- ^{14}C , melting at $173\sim 175^\circ\text{C}$, was 69.1% (0.792 g) as chemical and 68.5% (15.5 mCi) as radiochemical. Radio-thin-layer chromatography on Silica gel G with benzene-ethyl acetate (4:1) showed good radiochemical purity of the product (Fig. 3).

Δ^1 -MTI- ^{14}C (VI). Δ^1 -TPI- ^{14}C (0.729 g, 14.2 mCi), 1.46 mg of sodium carbonate and 0.787 g of formalin (37% conc.) were dissolved in 4 ml of water. The solution was heated to $60\sim 65^\circ\text{C}$ with stirring for 3.5 hr. As the reaction proceeded, the solution became turbid and finally separated to two layers. After cooling, the upper layer was extracted with toluene and dried with Drierite. White crystals were precipitated on adding *n*-hexane dropwise to the ice-cooled extract. The crystals were collected by filtration then dried at room temperature *in vacuo*. Δ^1 -MTI- ^{14}C was obtained in both chemical and radiochemical yield of 75% (0.656 g, 10.6 mCi), mp $81.5\sim 83.5^\circ\text{C}$. Radio-thin-layer chromatogram, on Silica gel G with benzene-methanol (50:1), of the product also showed good radiochemical purity (Fig. 4).

FIG. 4. Radio-thin-layer Chromatogram of Δ^1 -MTI- ^{14}C .

Adsorbent; Silica gel G, solvent; benzene-methanol (50:1). Full scale; 3×10^6 cpm, scanning speed; 12.5 mm/min.

Phthalthrin- ^{14}C (VII). To a solution of 0.656 g of Δ^1 -MTI- ^{14}C in 5 ml of toluene was added 0.431 g pyridine then cooled with ice-bath. To this solution, an ice-cooled *trans*-chrysanthemoyl chloride dissolved in 5 ml of dry toluene was added dropwise under an

efficient stirring, and the reaction mixture was kept at this temperature for another half hour with moderate stirring. Then the mixture was maintained at room temperature overnight to complete the condensation reaction. The precipitate, pyridine hydrochloride, was filtered and filtrate was washed with three portions of 5 ml of diluted hydrochloric acid, saturated solution of sodium chloride until the washing was neutral to litmus and dried on anhydrous sodium sulfate. The solvent was then removed under reduced pressure and resulting oily product was recrystallized from *n*-hexane. White crystals of phthalthrin- ^{14}C (0.802 g, 6.9 mCi) melting at $71\sim 73^\circ\text{C}$, were obtained in 66.7% yield. Radio-thin-layer chromatography on Silica gel G with two different solvent system (upper layer of *n*-hexane-ethyl ether-chloroform-acetic acid-ethanol-water; 40:10:10:5:1:1 and benzene-methanol; 50:1) showed good radiochemical purity (Fig. 5). And none of chemical impurities was found by visualization with iodine or UV on the chromatogram.

FIG. 5. Radio-thin-layer Chromatogram of Phthalthrin- ^{14}C .

Adsorbent; Silica gel G, solvent: upper layer of *n*-hexane-ethylether-chloroform-acetic acid-ethanol-water (40:10:10:5:1:1). Full scale; 1×10^6 cpm, scanning speed; 12.5 mm/min.

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APPENDIX

Self-decomposition data for phthalthrin- ^{14}C and Δ^1 -TPI- ^{14}C .

Phthalthrin- ^{14}C (8.6 mCi/g) and Δ^1 -TPI- ^{14}C (19.6 mCi/g) stored at 5°C in nitrogen atmosphere for about one year, were chromatographed without further treatment on Silica gel G with the said solvent systems.

On radioactivity scanning it was noted considerable decomposition of phthalthrin- ^{14}C due evidently to self radiolysis, since practically no decomposition was recognized about the non-labelled one under the above described conditions, as is seen in Appendix Fig. 1. The radioactivity peaks preceding to phthalthrin- ^{14}C on the chromatogram were identified with Δ^1 -TPI- ^{14}C

Appendix FIG. 1. Radio-thin-layer Chromatogram of Stored Phthalthrin- ^{14}C .

Adsorbent; Silica gel G, solvent; upper layer of *n*-hexane-ethyl ether-chloroform-acetic acid-ethanol-water (40:10:10:5:1:1). Full scale; 3×10^5 cpm, scanning speed; 12.5 mm/min.

Appendix FIG. 2. Radio-thin-layer Chromatogram of Stored Δ^1 -TPI- ^{14}C .

Adsorbent; Silica gel G, solvent; benzene-ethyl acetate (4:1). Full scale; 3×10^5 cpm, scanning speed; 12.5 mm/min.

and Δ^1 -MTI- ^{14}C from the *R_f*-values. This figure informs also that the undecomposed phthalthrin- ^{14}C occupies about 76% of the whole area of the peaks. Employing the self-radiation dose for labelled compounds of given specific activity, reported by Tolbert,⁸⁾ and assuming no more product other than those in the figure, the absorbed dose is estimated to be about 6.4 Mrad and G-value to be 100, approximately.

It is noteworthy that, regarding as Δ^1 -TPI- ^{14}C , as shown in Appendix Fig. 2, scarcely any decomposition product was detected on scanning the chromatogram. These facts suggest that the self-radiolysis of phthalthrin- ^{14}C mainly results from degradation of the molecule at N-C position forming Δ^1 -TPI- ^{14}C and residual moiety.

8) B. M. Tolbert, *Nucleonics*, **18** (8), 74 (1960).