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**Shigeyuki Takeyama, Teruhisa Noguchi, Yoshiaki Miura, and Morizo Ishidate :**

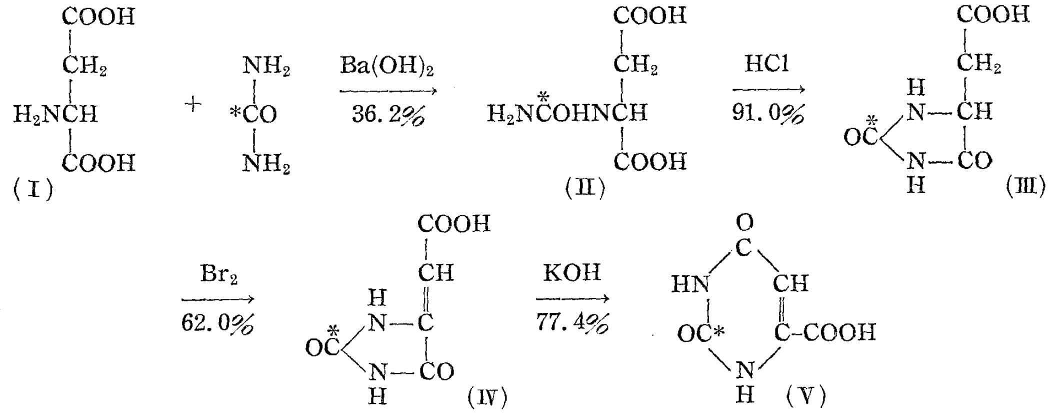
# Synthesis of Ureido[$^{14}C$]succinic Acid and Orotic[2-$^{14}C$] Acid from Urea[$^{14}C$].

(*Medical Faculty, University of Tokyo**)

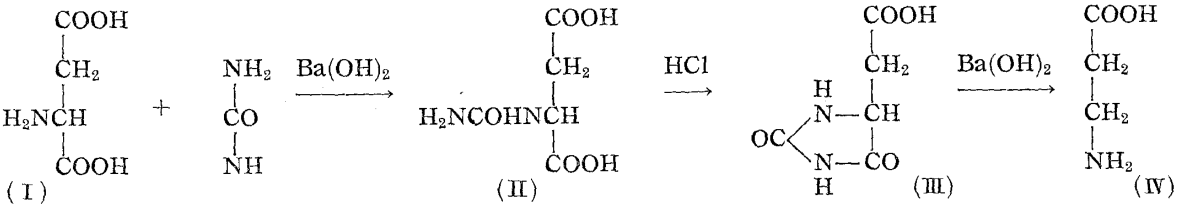
Orotic[2-$^{14}C$] acid, which was necessary for the series of studies being pursued in this laboratory, was synthesized by an improved method starting with DL-aspartic acid (I) and urea[$^{14}C$] in a yield of 15.7% on urea[$^{14}C$].

Since Biscaro and Belloni[1] found orotic acid in milk, various methods of its synthesis have been found[2]. Among them, the method used by Nyc and Mitchell[3] is applied most frequently for the synthesis of orotic[$^{14}C$ or $^{15}N$] acid, since it was found to be an intermediate of biosynthesis of nucleic acid pyrimidines *in vivo* and *in vitro*. In the present work, the authors modified this method for the synthesis of ureido[$^{14}C$]-succinic acid (II) with urea[$^{14}C$] as a starting material instead of potassium cyanate[$^{14}C$] in the original method, which is less available than urea[$^{14}C$].

The route of the synthesis was as follows :



The first step was originally reported by Lippich[4] in 1908 and required a large excess of urea. In 1933, Wada[5] demonstrated a method of synthesizing β-alanine (VI) starting with aspartic acid and a one-half mole excess of urea in barium hydroxide solution in an yield of 77% from aspartic acid, through ureidosuccinic acid and 5-carboxymethylhydantoin (III) without isolating them, as follows :



Since economic use of isotopic materials is required in this kind of synthesis, the authors attempted to find conditions which would produce the best yield of ureidosuccinic acid from urea[$^{14}C$]. The results of a series of experiments are summarized in Table I indicating that the best ratio of urea and barium hydroxide to aspartic acid

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* Motofujicho, Hongo, Bunkyo-ku, Tokyo (竹山茂之, 野口照久, 三浦義彰, 石館守三).

[1] G. Biscaro, E. Belloni : Ann. soc. chim. Milano, **11**, Nos. 1 and 2(1905) (Chem. Zentr., **2**, 64(1905)).

[2] E. Chargaff, J. N. Davidson : "The Nucleic Acids," Academic Press Inc., New York, **1**, 101(1955).

[3] J. F. Nyc, H. K. Mitchell: J. Am. Chem. Soc., **69**, 1382(1947).

[4] F. Lippich : Ber., **41**, 2958, 2966(1908).

[5] M. Wada : Biochem. Z., **260**, 47(1933).

TABLE I.

| Expt. No. | Urea (moles) / Aspartic acid (moles) | $Ba(OH)_2$ (moles) / Aspartic acid (moles) | Reaction time (hrs.) | Yield based on aspartic acid (%) | Yield based on urea (%) |
|---|---|---|---|---|---|
| 1 | 1.5 | 0.8 | 3 | 44.5 | 29.8 |
| 2 | 1.5 | 0.8 | 5 | 48.8 | 32.7 |
| 3 | 1.5 | 1.0 | 3 | 43.2 | 28.9 |
| 4 | 1.5 | 0.57 | 3 | 33.2 | 22.2 |
| 5 | 1.0 | 0.8 | 4.5 | 39.4 | 39.4 |
| 6 | 2.0 | 0.8 | 3 | 57.5 | 28.8 |
| 7 | 3.0 | 0.8 | 3 | 63.5 | 21.0 |
| 8 | 1.5 | 0.8 | 8 | 45.6 | 30.2 |
| 9 | 0.83 | 0.8 | 5 | 32.4 | 38.6 |
| 10* | 1.0 | 0.8 | 4.5 | 36.3 | 36.3 |

* Urea was divided into three portions and added at intervals of 1 hr.

was 1 to 0.8, and that the reaction time of longer than 5 hours was unnecessary for better yield. The conditions of experiment No. 5 were therefore adopted for the isotopic synthesis of ureido[$^{14}$C]succinic acid.

The synthesis of orotic acid from ureidosuccinic acid was carried out according to the original method of Nyc and Mitchell3) except the third step in which the amount of bromine used was only 32% of that theoretically required.* A mixture of 5-carboxymethylhydantoin and 0.27 mole excess bromine in glacial acetic acid gave 5-carboxymethylidenehydantoin (IV) in 60~70% yield.

The overall yield of orotic[2-$^{14}$C] acid from urea[$^{14}$C] obtained by the process described above was 15.7%. The product containing one mole of water was identified by the decomposition point of 320°(uncor.) and the ultraviolet absorption spectrum which was in good accord with that described by Nyc and Mitchell6). The analytical data of non-labeled orotic acid synthesized by the same method are given in the Experimental section. In the descending paper chromatography developed with a solvent system of butanol, ethanol, formic acid, and water (5:1.5:1:2.5), orotic[2-$^{14}$C] acid showed one radioactive spot at Rf 0.44 as described by Leone *et al.*7)

The procedures of all steps can be carried out without special technique and apparatus from the viewpoint of the isotopic synthesis. Besides the above, ureido-[$^{14}$C]succinic acid, which is also utilized as a precursor of nucleic acid pyrimidines, can be obtained. The incorporation of these isotopic precursors using regenerating pigeon liver slices and homogenates are now being examined in this laboratory and preliminary data showed a very successful incorporation.

The authors would like to express their appreciation to the members of the Tokyo Research Laboratory of Gohei Tanabe & Co. Ltd. for the analytical data.

## Experimental

**Ureido[$^{14}$C]succinic acid (II)**—1.65 g. of aspartic acid, 0.75 g. of urea with 1 mc. of radioactivity and 3.08 g. of $Ba(OH)_2 \cdot 8H_2O$ dissolved in 37.5 cc. of water were refluxed for 4.5 hrs., and acidified with 3 cc. of conc. HCl. After standing overnight, the resulting crystals were collected and recrystallized from water. Yield, 0.797 g.(36.2%) of m.p. 178~180°. Specific activity was 77,100 counts/min./micromole with Q-gas flow counter.

**5-Carboxymethylhydantoin[2-$^{14}$C] (III)**—0.75 g. of (II) was dissolved in 2.5 cc. of 20% HCl and the mixture was evaporated nearly to dryness on a steam bath. The residue was recrystallized from water. Yield, 0.612 g.(91.0%) of m.p. 210~215°.

**5-Carboxymethylidenehydantoin[2-$^{14}$C] (IV)**—A mixture of 0.612 g. of (III), 0.79 g. of bromine and 2.5 cc. of glacial acetic acid was heated at 100° for 2 hrs. in a sealed tube. The precipitated product

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* This may have been a typographical error.

6) J.F. Nyc, H.K. Mitchell: J. Am. Chem. Soc., **69**, 674(1947).

7) E. Leone, E. Scals: Boll. soc. ital. biol. sper, **26**, 1223(1950).

was filtered off and suspended in 7.7 cc. of boiling water for 20 mins. Water was added to the boiling suspension until all of the solid had dissolved. On cooling, (IV) crystallized out. Yield, 0.374 g. (62.0%).

**Orotic〔2-$^{14}$C〕 Acid (V)**—0.374 g. of (IV) was dissolved in 12.5 cc. of 1*M* KOH and allowed to stand in a constant temperature bath at 64° for 2 hrs. On acidifying the solution with conc. HCl, orotic-〔2-$^{14}$C〕 acid·$H_2O$ precipitated out and was recrystallized from water. Yield, 0.323 g. (77.4%) of m.p. 320°(decomp.). Specific activity of the product was 72,400 counts/min./micromole by Q-gas flow counter.

Non-labeled orotic acid, synthesized by the same foregoing method, gave the following analytical values: *Anal.* Calcd. for $C_5H_4O_4N_2 \cdot H_2O$: C, 34.46; H, 3.47; N, 16.15. Found: C, 34.70; H, 3.56; N, 16.03.

## Summary

Ureido〔$^{14}$C〕succinic acid and orotic〔2-$^{14}$C〕 acid, which are metabolic intermediates for nucleic acid pyrimidines, were synthesized by an improved method of Nyc and Mitchell starting with aspartic acid and urea〔$^{14}$C〕, which is more easily available than potassium cyanate〔$^{14}$C〕. The yield of orotic〔2-$^{14}$C〕 acid was 15.7% based on urea〔$^{14}$C〕.

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## **Tatsuo Kariyone and Keiichiro Hayashi**: Studies on the Microanalysis of Essential Oil Components. I. The Paper Chromatography of Terpene Alcohol by using Anthranilate.

(*Pharmaceutical Institute, Medical Faculty, University of Kyoto**)

All but a few of the reports in the past few years on the application of paper chromatography of alcohols were of compounds with larger carbon number such as terpene alcohol, obtaining well-separated spots on paper, while all these methods resulted in fine chromatograms with lower alcohols.[1,2]

In the present experiment, paper chromatography of terpene alcohols was carried out, using anthranilates which reveals dark violet spots under ultraviolet light or yellow coloration by spraying Ehrlich's reagent and a few micrograms of sample can be readily detected. Since esters of anthranilic acid are distributed widely among plants, e.g. in grape juice or as a component of miscellaneous essential oils such as neroli-bigarade, jasmin, ylang-ylang, and sweet orange oils, and since the synthetic anthranilates are also used widely in perfumes, their detection is becoming more important in recent years.

The method of Verley[3] or Roviralta[4] was applied after minor improvement by the authors, in which the esters of corresponding alcohols were obtained in higher yield by ester-exchange reaction between methyl anthranilate and alcohols using metallic sodium or sodium hydroxide. While the reaction was complete in three hours in the cases of borneol, citronellol, menthol, benzyl, and decyl alcohol, the others, among them geraniol, had to be heated at least five or six hours. The sample mix-

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* Yoshida-Konoe-cho, Sakyo-ku, Kyoto(刈米達夫, 林敬一郎).

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3) A. Verley: Bull. soc. chim. 〔4〕, **41**, 788(1927).

4) R. Roviralta: A. Stpul. Span. 210,549(Dec. 39, 1953).