Notes

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Sadatake Kato and Kunio Kurata: Application of a New Method to Synthesis of Radioactive Sodium 3,5-Diiodofluorescein (3,5-^{131}I).

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In a chemical preparation of a radioactive organic compound by means of isotopic exchange reaction or chemical synthesis, the improvement of the radioactive yield is most significant not only for saving the expensive radioisotope but for the diminution of the involved radiation hazards during the reaction and the waste disposal. In the isotopic exchange reactions or additional reactions to unsaturated bonds, this can be achieved by increasing the specific activity of the original radioisotopes. However, in a substitutional reaction such as $RH+I^*_2 \rightarrow RI^*+HI^*$, where I^* indicates radioisotope, the maximum radiochemical yield is theoretically 50% regardless of the specific activity of the radioisotope. In this paper, a new device for increasing the yield is studied with synthesis of radioactive sodium 3,5-diiodofluorescein (3,5-^{131}I). In this method, the radioactive iodine was at first added with very small amount of the carrier iodine to sodium fluorescein (equation 1 in Table I) and the produced radioactive hydroiodic acid was mostly converted into radioactive iodine by exchange reaction with larger amount of stable iodine which was added after the reaction (equation 2 in Table I), and reacted again with sodium fluorescein (equation 3 in Table I). By the repetition of the steps, the theoretical radiochemical yield can be infinitively increased. For example, by a single exchange reaction with stable iodine of nonuple the quantity of the iodide produced, the theoretical yield is 72.5% and by a single repetition of the step with the same proportion, the theoretical yield is increased as much as 85%.

Table I. Application of the Method to Synthesis of 3,5-Diiodofluorescein (^{131}I)

No.	Equations			Yield[a]
(1)	$20FH_2$[b] $+40{}^{131}I_2$	$\longrightarrow$	$20F{}^{131}I_2$[c] $+40H{}^{131}I$	50
(2)	$40H{}^{131}I+180I_2$	$\longrightarrow$	$18{}^{131}I_2+4H{}^{131}I+162I_2+36HI$	
(3)	$90FH_2+18{}^{131}I_2+162I_2$	$\longrightarrow$	$9F{}^{131}I_2+81FI_2$[d] $+18H{}^{131}I+162HI$	
total	$110FH_2+40{}^{131}I_2+180I_2$	$\longrightarrow$	$29F{}^{131}I_2+81FI_2+22H{}^{131}I+198HI$	72.5

a) Maximum theoretical yield based on radioactivity
b) Sodium fluorescein
c) Sodium 3,5-diiodofluorescein (^{131}I)
d) Sodium 3,5-diiodofluorescein

This deviced method can also be applied efficiently to other substitutional reactions provided the following conditions are satisfied.

(1) The reaction velocity of the substitution of the radioisotope is far more rapid than the velocity of the reaction between the radioactive atoms in the labeled compounds and the other isotopic atoms in the reaction system.

(2) The velocity of the isotopic exchange reaction between the reactive and the inert form of the isotope is far more rapid than that of the substitution reaction of the isotope.

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Experimental

Measurement of Radioactivity——Liquid samples were counted either with a well type ionization chamber (Mod. 1-4050/201, Ohkura Elec. Co.) or with a well type scintillation counter (Mod. EA 14, Kobe Kogyo Co.) and compared with a standard solution under the same geometrical conditions. To determine the percentages of radioactivities of each spot on parer chromatogram strips, the strips were scanned with a paper chromatogram scanner (Aloka, Mod. PCS 2) and areas of each peak were measured with a planimeter.

Preliminary Experiments——Several preliminary experiments were done to determine the applicability of the deviced method to this reaction.

Exp. 1. Exchange Reaction between Iodine and Iodide——To a solution of 0.1 g. of stable iodine in 10 ml. of $CHCl_3$, 10 ml. of $1/15M$ phosphate buffer, pH=7, containing a tracer amount of Na ^{131}I was added. 99.4% of the total radioactivity was transferred to $CHCl_3$ layer after 1 min. stirring.

Exp. 2. Exchange Reaction between Iodine Atoms of 3,5-Diiodofluorescein and Iodine or Iodide——A solution of 8 mg. of sodium diiodofluorescein and 4 mg. of sodium radioiodide (^{131}I) in 1 ml. of $1/15M$ phosphate buffer, pH=7, was added to a solution of 13 mg. of radioactive iodine in 3 ml. of $CHCl_3$ and stirred for 10 min. The aq. layer was separated and washed with 3 ml. $CHCl_3$. Diiodofluorescein was recollected from the layer with little radioactivity.

Exp. 3. Reaction Velocity of Iodination of Sodium Fluorescein——To a round bottom flask containing 1 ml. of 0.5% radioactive iodine in $CHCl_3$, 1 ml. of 0.5% sodium fluorescein in $1/15M$ phosphate buffer of pH=7 was added quietly and began stirring. After an exact period of stirring, the reaction was stopped by adding 2 ml. of conc. NaOH to the mixture. The rate of the reaction for the stirring periods were determined by ascending paper chromatography of the aq. layer using 75% MeOH as developer.

The control experiment without sodium fluorescein showed almost all radioactivity was in the aq. layer as iodide form in this experimental condition. The result is shown in Fig. 1.

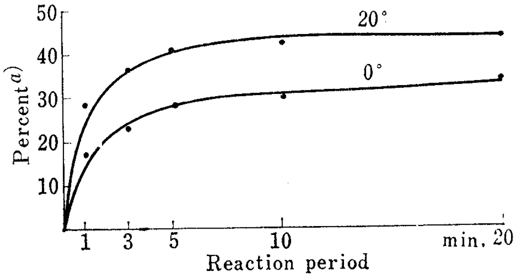

Fig. 1. Iodination of Fluorescein

a) Percentage of radioactivity as 3,5-diiodofluorescein (^{131}I)

The Condition of the Following Experiments

Apparatus——The reactions were done stirring in a round bottom flask with a glass stopper, placed in a bath on a magnetic stirrer.

Reagents——Radioactive iodine solution : 0.5% iodine in $CHCl_3$ solution containing a tracer amount of ^{131}I. Iodine solution : 0.5% iodine solution in $CHCl_3$. Fluorescein solution : 0.5% solution of sodium fluorescein in $1/15M$ phosphate buffer, pH=7.

Identification——Rf values of the related compounds in ascending paper chromatography using the mixture of $BuOH:H_2O:EtOH$:conc. NH_4OH (200:88:40:2) are about as follows; yellow spot of sodium fluorescein, 0.35; orange spot of sodium diiodofluorescein, 0.39; pink spot of sodium tri- or tetra-iodofluorescein (?), 0.46; iodide, 0.52.

Synthesis of Radioactive Diiodofluorescein (3,5-^{131}I)

Exp. 4. Ordinary Method1)——13.5 ml. of the radioactive iodine solution was added to 10 ml. of the fluorescein solution. After 20 min. stirring at room temperature, the aq. layer was separated and washed with $CHCl_3$. To the aq. layer, 10 ml. of 10% $KHSO_4$ and 10 ml. of AcOEt were added and radioactive diiodofluorescein was extracted into AcOEt, which was washed repeatedly with dil. HCl until the radioactivity in the acid solution became neglegible. 46% of the total activity used was found in AcOEt layer, while 52% of the activity in aq. layer of the reaction mixture, 0.7% in combined $CHCl_3$ layer and 1.3% in acid layer were determined.

Exp. 5. Application of the Method at Room Temperature——To 10 ml. of the fluorescein solution, 0.1 ml. of the radioactive iodine solution was added at first. After 10 min., 1 ml. of the iodine solution was added to the mixture. Finally, after more 10 min., 12.4 ml. of the iodine solution was again added to the mixture and continued stirring for another 10 min. Then, the reaction mixture was treated as Exp. 4 and 50.4% of the total activity was found in AcOEt layer.

Exp. 6. Modification of the Method——To 0.1 ml. of the fluorescein solution, 0.1 ml. of the radioactive iodine solution was added. After 10 min., the temperature was decreased to 0° and 1 ml. of the

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1) J. Vigne, J. Fondarai : Bull. Soc. Chim. France, **1953**, 331, (1953).

iodine solution was added and kept for 3 min. After raising the temperature to 20°, 1 ml. of the fluorescein solution was added and reacted for 10 min. The temperature was again decreased to 0°, 12.4 ml. of the iodine solution was added and kept for 3 min., and then 8.9 ml. of the fluorescein solution was added and reacted for another 10 min. at 20°. 70.0% of the total activity was found in AcOEt layer.

Discussion

By the Exp. 1 and Exp. 2, it was shown that this deviced method can be applied to prepare sodium radio 3,5-diiodofluorescein (3,5-^{131}I) to increase the radioactive yield. However, in Exp. 5, where the theoretical maximum yield is 86.5%, the yield was not very much improved. This is mostly owing to the relatively high velocity of the reaction between fluorescein and iodine at room temperature as indicated in Exp. 3. In Exp. 6, the yield was remarkably improved by suppressing the iodination by lowering the temperature during the exchange reaction between iodine isotopes.

Summary

A new method to increase radioactive yield in substitutional reaction is deviced and applied to preparation of sodium radio 3,5-diiodofluorescein (3,5-^{131}I). In this method, produced radioisotope of inert form is converted into active form by isotopic exchange reaction and reused for the reaction. The radioactive yield was increased from 43% to 70%.

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Shoji Shibata, Tsutomu Furuya,[*1] **and Hidefumi Iizuka** : Gas-liquid Chromatography of Lichen Substances. I. Studies on Zeorin.

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The microchemical techniques have been introduced in lichenology to overcome the difficulty in morphological differentiation which frequently arises among lichens.

The spot color reactions on lichens, the microscopical detection of crystal forms of the extracted constituents and the paper[1~3] and thin-layer chromatographies[4] of the extracts have usefully been applied for such a purpose. Gas-liquid chromatography would also be the most useful tool for chemotaxonomical studies of lichens which can be performed using only a minute sample.

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4) *a*) M. Nuno : Journ. Jap. Bot., 38, 196 (1963); *Ibid.*, **39**, 313 (1964). *b*) Y. Asahina : Journ. Jap. Bot., **39**, 209 (1964). *c*) Y. Asahina, M. Nuno : *Ibid.*, **39**, 313 (1964).