

PREPARATION OF TRITIUM-LABELED 2,4-DICHLOROPHENYL PYRROLIDINECARBOXYLATE

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SUMMARY

2,4-Dichlorophenol was labeled with tritium at 6 position by the catalytic reduction of 2,4-dichloro-6-iodophenol. The condensation of 2,4-dichlorophenol-6-³H with N-chlorocarbonylpyrrolidine produced 2,4-dichlorophenyl pyrrolidinecarboxylate-6-³H. Distribution of tritium at 6 position in the 2,4-dichlorophenol-6-³H was 95.9%.

INTRODUCTION

2,4-Dichlorophenyl pyrrolidinecarboxylate is one of potent herbicides which are used in paddy field. In order to investigate the metabolic fates of this compound in plants and mammals, we were urged to prepare the compound which was labeled with tritium. Among several methods of tritiation available, catalytic reduction of 2,4-dichloro-6-iodophenol with tritium gas is most suitable.

Various conditions for catalytic reduction of 2,4-dichloro-6-iodophenol on palladium carbon were studied. The results are shown in Table I. The effects of reaction time and amount of hydrogen on the reduction are illustrated in Fig. 1 and 2, respectively.

The rate of reduction increases with the increase of molar ratio of hydrogen gas to the phenol. We selected the ratio 2.5 because the

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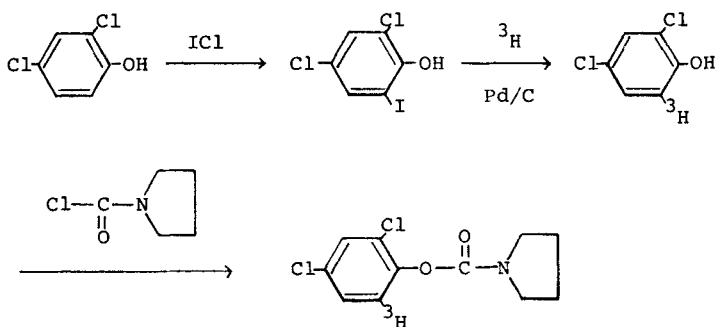


Table I. Catalytic reduction of 2,4-dichloro-6-iodophenol to 2,4-dichlorophenol.

Pd/C	(mg)	20	20	20	*20
Reaction time (hr.)		0.25	0.5	0.5	0.5
2,4-Dichloro-6-iodophenol	(mmol)	0.1	0.1	0.1	0.1
Hydrogen	(mmol)	0.25	0.25	0.25	0.25
KOH	(mmol)	0	0	0.16	0.16
Methanol	(ml)	1.0	1.0	1.1	5.1
Yield	(%)	4.3	5.8	12	11

* Preactivated catalyst

amount of tritium gas was very small (0.8 ml, 0.035 mmol). Preactivation of palladium carbon with hydrogen showed no serious effects on the reduction with hydrogen, but we still used the catalyst after the preactivation to avoid the possibility that a part of tritium gas may be absorbed on the catalyst without such treatment.

2,4-Dichloro-6-iodophenol dissolved in methanol containing potassium hydroxide was reduced with tritium gas over the catalyst under stirring for 4 hr. The radiochemical yield of crude product obtained was 6.61%. Pure 2,4-dichlorophenol- ^3H was separated from the crude product by distillation and fractional extraction. From 2 Ci of trit-

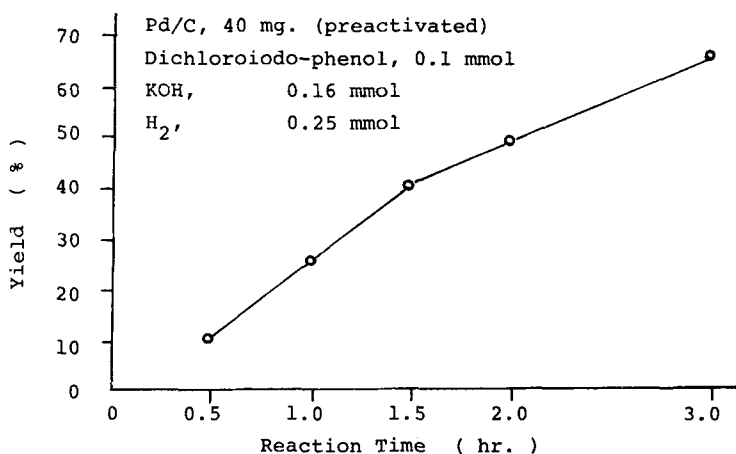


Fig.1. Effect of reaction time on the catalytic reduction.

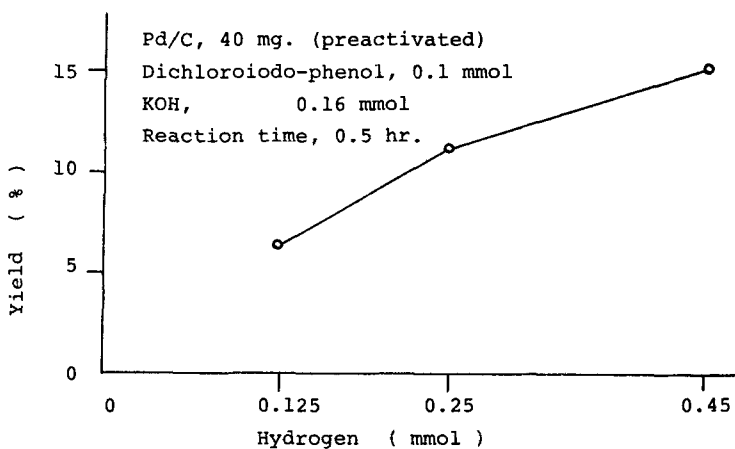


Fig.2. Effect of hydrogen amount on the catalytic reduction.

ium, 56 mCi of 2,4-dichlorophenol-³H was obtained. In order to confirm the labeled position of the 2,4-dichlorophenol-³H¹⁾, the compound was iodinated back to the starting 2,4-dichloro-6-iodophenol whose radioactivity was assayed. It was found that 95.9% of radioactivity was distributed at 6 position of benzene ring. Then, the 2,4-dichlorophenol-6-³H (5.4 mCi) was condensed with N-chlorocarbamoylpyrrolidine in methyl ethyl ketone in the presence of potassium hydroxide under

refluxing for 4 hr. 2,4-Dichlorophenyl pyrrolidinecarboxylate-6-³H obtained was purified by column chromatography. The yield of the carboxylate was 1.14 mCi and its radiochemical purity based on TLC was 97.2%. The radiochemical purity and specific activity of the labeled compound are sufficient for various tracer experiments.

EXPERIMENTAL

Radioactivity measurement was made with a Packard Liquid Scintillation Counter Model 3380.

1) Synthesis of 2,4-dichloro-6-iodophenol²⁾.

A mixture 3.9 g of 2,4-dichlorophenol, 105 g of sodium chloride, 213 g of 70% perchloric acid, 2 g of iodine chloride and 3000 ml of water was refluxed for 30 min. The precipitate produced was collected and recrystallized from n-pentane; white needles, mp. 60°, yield 1.2 g.

2) Catalytic reduction of 2,4-dichloro-6-iodophenol with hydrogen.

A solution of 16.3 mg (0.1 mmol) of 2,4-dichloro-6-iodophenol and 9 mg (0.16 mmol) of KOH in 1 ml of methanol and 20 mg of 5% palladium carbon catalyst were placed in a small flask. The reaction flask and a bulb containing certain amount of hydrogen gas were attached to a vacuum manifold. After cooling the flask with liquid nitrogen, the entire system was evacuated and the reaction system was isolated from the main manifold. Then, the hydrogen gas was allowed to react with the well-stirred mixture for certain time at room temperature. After the reaction, the catalyst was removed and the solution was subjected to the ECD-GLC analysis of 2,4-dichlorophenol.

3) Preparation of 2,4-dichlorophenol-6-³H.

To a suspension of 5% palladium carbon preactivated by hydrogen in 4 ml of methanol, were added 29.3 mg (0.1 mmol) of 2,4-dichloro-6-

iodophenol, 1 ml of methanol and 0.1 ml of 9% KOH-methanol solution. The flask containing the above mixture, an ampoule of tritium gas (2 Ci, 0.8 ml) purchased from Radiochemical Center Amersham, England and a bulb containing hydrogen gas were attached to a vacuum manifold. After the reaction mixture was frozen with liquid nitrogen, the entire system was evacuated. Then the reaction system was isolated from the manifold and the ampoule was opened at the break-seal and the tritium gas was allowed to react with well-stirred reaction mixture for 1 hr. Finally, 11.2 ml of hydrogen gas was introduced into the reaction flask and stirred for additional 3 hr. After the reaction, unreacted hydrogen and tritium were transferred from the reaction flask to a reservoir. The reaction mixture was filtered to remove the catalyst and was subjected to assays. Total radioactivity of the reaction mixture was 132.2 mCi (6.61%) and the chemical yield of 2,4-dichlorophenol based on ECD-GLC analysis was 5.51 mg (34.5%).

The methanol solution containing 2,4-dichlorophenol-6-³H was collected by vacuum distillation of the above reaction mixture at about 25°. To the residue of the distillation was added 24 ml of benzene and the resulting solution was washed with 20 ml of 0.05 N-HCl and 20 ml of water, successively. Radioactivities of each fraction are as follows : recovered methanol solution, 122.5 mCi ; benzene layer, 8.94 mCi ; aqueous layer, 0.43 mCi. From the ECD-GLC analysis of each fraction, a large portion of 2,4-dichlorophenol was found in the methanol fraction and 2,4-dichloro-6-iodophenol was detected only in the benzene layer. In order to purify 2,4-dichlorophenol-6-³H, a mixture of the above methanol solution and 100 ml of 0.5 N-HCl was shaken with 100 ml of n-hexane. The hexane layer was washed with another 100 ml of 0.5 N-HCl, dried over sodium sulfate and the solvent was removed. Total activity of 2,4-dichlorophenol in this fraction was about 30 mCi. The combined acidic layer was extracted with benzene and about 15 mCi of 2,4-dichlorophenol-6-³H was recovered. Specific activity of 2,4-dichlorophenol-6-³H obtained was 1.91 Ci/mmol.

4) 2,4-Dichlorophenyl pyrrolidinecarboxylate-6-³H (phenopylate-6-³H).

To 5.4 mg (0.03 mmol) of sodium 2,4-dichlorophenolate-6-³H (5.4 mCi, 180 mCi/mmol) were added 1.6 ml of methyl ethyl ketone, and 3.9 mg (0.03 mmol) of N-chlorocarbonylpyrrolidine³⁾ obtained by the reaction of pyridine and phosgene in the presence of triethylamine. The mixture was refluxed for 4 hr. After the addition of 20 ml of benzene to the mixture, the solution was washed successively with 20 ml of 0.5N-NaOH and water, and dried over sodium sulfate. Radioactivities of benzene and water layer were 3.6 and 1.8 mCi, respectively. The benzene layer which was concentrated to 5 ml, was transferred to a chromatographic column (2 cm O.D.) which was packed with Florisil, and eluted successively with 50 ml of benzene and 100 ml of benzene-ether mixture (4:1). First 50 ml of the fraction eluted with benzene-ether mixture was collected as the fraction containing the phenopylate.

Two μ l of the fraction was spotted on a silica gel TLC plate and developed with n-hexane-acetone-epichlorohydrin (75:20:5) mixture. After the identification of the spot of the phenopylate by UV light, the scraped silica gels of the fraction containing the phenopylate and the other fraction were extracted with methanol, separately. The radioassays of those extracts showed that 88.4% of radioactivity was distributed on the fraction of the phenopylate.

The solution collected as the fraction containing the phenopylate was concentrated to 20 ml, washed successively twice with 0.5 N-NaOH, once with 0.5N-HCl and water, and dried over sodium sulfate.

Radiochemical purity of the phenopylate in this solution was 97.2% by the method mentioned above. Total activity of the phenopylate purified was found to be 1.14 mCi and its chemical yield based on ECD-GLC analysis was 10.25 mg.

5) Intramolecular distribution of tritium in 2,4-dichlorophenol-6-³H.

2,4-Dichloro-6-iodophenol was derived from 2,4-dichlorophenol-6-³H by the procedure described above. Specific activities of 2,4-

dichlorophenol-6-³H and 2,4-dichloro-6-iodophenol were 0.116 and 0.005 μ Ci/mmol, respectively. Therefore, 95.9% of tritium in the 2,4-dichlorophenol-6-³H was distributed at 6 position and the rest would have been at 3 and/or 5 positions.

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