

## TRITIATION OF BENZOIC ACID BY ${}^6\text{Li}(n, \alpha){}^3\text{H}$ REACTION WITH ENRICHED LITHIUM-6

Kunio Oohashi, Norio Nogawa\*, Yoshihiro Makide\*,  
Naotake Morikawa\*, and Misiroku Izumo\*\*

Laboratory of Radiopharmaceutical Chemistry,  
Faculty of Pharmaceutical Sciences, Chiba University,  
Yayoi, Inage-ku, Chiba 263-0022, Japan

\*Radioisotope Center, The University of Tokyo,  
Yayoi, Bunkyo-ku, Tokyo 113-0032, Japan

\*\*Japan Atomic Energy Research Institute,  
Tokai-mura, Naka-gun, Ibaraki-Pref. 319-1195, Japan

### SUMMARY

Lithium benzoate containing 95 atom% lithium-6 (abbreviation: enriched) was tritiated by neutron irradiation in a pile. The degree of tritium incorporation to the benzene ring was 6.8 to 7.6 times that for the benzoate of natural isotopic abundance (abbreviation: natural abundance) according to the sample size, i.e., the geometries of irradiation samples were compared under the same thermal neutron fluence. The computed self-shielding effect for incident neutrons was negligible in the irradiation of the latter benzoate, while in the case of the former one rather large effects were observed in thick samples used in the present experiment. The tritium distribution in the benzene ring showed a slight ortho orientation in the irradiation of enriched  ${}^6\text{Li}$  compound, but was almost uniform in the other compounds.

Key words: Thermal neutron fluence, Self-shielding effect, Tritium,  
Lithium-6,  ${}^6\text{Li}(n, \alpha){}^3\text{H}$ , Lithium benzoate

### Introduction

The major concerns respecting recoil tritium atoms from nuclear reactions have been centered on study of the reaction mechanism. The nuclear recoil techniques, on the other hand, have been employed for labeling of such complex compounds as biological substances<sup>1-4</sup>. The tritium

atom can be conveniently formed by the nuclear processes,  ${}^6\text{Li}(n, \alpha){}^3\text{H}$  and  ${}^3\text{He}(n, p){}^3\text{H}$ . The techniques are subject to a major drawback in that the specific activity of the products is usually too low to be of use as a tracer for various purpose. This nuclear recoil method, however, is useful for labeling of organic and biological substances unless very high specific activities are required. We previously studied the tritium labeling of triphenylmethane by both of the above nuclear reactions with regard to the labeling efficiency and for use as a radioactivity standard<sup>3</sup>. Tetrodotoxin was also tritiated by the  ${}^3\text{He}(n, p){}^3\text{H}$  reaction. The purified [ ${}^3\text{H}$ ]tetrodotoxin showed a specific activity of  $3 \text{ MBq g}^{-1}$  and was able to be used to investigate the anatomical distribution of tetrodotoxin in pufferfish<sup>4</sup>.

Recently, lithium carbonate containing 95 atom% lithium-6 has been available. In the present work, the tritium labeling by irradiation of the enriched lithium-6 was studied with benzoic acid from the viewpoint of labeling techniques. The self-shielding effect on the incident neutron was estimated for both the natural abundance and enriched systems in order to understand the degree of the labeling.

### Experimental

Lithium benzoate was prepared by the reaction of purified benzoic acid with lithium carbonate in an aqueous solution. Lithium carbonate containing lithium-6 in a natural isotopic abundance was obtained from Nihon Rikagaku Yakuhin Co. Ltd. A mass spectrometric analysis showed that the isotopic abundance of lithium-6 was 7.42 atom%. Calcd for  $\text{C}_7\text{H}_5\text{O}_2\text{Li}$  (in 7.42 atom%  ${}^6\text{Li}$ ): C: 65.65, H: 3.94. Found: C: 65.64; H: 4.00. Lithium carbonate containing enriched lithium-6 was supplied from Isotec Inc. and the nominal isotopic abundance was 95 atm%. Calcd for  $\text{C}_7\text{H}_5\text{O}_2\text{Li}$  (in 95 atom%  ${}^6\text{Li}$ ): C: 66.11; H: 3.96. Found: C: 65.98; H: 3.98.

Quartz ampoules of 20 mm o. d. and 80 mm length were used for irradiation. In each quartz ampoule was placed about 0.5 g of lithium benzoate to obtain the intramolecular tritium distribution. The ampoules were sealed under an air pressure of ca. 20 mPa ( $10^{-4}$  mmHg). One  ${}^{59}\text{Co}$  activation detector was attached to the outer surface of each ampoule. To estimate the self-shielding effect, an additional two sets of samples containing, respective, five different amounts of natural or enriched lithium-6 benzoates were prepared by similar procedures.

Irradiation was carried out in the T-pipe of the JRR-4 reactor for the runs of intramolecular tritium distribution (nominal thermal neutron fluence rate:  $6 \times 10^{13} \text{ cm}^{-2} \text{ sec}^{-1}$ , nominal cadmium ratio: 4) and through

the pneumatic tube of the JRR-3 (nominal thermal neutron fluence rate:  $6 \times 10^{13} \text{ cm}^{-2} \text{ sec}^{-1}$ , nominal cadmium ratio: 26) at Japan Atomic Energy Research Institute.

After irradiation, the natural  $^6\text{Li}$ -containing benzoate was light brown, whereas the enriched one was dark brown. The irradiated lithium benzoate was added to about 200ml of water, extracted with ether to remove possible organic impurities, acidified, and extracted again with ether. The recovered tritiated benzoic acid was treated with activated charcoal, sublimed, followed by repeated crystallization from water to be a constant count as described in the previous paper<sup>(6)</sup>. Labile tritium at the carbonyl position is supposed to have been removed by exchange with hydrogen of water. In the case of the samples to obtain the intramolecular tritium distribution, the irradiated lithium benzoate was removed from the quartz ampoule, dissolved in aqueous alkali, and submitted to reverse isotope dilution analysis in order to obtain the chemical yield of tritium-labeled benzoic acid.

The thermal neutron fluence rate for the irradiated sample was determined from the intensity of the gamma rays from  $^{60}\text{Co}$  produced in the  $^{59}\text{Co}$  activation detector ( $^{59}\text{Co}$ -Al alloy containing 0.475% of  $^{59}\text{Co}$ ). Gamma rays were counted with a Ge(Li) semiconductor detector (Canberra Corp.). The overall uncertainty of the computed thermal neutron fluence is within 7.6%.

The specific activity of tritium-labeled benzoic acid from each sample was determined by using [ $^3\text{H}$ ]n-hexadecane obtained from Amersham International plc as a radioactivity standard. To determine the intramolecular tritium distribution in the benzene ring of the irradiated enriched  $^6\text{Li}$ -containing benzoate, the recovered and purified benzoic acid was converted into p-bromoacetanilide, 2,4,6-tribromoaniline, and 3,5-dinitrobenzoic acid as described in Fig. 1. Tritium is not contained, of course, in the amino positions as well as in the carboxyl position.

Radioactivity of tritium was measured in ACS II scintillator from Amersham by liquid scintillation system (Aloka 3050 or Packard 2000 CA/LL). Each sample was counted for a period sufficient to reduce the statistical error to 0.5% or less. The specific activity of tritium-labeled compounds was determined from the count rate by making quenching corrections as described in the previous paper<sup>(6)</sup>

### Results and discussion

Lithium-6 has a rather large cross section of 945 barn for the  $^6\text{Li}(n, \alpha)^3\text{H}$  reaction and the present irradiation samples were thick targets. They

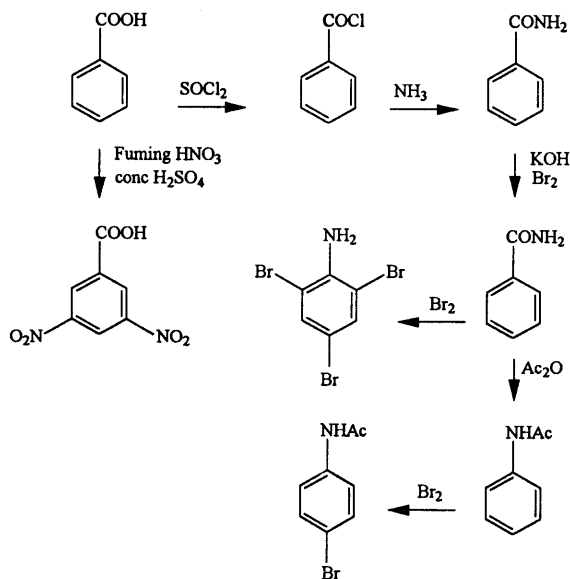


Fig. 1 Synthetic scheme of derivatives

contain ca. 20 mg of lithium-6 in 0.5 g of lithium benzoate in the  $^6\text{Li}$  enriched system and have the dimensions of about  $1\text{ cm}^3$ . The self-shielding effects were computed for the above-mentioned two sets of samples using a version of the DOT 3.5 code. The two sets consisted of five pairs of samples having similar sample sizes, i.e., similar geometry for the incident neutrons. Table 1 shows the computed self-shielding effects and the specific activities of benzoic acid recovered from the irradiated lithium benzoates.

Table 1 Self-shielding effect and specific activity of benzoic acid from irradiated lithium benzoate

Sample	Weight of PhCOOLi (mg)	Thermal neutron fluence rate $\times 10^{13}$		Self-shielding effect <sup>3)</sup>	Specific activity of PhCOOH ( $\times 10^8\text{ Bq mol}^{-1}$ )	
		Incident <sup>1)</sup>	Inner <sup>2)</sup>		Measured	Corrected <sup>4)</sup>
Natural Li-6 -containing	206	4.47	4.36	0.975	1.41	1.41
	292	4.43	4.30	0.971	1.40	1.41
	496	4.26	4.11	0.964	1.40	1.47
	697	4.24	4.08	0.961	1.43	1.51
	901	4.44	4.25	0.957	1.40	1.41
Enriched Li-6 -containing	202	4.21	3.27	0.777	10.1	10.7
	304	4.04	3.01	0.745	9.29	10.3
	501	3.77	2.56	0.679	8.47	10.0
	697	3.66	2.40	0.656	8.01	9.78
	895	3.53	2.25	0.637	7.55	9.56

1) Thermal neutron fluence rate incident to the surface of each quartz ampoule.

2) Thermal neutron fluence rate averaged over the whole volume of lithium benzoate in each quartz ampoule.

3) The ratio of inner thermal neutron fluence rate to incident one.

4) The values corrected as  $(4.47 \times 10^{13} / \text{incident fluence rate}) \times$  (corresponding specific activity).

The "inner neutron" in the 4th column expresses the averaged thermal neutron fluence rate which was calculated by the weighted mean method with thickness over the whole volume of lithium benzoate in each quartz ampoule. In the case of the natural  ${}^6\text{Li}$ -containing system, the inner fluence rate was close to unity almost independently of the thickness of sample, whereas on passing the enriched- ${}^6\text{Li}$  containing sample the fluence rate was greatly reduced and in addition with increasing thickness of the sample. That is, large self-shielding effects were observed in the enriched system. The measured specific activities were corrected for the incident thermal neutron fluence rate. The corrected values fairly paralleled the shield effect of the corresponding samples. The values divided by the corresponding inner fluence rate were approximately constant in both systems, respectively, although the values were slightly higher for the thicker samples in the enriched system. This suggests that the estimated values for the self-shielding effect are valid.

Shown in Table 2 are the irradiation conditions, specific activities of tritium-labeled benzoic acid, and chemical yields obtained by the reverse dilution analysis in the tritium distribution runs.

Table 2 Irradiation conditions of lithium benzoate and specific activity and chemical yield of [ ${}^3\text{H}$ ]benzoic acid

Run No.	Isotopic abundance of ${}^6\text{Li}$ (atom%)	Irradiation time (sec)	Thermal neutron fluence ( $\text{n cm}^{-2}$ )	[ ${}^3\text{H}$ ]Benzoic acid Specific activity ( $\times 10^8 \text{ Bq mol}^{-1}$ )	Chemical yield (%)
			$\times 10^{15}$		
1	95	60	2.23	6.47	93
2	95	300	2.20	6.61	95
3	7.42	300	13.1	5.08	100
4	7.42	300	12.4	5.12	99

The enriched system had a somewhat lower chemical yield compared to the natural isotopic abundance system. Lithium benzoate is decomposed by recoil tritium atoms and alpha particles as well as by background pile radiation during the irradiation, and in addition presumably by the heat from the nuclear reaction. The decomposition may result in degradation products with very high specific activity<sup>2)</sup>. Between two lithium-6 systems the ratio of specific activity is about 7, which is similar to the values shown in Table 1, in case of comparison under the equivalent neutron fluence. As seen above, in contrast with the natural abundance system, the enriched system underwent radiation damage in the short irradiation time. Such situation prevents the application of the latter system to labeling. To

reduce the radiation damage, approaches to labeling under milder conditions are likely required. The total amount of tritium produced by the nuclear reaction in each quartz ampoule was not determined experimentally, but the calculated values suggested that the extent of tritium incorporation to benzoic acid was about 30% in the enriched  $^6\text{Li}$  system.

The tritium distribution in the benzene ring was examined in the enriched system. The distribution was calculated from the specific activities of benzoic acid, p-bromacetanilide, and 2,4,6-tribromoaniline in Table 3 as described in the previous paper<sup>6)</sup> and shown in Table 4 for comparison, together with those from both a mixture of benzoic acid with lithium carbonate and lithium benzoate in the natural isotopic abundance system<sup>7)</sup>. The extent of tritium labeling at the carboxyl position was not estimated. Labile tritium at the position was removed prior to the distribution runs.

Table 3 Specific activities of tritiated benzoic acid and its derivatives

Compound	Specific activity ( $\times 10^8 \text{Bq mol}^{-1}$ )	
	run 1	run 2
Benzoic acid	6.47	6.61
P-Bromoacetanilide	5.24	5.32
2,4,6-Tribromoaniline	2.30	2.34
3,5-Dinitrobenzoic acid	4.08	4.09

The distribution was reproduced within the experimental error described in the footnote below Table 4 in duplicate runs and it indicated a slight orientation as seen in a moderated system<sup>8)</sup> as compared to a uniform one in the system containing lithium-6 in natural abundance<sup>7)</sup>. However, the reason for the difference in the distribution between the two systems is not obvious.

Table 4 Tritium distribution in the benzene ring of benzoic acid

Run No. or chemical system	tritium distribution		
	o (%)	m (%)	p (%)
1	45	36	19
2	45	35	20
PhCOOLi	40	39	21 <sup>7)</sup>
PhCOOH + $\text{Li}_2\text{CO}_3$	40	39	21 <sup>7)</sup>

Errors are estimated to be within  $\pm 1\%$  at each carbon position.

## References

- 1) Evans E. A., "Tritium and Its Compounds", 2nd Ed. Butterworths, London, 430(1974)
- 2) Tang Y. -N., "Volume 4. Tritium in Organic Chemistry" in "Isotopes in Organic Chemistry", Ed. By E. Buncl and C. C. Lee, Elsevier, 85(1978)
- 3) Oohashi K., Ohmuro M., Maruyama H., Nogawa N., Matuoka H., Moki T., Moriya T., and Morikawa N., The 29th Symposium on Radiochemistry (in Japan), Abstract papers, 187(1985)
- 4) Watabe S., Sato Y., Nakaya N., Nogawa N., Oohashi K., Noguchi T., Morikawa N., and Hashimoto K., *Toxicon*, 25: 1283(1987)
- 5) Nogawa N., Oohashi K., Matuoka H., Hoizumi K., Moki T., Moriya T., and Morikawa N., *Radioisotopes*, 36: 157(1987)
- 6) Saito T., Oohashi K., and Morikawa N., *ibid.*, 30: 596(1981)
- 7) Morikawa N., Oohashi K., and Shimamura O., *Chem. Commun.*, 899(1966)
- 8) Oohashi K. and Morikawa N., The 20th Symposium on Radiochemistry (in Japan), Abstract Papers, 54(1976)