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Studies on Hydrogen Exchange. X.[1] Tritium-Labeling of Carcinogenic 4-Nitroquinoline 1-Oxide and Related Compounds

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This paper describes the tritium-labeling of some quinoline derivatives which are of urgent use as tracers in the study of carcinogenic mechanism of 4-nitro- and 4-hydroxyamino-quinoline 1-oxides which are well known to be potent carcinogens.

In general, tritium-labeling can be synthetically achieved by i) Wilzbach method (or tritium gas exposure method), ii) ionic hydrogen-tritium exchange method, iii) catalytic hydrogen-tritium exchange method using the VIII transition metals such as platinum, iv) hydrolysis of organo metal compounds such as C-MgX and C-Li with tritium, v) reductive dehalogenation or desulfurylation of C-halogeno or sulfur compounds, and other chemical synthetic ways. In the present study, quinoline derivatives related to carcinogenesis of 4-nitroquinoline 1-oxides were tritiated by the first and second methods.[3]

Acid-Catalyzed Hydrogen-Tritium Exchange

The acid-catalyzed hydrogen exchange which involves an electrophilic attack of tritium cation ($^3H^+$ or $^3H_3O^+$) can generally be applied to labeling aromatic compounds which are activated toward electrophilic reactions. Although quinoline ring is not so reactive to electrophilic substitution reaction, some of the ring hydrogens are known to be exchangable with the active hydrogens in the reaction medium under rather drastic conditions; in strongly acidic medium at a high reaction temperature.[4]

In order to determine the reaction condition to be chosen for the hydrogen-tritium equilibrium, hydrogen-deuterium exchange was investigated of some quinoline 1-oxides by treatment with sulfuric acid-d_2 at 150—260°. Deuteration reaction was conveniently traced by nuclear magnetic resonance spectroscopy, assignment of the signals being done by analyzing the signal shape without ambiguity.[4]

Quinoline 1-oxide reached to the exchange equilibrium by heating in 90% or more concentrated sulfuric acid-d_2 at 260° for more than 8 hours. Hydrogen at 7-position resisted exchange to a considerable extent and those at 2- and 4-positions were completely inactive. Fortunately, no appreciable decomposition nor side reactions such as sulfonation nor deoxygenation occurred in this process under the condition chosen above. 3-Methylquinoline 1-oxide was deuterated by heating in 90% or more concentrated sulfuric acid-d_2 at 200° for 6 hours. Hydrogens at 5- and 8-positions underwent deuteration, followed by those at 6- and 7-positions. Hydrogens at 2- and 4-positions and in the methyl group were never deuterated under more drastic conditions than that described. Recovery of deuterated 3-methylquinoline 1-oxide was found to be satisfactory. With 6-chloroquinoline 1-oxide, deuterium exchange occurred at 5-position and then at 8-position under the similar reaciton condition to the former. Electrophilic deuteration of this compound seemed to proceed with smaller reaction rate than quinoline 1-oxide and 3-methylquinoline 1-oxide.

1) Part IX: Y. Kawazoe and Y. Yoshioka, *Chem. Pharm. Bull.* (Tokyo), **16**, 715 (1968).
2) Location: *Tsukiji, Chuo-ku, Tokyo.*
3) Highly tritiated 4-nitroquinoline 1-oxide was recently synthesized by S. Baba of the Tokyo College of Pharmacy by catalytic dehalogenation of halogenoquinoline with tritium gas, followed by N-oxidation and then nitration.
4) Y. Kawazoe and M. Ohnishi, *Chem. Pharm. Bull.* (Tokyo), **15**, 826 (1967).

Tritiation of quinoline 1-oxide and 3-methyl derivative were carried out using about 90% sulfuric acid containing tritium oxide, as illustrated in Chart 1.

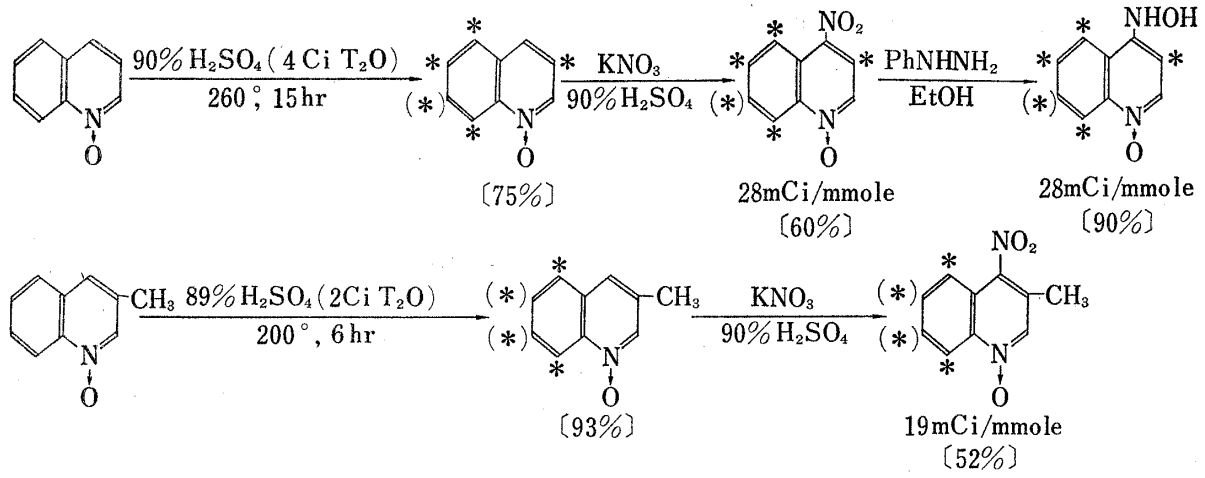

Chart 1. Syntheses of ^{3}H-Labeled Quinoline 1-Oxide Derivatives

*: exchange equilibrium of exchangable hydrogens was completed
(*): exchange equilibrium of exchangable hydrogens was incompleted
[ ]: chemical yield

Six grams of anhydrous quinoline 1-oxide was dissolved in a mixture of 14 g of concentrated sulfuric acid (>95%), 2.3 g of 60% fuming sulfuric acid, and 0.8 ml of tritium-containing water (4 Ci). The mixture was warmed in a sealed tube at 260° ±5° for 16 hours. The reaction mixture was poured into ice, made alkaline with sodium carbonate, and extracted with chloroform. From the chloroform extract, tritiated quinoline 1-oxide was isolated by alumina column chromatographic separation. About 4.0 g of tritiated quinoline 1-oxide thus prepared was nitrated by warming with 2.0 g of potassium nitrated in 15 ml of 90% sulfuric acid at 75° for 2.5 hours. The reaction mixture was treated in an usual manner to afford about 2 g of 4-nitroquinoline 1-oxide, whose specific radioactivity was 28 mCi per mmole. Its isotopic purity was proved to be satisfactory by paper chromatography and successive recrystallizations. The tritiated 4-nitroquinoline 1-oxide thus prepared was derived into the corresponding 4-hydroxyaminoquinoline 1-oxide by reduction with phenylhydrazine.[5] Thus, a half gram of the labeled 4-nitroquinoline 1-oxide was dissolved in 10 ml of ethanol containing 2 ml of phenylhydrazine and warmed at 70° for 2 hours, when yellow precipitates came out. The precipitates were recrystallized as the hydrochloride from a mixture of 5 ml of methanol and 10 ml of water containing 5 ml of concentrated hydrochloric acid. The specific radioactivity of this product was 28 mCi per mmole, indicating that the tritium-incorporated 4-nitroquinoline 1-oxide had not been lost at all during the reduction procedure.

3-Methyl-4-nitroquinoline 1-oxide was labeled in a similar way to the above. One and half grams of anhydrous 3-methylquinoline 1-oxide were dissolved in a mixture of 10 g of concentrated sulfuric acid and 0.4 ml of tritium containing water (2 Ci) and warmed in a sealed tube at 200° ±2° for 6 hours. The reaction mixture was poured into ice, made alkaline with sodium carbonate, and extracted with chloroform. The chloroform extract (about 1.4 g), after complete evaporation of the solvent *in vacuo*, was nitrated in an usual way[6] to afford crude 3-methyl-4-nitroquinoline 1-oxide, which was purified by eluting through an alumina column with benzene, followed by recrystalization from methanol. Yellow needles which weighed 1.0 g melt at 180—181° and its specific radioactivity was 19 mCi per mmole.

5) E. Ochiai and H. Mitarashi, *Ann. Rept. ITSUU Lab.*, **13**, 19 (1963).
6) Y. Kawazoe and M. Tachibana, *Chem. Pharm. Bull.* (Tokyo), **15**, 1 (1967).

Table I. Tritiation of 4-Nitroquinoline 1-Oxide by Tritium Gas Exposure Method[a]

Run No.	Condition	Specific activity (mCi/mmole)
1	1 Ci, 15 days	0.135
2	2 Ci, 15 days	0.225
3	2 Ci, 15 days	0.335

a) Exposed to tritium gas for 15 days at room temperature.

Tritium-Labeling by Tritium Gas Exposure Method (Wilzbach Method)

Tritium gas exposure method is known to be unsatisfactorily applied to tritiation of 4-nitroquinoline 1-oxide.[7] As shown in Table I, 4-nitroquinoline 1-oxide was tritiated at as a low level as less than a half mCi per mmole by exposure of the powdered sample to 1 Ci tritium gas for 15 days at room temperature. Then, gas exposure was done with 4-nitroquinoline 1-oxide coated on an appropriate amount of neutral active alumina.[8] The results are shown in Table II.[9] It was clearly demonstrated that the specific activity was raised upto more than 10 mCi per mmole, multiplied by 50 to 100 compared with those when no catalyst was used. As can be seen in the table, efficiency of the alumina–catalyst depends on the mixing ratio of 4-nitroquinoline 1-oxide to the catalyst. As far as the present experiments are concerned, the highest labeling was achieved when the ratio was "1:8".

Tritiation of 4-nitropyridine 1-oxide was also effectively catalyzed by alumina as shown in Table II.

Table II. Alumina-Catalyzed Tritiation of 4-Nitroquinoline 1-Oxide and 4-Nitropyridine 1-Oxide by Tritium Gas Exposure Method[a]

Run No.		Carrier	Ratio (w/w) (compd. to carrier)	Curries of $^3H_2$ used	Specific activity (mCi/mmole)
4	4-nitroquinoline 1-oxide	Al_2O_3	4/ 1	1	0.076
4	4-nitroquinoline 1-oxide	Al_2O_3	1/ 1	1	0.24
4	4-nitroquinoline 1-oxide	Al_2O_3	1/ 5	1	1.60
5	4-nitroquinoline 1-oxide	none	—	2	0.225
5	4-nitroquinoline 1-oxide	Al_2O_3	1/ 5	2	10.9
5	4-nitroquinoline 1-oxide	Al_2O_3	1/ 8	2	15.2
5	4-nitroquinoline 1-oxide	Al_2O_3	1/10	2	12.5
5	4-nitropyridine 1-oxide	none	—	2	0.493
5	4-nitropyridine 1-oxide	Al_2O_3	1/ 5	2	0.48

a) One to two hundreds miligrams of the compound to be tritiated were coated on alumina and exposed to tritium gas for 15 days at room temperature.

Experimental

Wilzbach Tritiation——In each run of experiments, the samples to be tritiated were placed in respective micro tubes, which were put together in a special breakable ampoule and exposed to tritium gas with an usual Wilzbach tritiation apparatus.[10]

Measurement of Radioactivity——Radioactivity was measured with a liquid scintillation counter, Beckmann DPM–100. The dpm values were calibrated from the observed cpm values by automatic external

7) Private communication from Dr. Tomoyoshi Komai of National Institute of Health (Japan).
8) Aluminium oxide standardized for chromatographic adsorpton analysis according to Brockmann (E. Merck AG, Darmstadt).

standard (AES) method. The scintillators used were a) dioxan–toluene–ethylcellosolve (75:15:10) containing 4.0 g/liter of PPO, 0.4 g/liter of dimethyl–POPOP, and 100 g/liter of naphthalene, b) toluene containing 4.0 g/liter of PPO and 0.3 g/liter of dimethyl–POPOP.

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9) Reproducibility was not so good probably due to variation in the activity of alumina catalyst. Details are now being investigated in our laboratory.
10) S. Okada and O. Tamemasa, *Radioisotopes* (Tokyo), **14**, 42 (1965).

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Prostaglandin E_2 like Activity in Monkey Lung[1)]

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Recently, the occurrence of derivatives of prostaglandin (PG) has been demonstrated in the lungs of some species, such as sheep,[3)] pigs,[3a)] cattle,[4)] guinea pigs,[3a)] monkies,[3a)] and humans.[3a,5)] Prostaglandin F (PGF) series, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) was found and identified in the lungs of all species, whereas prostaglandin E (PGE) series, prostaglandin E_2 (PGE_2) was proved to be present in only two species, namely sheep[3a)] and humans.[5)] The amount of the above compound in these species was found to be approximately one percent of the $PGF_{2\alpha}$ content. It seems of interest to investigate the occurrence and the contents of the PGE series compounds in the lungs of other species.

The present paper reports the occurrence of a compound which has a PGE_2 like activity in the lung of monkies (*Macaca irus*), in addition to $PGF_{2\alpha}$, the presence of which in monkey lungs has been previously reported by Äggård.[3a)]

An acidic lipid substance, which stimulates smooth muscle and reduces blood pressure, was separated from monkey lung by the use of organic solvent distribution, column chromatography and thin-layer chromatography (TLC). Treatment of this lipid compound is briefly outlined in Chart 1.

Fig. 1 shows the distribution of smooth muscle stimulating activity of the active component in fraction X on silver nitrate silicagel plate, which was found to be useful for the separation of the derivatives of PG with different degrees of unsaturation to each other.

1) Part of this work was presented at the Meeting of the Chugoku–Shikoku Branch, Pharmaceutical Society of Japan, Nov. 1968.
2) Location: *No. 78, Shomachi–1–chome, Tokushima.*
3) *a*) E. Äggård, *Biochem. Pharmacol.*, **14**, 1057 (1965); *b*) S. Bergström, F. Dressler, L. Krabisch, R. Ryhage and J. Sjövall, *Arkiv. Kem.*, **20**, 63 (1962); *c*) E. Äggård and B. Samuelsson, *Acta Physiol. Scand.*, **59**, suppl., **213**, 170 (1963).
4) B. Samuelsson, *Biochem. Biophys. Acta* (Amst.), **84**, 707 (1964).
5) S.M.M. Karim, M. Sandler, and E.D. Williams, *Brit. J. Pharmacol.*, **31**, 340 (1967).