Note

Synthesis of High Specific Activity 4-Hydroxyaminoquinoline 1-oxide [$6\text{-}^3\text{H}$][†]

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It was reported that ^3H -4-hydroxyaminoquinoline 1-oxide (4-HAQO) was synthesized from ^3H -quinoline 1-oxide which was tritiated with 90% sulfuric acid containing tritium oxide¹⁾ and that ^{14}C -4-HAQO was formed from ^{14}C -4-nitroquinoline 1-oxide (4-NQO) by the use of diaphorase in the presence of NADH²⁾. These labeled compounds have been used mainly *in vitro* to study the mechanisms of the carcinogenesis of 4-NQO and 4-HAQO²⁻⁴⁾. Recent reports have shown that pancreatic carcinomas can be induced by a single injection of 4-HAQO in rats^{5,6)} and this system provides a useful experimental model for studying pancreatic carcinogenesis. However, the labeled 4-HAQO previously synthesized does not possess a specific activity high enough to study the carcinogenesis *in vivo*. Therefore, it has become desirable to synthesize 4-HAQO with higher specific activity. Baba et al.⁷⁾ have shown that highly tritiated 4-NQO could be synthesized by catalytic dehalogenation of halogenoquinoline with tritium gas, followed by N-oxidation and then nitration. We have studied the synthesis of 4-HAQO [$6\text{-}^3\text{H}$] (I) with high specific activity starting from 6-bromoquinoline (II).

Tritiation of II was carried out using 185 GBq (5 Ci) of tritium gas, as illustrated in Fig. 1. Quinoline [$6\text{-}^3\text{H}$] (III) was obtained by catalytic

dehalogenation of II. N-Oxidation of a mixture of III and unlabeled quinoline added as a carrier with *m*-chloroperbenzoic acid gave quinoline 1-oxide [$6\text{-}^3\text{H}$] (IV) in a good yield (85%). Quinoline 1-oxide [$6\text{-}^3\text{H}$] was then nitrated with potassium nitrate in sulfuric acid, and the crude 4-NQO [$6\text{-}^3\text{H}$] obtained was isolated by column chromatography. 4-NQO [$6\text{-}^3\text{H}$] (V) thus prepared was converted into 4-HAQO [$6\text{-}^3\text{H}$] by reduction with phenylhydrazine. 4-HAQO [$6\text{-}^3\text{H}$] was recrystallized as hydrochloride (I) from a mixture of methanol and water containing concentrated hydrochloric acid. The overall radiochemical yield for this three-step synthesis of I from III amounted to 10.6%. The specific activity of this product was 4.60 GBq (124 mCi) per mmol, and its radiochemical purity was 97%. It is believed that the increase of the amount of tritium gas in catalytic dehalogenation and/or the decrease of that of the carrier added in N-oxidation reaction can result in an increased specific activity. Inactive product similarly synthesized from 6-bromoquinoline as shown in

Fig. 1 Synthesis of 4-hydroxyaminoquinoline 1-oxide [$6\text{-}^3\text{H}$]

[†] 高比放射能4-ハイドロキシアミノキノリン1-オキサイド [$6\text{-}^3\text{H}$] の合成. 森幸雄, 豊吉一美, 伝田阿由美*, 小西陽一*. 岐阜薬科大学, 502 岐阜市三田洞東5-6-1, *奈良県立医科大学附属ガンセンタ一腫瘍病理講座, 634 橿原市四条町 840

Fig. 1 melts at 199°C (analysis calculated for $C_9H_8N_2O_2 \cdot HCl$: C, 50.84; H, 4.27; N, 13.17; found: C, 50.68; H, 4.53; N, 13.10).

Baba et al.⁷⁾ reported that the loss of tritium in tritiated 4-NQO [3.52 GBq (95 mCi)/mmol] synthesis under almost the same conditions was only 2% and the labeled position was ascertained by the infrared and nuclear magnetic resonance spectra of deuterated quinolines which were prepared under the same conditions as for the tritiated compound. It was also reported that the tritium incorporated 4-NQO [1.04 GBq (28 mCi)/mmol] had not been lost at all during the reduction procedure with phenylhydrazine⁴⁾. Consequently, these reports indicate that 4-HAQO [6-³H] synthesized under these conditions is tritiated exclusively at the desired position. Accordingly, it was concluded that the use of I permits detailed studies on the mechanism of carcinogenesis by a single injection of 4-HAQO.

1. Chemicals

6-Bromoquinoline was purchased from Eastman Kodak Co. Rochester, N.Y., and phenylhydrazine from Tokyo Kasei Co. Tokyo. Quinoline, *m*-chloroperbenzoic acid, and alumina for column chromatography were purchased from Wako Pure Chemical Co. Osaka. All other reagents used were of reagent grade.

2. Quinoline [6-³H] (III)

Twenty one milligrams of 6-bromoquinoline (II) was dissolved in 2 ml of ethanol, and 150mg of 5% palladium on alumina catalyst and 185 GBq (5 Ci) of tritium gas were added. The reaction mixture was stirred overnight at room temperature. Labile tritium was removed in vacuo using ethanol as a solvent. After filtration from the catalyst, the product was dried in vacuo and dissolved in 10 ml of ethanol, and 0.7 g of quinoline was added as a carrier. The radiochemical yield of III from II was 83.29 GBq (2.25 Ci, 45%).

3. Quinoline 1-oxide [6-³H] (IV)

After evaporation of the ethanol, III was dissolved in 25 ml of benzene and 1 g of 70% *m*-chloroperbenzoic acid was added slowly. The

reaction mixture was stirred for 2 hours at room temperature and dried under reduced pressure. Ether and water were added to the resultant residue with continuous stirring. *m*-Chlorobenzoic acid and unreacted *m*-chloroperbenzoic acid were extracted to the ether layer, which was further extracted with water. The first and second aqueous layers were combined and evaporated under reduced pressure at below 50°. Ethanol was added to the residue (Quinoline 1-oxide [6-³H]) obtained thus, and again evaporated to dry the product.

4. 4-Nitroquinoline 1-oxide [6-³H] (V)

IV was dissolved in 10 g of 85% sulfuric acid and warmed with a hot water at 65°, and 1 g of potassium nitrate was added slowly. The reaction mixture was stirred for 3 hours at 65° and then poured into ice cold water. The resultant precipitates of the crude V were filtered and chromatographed on an alumina column (1.5×8 cm) with an eluent of CCl_4-CHCl_3 (1:2). The eluted yellow fraction was evaporated and the purified V was obtained in 700 mg yield.

5. 4-Hydroxyaminoquinoline 1-oxide [6-³H]

Then, V was dissolved in 12 ml of ethanol containing 2.8 ml of phenylhydrazine, and warmed at 70° for 2 hours. The resultant yellow precipitates of the product were filtered and recrystallized as a hydrochloride (I) from a mixture of 7 ml of methanol and 10 ml of water containing 7 ml of concentrated hydrochloric acid. The yield of I was 406 mg with 8.79 GBq (237.56 mCi) radioactivity, and its chemical purity was found to be 97% by radioscanography of paper chromatogram (Whatman No. 1, ethyl acetate-ethanol-acetic acid=5:4:1 and ethyl acetate-butanol-water=5:4:1, *R_f* values 0.78 and 0.60, respectively, identical with an unlabeled authentic sample).

In addition, we utilized the tritium labeling service of New England Nuclear Co., Boston, Mass. in the catalytic dehalogenation of 6-bromoquinoline.

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