

SYNTHESIS OF ^{14}C -LABELED INOSINE 5'-PHOSPHATE

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SUMMARY

^{14}C -Labeled inosine 5'-phosphate was synthesized by direct phosphorylation of inosine-8- ^{14}C with phosphoryl chloride followed by hydrolysis of the resulting phosphorodichloridate. Isolation and recrystallization of its disodium salt afforded pure material in 66.8% overall chemical yield.

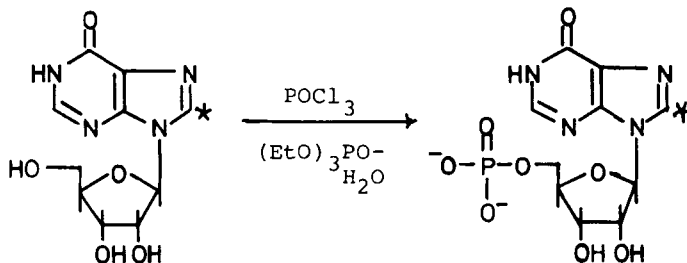
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Various procedures on 5'-phosphorylation of nucleosides have been devised in view of the biological importance of resulting nucleotides.⁽¹⁻⁵⁾ The application of these procedures to the synthesis of nucleotides labeled by a radioisotope is sometimes unsuitable because work-up of a reaction mixture is inadequate for handling a radioisotope. Consequently, it is required to modify these procedures, especially in isolating product.

The phosphorylation of 2',3'-O-isopropylidene nucleosides is generally considered to be a common procedure for the synthesis of nucleoside 5'-phosphate, and is especially good for labeling by isotopic phosphorus, since inosine 5'-phosphate- ^{32}P is now commercially available. Thus contamination by isomeric nucleotides and the loss of isotope is avoided. However, if it is adapted to the synthesis of ^{14}C -labeled nucleotides, one may encounter the some loss of isotope

because the conversion of nucleosides into their O-isopropylidene derivatives does not always result in high yields.^(6,7) Therefore, we undertook to examine direct phosphorylation of inosine in order to test its applicability for 5'-phosphorylation in the one step synthesis of inosine 5'-phosphate labeled with ^{14}C .

As is shown in Scheme 1, inosine-8- ^{14}C (sp.act.: 500 $\mu\text{Ci}/\text{mmole}$) was phosphorylated with phosphoryl chloride in a mixture of triethyl phosphate and water. The resulting product was hydrolyzed and adsorbed on a charcoal. Elution with ethanolic ammonium hydroxide gave ammonium salt of inosine-8- ^{14}C -5'-phosphate, which was made acid by using a cation exchange resin and then the disodium salt by neutralization. Recrystallization of the salt from 50% aqueous ethanol gave pure material, whose overall chemical yield was 66.8% and whose specific activity was 1.41 $\mu\text{Ci}/\text{mg}$ or 554 $\mu\text{Ci}/\text{mmole}$.



Scheme 1.

In this phosphorylation process, the formation of 2' and/or 3'-phosphate can be considered as a by-product. In fact, a trace amount of 2' (3')-phosphate, in a cold run, could be observed on a thin-layer chromatogram (a cellulose F254 plastic sheet supplied by Merck A.G., Darmstadt) developed by using an ascending solvent system of isopropanol-0.1M-boric acid-conc. NH_4OH (v/v; 40:20:10). On the chromatogram, the spot of 2' (3')-isomer ($R_f=0.60$) was completely separable from those of 5'-phosphate ($R_f=0.51$) and inosine ($R_f=0.69$).

The high purity of the final material, inosine 5'-phosphate, after recrystallization, was confirmed by thin-layer, paper partition, and high speed liquid chromatographic techniques.

EXPERIMENTAL

To a cold solution of 1.25 ml of triethyl phosphate containing 230 mg (1.5 mmole) of phosphoryl chloride and 9 μ l of H₂O (measured using a microsyringe) was added 134 mg (0.5 mmole) of labeled inosine (inosine-8-¹⁴C; sp.act., 500 μ Ci/mmmole) with magnetic stirring at 0°. Inosine undissolved at starting time dissolved gradually as the reaction proceeded and the solution became clear after stirring for 4 hrs at 0°. In order to convert the chloride to the acid, the reaction mixture was poured into 50 ml of ice-water and 40% NaOH solution (0.6 ml) was added, and then the solution was neutralized by adding 0.5 N NaOH solution dropwise until the pH maintained 7.0 steadily. At least 2 hrs were necessary for this neutralization. After standing overnight, the pH was brought to 1.0 with conc. HCl and 10 g of charcoal (Norit) were added, then the mixture was stirred for 3 hrs at room temperature. The inosinic acid adsorbed on charcoal was collected by filtration and washed with 200 ml of H₂O. The elution of the labeled inosine 5'-phosphate was carried out with 250 ml of a mixture of EtOH-H₂O-NH₄OH (30:60:10) by stirring for 4 hrs at room temperature. The charcoal was removed by filtration under suction and the filtrate was evaporated by the aid of a rotary evaporator to leave a pale yellow, viscous oil. The oil was dissolved in 4 ml of H₂O and passed through a cation exchange column (Amberlite IR-120, H⁺form, 1 x 10 cm). Further elution with 200 ml of H₂O gave an aqueous solution of labeled inosine 5'-phosphate, which was neutralized with 2.3 ml of 0.5 N NaOH solution (pH 7.0) and evaporated to afford the crude disodium salt. Recrystallization from 3 ml of 50% aqueous EtOH and drying under reduced pressure over P₂O₅ at 50° for 5 hrs gave 131 mg (yield, 66.8%) of 5'-inosinic acid-8-¹⁴C disodium salt.

The radiochemical purity was examined by thin-layer (described before) and paper partition chromatographic methods in ascending technique on Toyo Roshi No. 51 paper (5 x 40 cm) with two kinds of solvent (a; 2:79:19 v/v-i-PROH:satd. $(\text{NH}_4)_2\text{SO}_4:\text{H}_2\text{O}$, b; 7:1:2 v/v-i-PROH:conc. $\text{NH}_4\text{OH}:\text{H}_2\text{O}$). There was observed a single spot with which the Rf value for an authentic sample was consistent.

The radioactivity of the labeled compound was measured by a liquid scintillation counter (Nuclear Chicago Mark I) using a 2:1 mixture of toluene scintillator and Triton X100 as a scintillator. Quenching was determined by an external standard technique. There was obtained a specific activity of 1.41 $\mu\text{Ci}/\text{mg}$ or 554 $\mu\text{Ci}/\text{mmole}$. Its radiochemical yield was 74%. A 11% higher specific activity of the product than that of the starting material used would be resulted from the reason either that the starting material contained some unlabeled impurity or that its specific activity was actually 11% higher than advertised.

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