Synthesis of Pyridine-2-aldoxime-C^{14} Methiodide (2-PAM-C^{14} Iodide)

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A procedure is described for the synthesis of C-14 oxime-labeled pyridine-2-aldoxime methiodide. Picolinamide-C^{14} is prepared from 2-bromo-pyridine and potassium cyanide-C^{14}. This amide is used in the synthesis of 2-picolinyl-C^{14}-benzenesulfonyl hydrazide which, upon decomposition with heat and alkali, yields pyridine-2-aldehyde-C^{14}. Pyridine-2-aldoxime-C^{14} is readily obtained from the aldehyde. Treatment of the oxime with methyl iodide in benzyl alcohol yields the desired 2-PAM iodide.

INTEREST in 1-methyl-2-pyridinium aldoxime iodide or pyridine-2-aldoxime methiodide (2-PAM iodide) is largely a result of its effectiveness as an antidote for organic phosphate poisoning. The ever-increasing use of highly toxic organophosphorus compounds as agricultural insecticides as well as potential military nerve gases makes it a drug of importance. In addition, 2-PAM iodide has found important research application in the study of enzyme systems involving cholinesterase inhibitors.

2-PAM iodide labeled with C-14 makes possible the study of this drug and/or its metabolites within brain and other body tissues. Studies utilizing oxime-labeled as well as methyl-labeled PAM iodide are desirable. The lack of any published procedure for the synthesis of oxime-labeled 2-PAM, and the potential usefulness of this compound as a research tool, led us to develop the synthesis herein reported.

EXPERIMENTAL

Picolinamide-C^{14}.—Two major considerations entered into the choice of this compound as a starting point in the synthesis of C^{14}-labeled pyridine-2-aldehyde. First, because it can be prepared from cyanide-C^{14}, it affords a convenient means for the introduction of a labeled atom. Secondly, the amide can be used directly in the synthesis of the required hydrazide thus circumventing the problem of esterification of picolinic acid.

The preparation of picolinamide from 2-bromopyridine and potassium cyanide has been reported by Brode and Bremer (1). Inasmuch as these workers were concerned with optimum yield based upon bromopyridine, the procedure had to be redesigned with a view toward the achievement of optimum yield based upon cyanide.

A solution of potassium cyanide-C^{14} (390 mg., 6m*M*, 10 mc.) in water (1 ml.) was prepared in a heavy-walled Pyrex reaction tube. The tube and contents were kept cold by means of an ice water bath as cuprous chloride (250 mg.), added in small portions, was brought into solution with shaking and swirling. To the solution thus prepared were added, in order, 790 mg. (5 m*M*) of 2-bromopyridine dissolved in 1 ml. of ethanol, and 100 mg. of potassium carbonate. The tube was then sealed and heated for 40 hours at 180 to 200°.

The tube was allowed to cool to room temperature, chilled in an ice bath, then opened and the contents evaporated to dryness under reduced pressure. The residue was redissolved in hot dilute ammonium hydroxide. This ammoniacal solution was then made slightly acidic with hydrochloric acid and treated with hydrogen sulfide. Copper sulfide was filtered off, and the filtrate evaporated to dryness *in vacuo*. The residue was dissolved in a minimum volume of water, neutralized with potassium carbonate, and submitted to continuous extraction with ether for 2 hours. Crude picolinamide-C^{14} was obtained upon evaporation of the solvent. The product was purified by sublimation *in vacuo*, m.p. 105–106° [reported m.p., 106.3 to 106.8° (1)]. Yield: 182 mg. (25%), based on cyanide.

During the course of preliminary research with unlabeled materials, the product was further characterized by hydrolysis to the corresponding acid, isolated as the copper salt. The acid obtained by regeneration from the copper salt was chemically indistinguishable from an authentic purified specimen of picolinic acid.

Picolinic-C^{14} Acid Hydrazide.—The amide from the previous step was dissolved in absolute alcohol (5 ml.) and treated with 0.5 ml. of hydrazine hydrate (99–100%). This solution was heated under reflux for 48 hours on a steam bath. Solvent was then removed under reduced pressure. The product was purified by recrystallization from benzene. Yield: 169 mg. (82%), m.p. 98–99° [reported m.p. 100° (2)].

2-Picolinyl-C^{14}-benzenesulfonylhydrazide.—The above product in pyridine solution was treated with benzenesulfonyl chloride in accordance with the method of Niemann, Lewis, and Hays (3). The resultant benzenesulfonylhydrazide was purified by recrystallization from boiling alcohol. Yield: 230 mg. (68%), m.p. 202–203° [reported m.p. 202–203.5° (3)].

Pyridine-2-aldoxime-C^{14}.—Use was made of the reaction of McFadyen and Stevens (4) in the production of pyridine-2-aldehyde-C^{14} from the above benzenesulfonylhydrazide. Inasmuch as this reaction imposes rather severe conditions of temperature and alkalinity upon the relatively unstable aldehyde, it was deemed imperative that the

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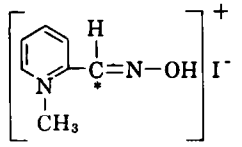
product be isolated from the reaction mixture as rapidly as possible. Accordingly, the reaction was carried out under vacuum, using an all-glass apparatus with appropriate ground glass, Apiezon (N) lubricated fittings. In essence, this apparatus consisted of a vacuum trap with a separable receiver and a side arm to accommodate a ground glass-jointed reaction tube.

With a mixture of the above benzenesulfonylhydrazide, anhydrous sodium carbonate (200 mg.) and glycerol (2 ml.) in the reaction tube, the system was evacuated to 10^{-5} mm. of Hg. The lower portion of the receiver-trap was surrounded by a liquid nitrogen bath while the reaction tube was gently heated with a micro burner. The reaction proceeded with a vigorous evolution of gas. The condensable fraction of the gas collected as an ice in the cold trap. The noncondensable portion was pumped into the exhaust system of an efficient hood which was equipped with a high-efficiency filter.[1]

When the evolution of gas had practically ceased, the evacuated trap was closed off and permitted to warm to room temperature. The separable receiver (a 1 × 10 in. test tube with ⅋ 24/40 outer joint) was used as a reaction vessel in the next operation.

To the water-clear liquid in the receiver tube was added hydroxylamine hydrochloride (20 mg.), sodium bicarbonate (25 mg.), and water (1 ml.). The resultant mixture was heated for 10 minutes on a steam bath. The oxime was isolated by continuous extraction with ether and purified by recrystallization from benzene-petroleum ether. Yield 20.2 mg (20%), m.p. 112–113° [reported m.p. 113.5° (5)].

Radiochemical purity of the pyridine-2-aldoxime-C^{14} was determined by means of paper chromatography, using a water-saturated benzene system and a water-saturated chloroform system. In each instance, an autoradiogram of the chromatographic strip showed a single band of radioactivity.



Pyridine-2-aldoxime-C^{14} Methiodide

Pyridine-2-aldoxime-C^{14} Methiodide.—Whereas pyridine-2-aldoxime methiodide has been prepared by heating the oxime with methyl iodide in a suitable solvent (6), this procedure proved unsatisfactory when applied to production of the radiochemically pure compound on a millimole scale. Thus, a scheme of synthesis was sought whereby side reactions could be minimized or eliminated by conducting the reaction at a lower temperature. The use of benzyl alcohol as a catalytic solvent (7) proved a satisfactory solution to the problem.

The oxime from the previous step was thoroughly mixed with 0.25 ml. of freshly distilled benzyl alcohol in a glass-stoppered test tube. To it was added 0.25 ml. of freshly distilled methyl iodide. The tube was securely stoppered and set aside for 1 week at room temperature. An additional 0.25-ml. portion of methyl iodide was then added and the mixture set aside for 4 or 5 days. In the meantime, bright yellow crystals of the methiodide had formed in the reaction mixture. This mixture was added, dropwise with stirring, to 100 ml. of anhydrous ether. The precipitate was collected on a sintered-glass filter, washed thoroughly with anhydrous ether, and dried in air. A sample for microanalysis was dried for several days *in vacuo*, at room temperature. Yield: 39.3 mg. (90%); m.p. 225–226° (decompn.) [reported m.p. 225–226° (decompn.) (8)].

Anal.—Calcd. for $C_7H_9IN_2O$: C, 31.8; H, 3.41; N, 10.6; I, 48.1. Found: C, 32.1; H, 3.45; I, 47.96; N, 10.5. I, 47.96.

Radiochemical purity of the pyridine-2-aldoxime-C^{14} methiodide was determined by means of paper chromatography, using a *n*-butanol:acetic acid:water (5:1:3) system, and a water-saturated *n*-butanol system. In each case an autoradiogram of the chromatographic strip showed a single band of radioactivity. Radiochemical yield 0.232 millicuries (2.3% based on potassium cyanide).

SUMMARY

1. Pyridine-2-aldoxime-C^{14} has been synthesized and used in the preparation of the corresponding methiodide.

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[1] This filter is rated as 99.98% efficient for removal of particles in the order of 0.4 μ in diameter and manufactured by Flanders Filters, Inc., Riverhead, N. Y.