Synthesis of [4-^{14}C]Theophylline

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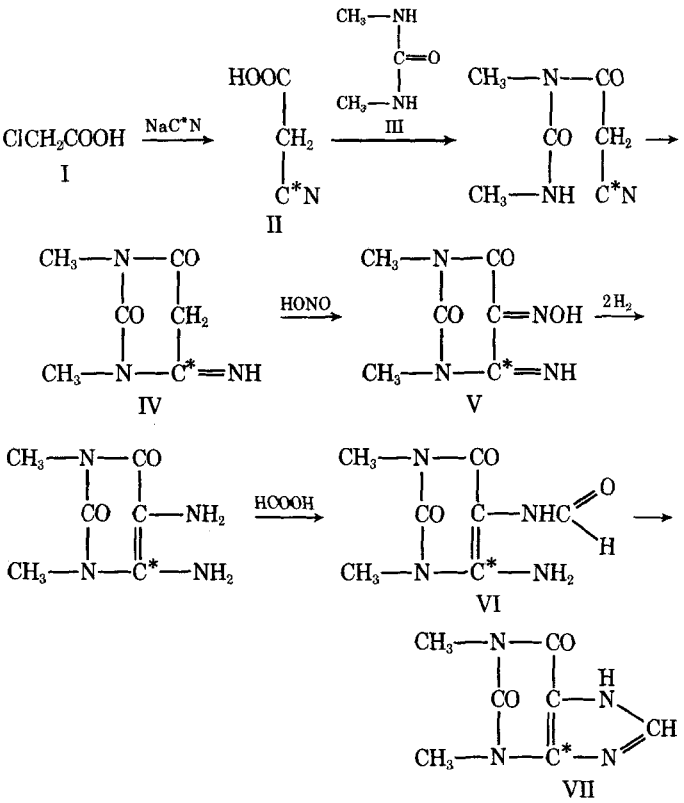
Abstract □ The synthesis of [4-^{14}C]theophylline is presented with the radiolabel introduced in the first step of the procedure. The label is carried stoichiometrically to theophylline, and yields are satisfactory. The position of the label at position 4 of the purine ring is attractive for discerning metabolic pathways of the drug.

Keyphrases □ Theophylline, radiolabeled—synthesis □ Radiolabeled compounds—[4-^{14}C]theophylline, synthesis □ Antiasthmatics—synthesis of [4-^{14}C]theophylline

Theophylline (3,7-dihydro-1,3-dimethyl-1*H*-purine-2,6-dione), used to alleviate symptoms of bronchial asthma, has been a popular drug for metabolic and pharmacokinetic studies (1–7). Problems inherent in determining its metabolic profile are compounded by the fact that other commonly ingested methylxanthines form metabolites in common with theophylline. In addition, some metabolites are similar to endogenous compounds.

The best method of distinguishing the metabolites of theophylline from those of its analogs or from other compounds is to insert a radiolabel in the purine ring. Theophylline synthesis has been accomplished (8–10), and a general method (8) was used (6) in [8-^{14}C]theophylline synthesis. This procedure calls for the insertion of the radiolabel during the last step of synthesis by adding [^{14}C]formic acid in excess to 1,3-dimethyl-4,5-diaminouracil. The reaction is stoichiometric with respect to the latter compound and results in yields of ~7.5% with respect to the label.

A high-yield synthetic procedure (Scheme I) was developed to introduce the carbon 14 label stoichiometrically to maintain a desired specific activity during synthesis and to avoid loss of the expensive ^{14}C-labeled intermediate.



Scheme I—Synthesis of [4-^{14}C]theophylline.

EXPERIMENTAL

Materials—The following reagent grade materials were used as supplied: chloroacetic acid, sodium carbonate, sodium cyanide, hydrochloric acid, ethanol, 1,3-dimethylurea, acetic anhydride, acetic acid, sodium nitrite, zinc dust, 90% formic acid, and sodium hydroxide. Sodium [^{14}C]cyanide[1] (54 mCi/mmole) was diluted with sodium cyanide to give a starting material with a specific activity of 0.72 mCi/mmole.

[^{14}C]Cyanoacetic Acid (II) (11)—A solution of 0.370 g of chloroacetic acid (I) in 0.6 ml of water at 50° was slowly made alkaline with 0.222 g of sodium carbonate. To the stirred solution at 0° was added dropwise 0.204 g of sodium [^{14}C]cyanide in 0.7 ml of ice water, keeping the temperature low. The clear solution was brought to room temperature for 30 min, heated to boiling for 5 min, cooled in an ice bath for 30 min, and slowly acidified with 0.47 ml of concentrated hydrochloric acid.

The clear solution was placed in a desiccator under vacuum until dry, and then the crystalline residue was taken up in 95% ethanol. The sodium chloride byproduct was removed by centrifugation and washed with several portions of alcohol. The combined alcoholic solutions were evaporated at 45° under reduced pressure, leaving a clear viscous concentrate of cyanoacetic acid (II) in alcohol. This material was stored in a desiccator until the last of the alcohol had evaporated and the product had crystallized.

[4-^{14}C]-1,3-Dimethyl-4-amino-2,6-pyrimidinedione (IV) (12)—To the cyanoacetic acid (II) were added 0.352 g of 1,3-dimethylurea (III), 0.82 ml of acetic anhydride, and 1 ml of acetic acid, and the mixture was heated at 60° for 3 hr. The clear solution became slightly orange. The acetic acid and excess anhydride then were removed as well as possible under reduced pressure without raising the temperature, leaving a burnt-orange viscous residue. This residue was dissolved in 8 ml of water and made alkaline by the slow addition of sodium carbonate and boiling for 2 hr. On cooling, a pale-yellow crystalline precipitate was formed. The yield was 0.375 g or 58% based on sodium cyanide.

[4-^{14}C]-1,3-Dimethyl-4-amino-5-nitroso-2,6-pyrimidinedione (V) (9, 10, 13)—The dried compound (IV) was dissolved in 8 ml of hot water, and 0.2 g of sodium nitrite was added with stirring. A mixture of 0.85 ml of acetic acid, 0.86 ml of water, and 2.6 g of ice was added in small portions with stirring. The solution turned deep red, and tiny dark-red crystals appeared. The solution was left in the refrigerator overnight to allow the complete precipitation of crystalline product. The product then was filtered out and dried in a desiccator. The yield was 0.420 g or 55% with respect to sodium cyanide.

[4-^{14}C]-1,3-Dimethyl-4-amino-5-formamido-2,6-pyrimidinedione (VI) (10, 14)—The nitrosated compound (V) was mixed with 0.36 g of zinc dust and placed in a 100-ml round-bottom flask equipped with a reflux condenser. Five milliliters of 90% formic acid was added to the mixture, and the apparatus was swirled continuously as the mixture heated spontaneously. The solution changed from oxblood to yellow over several minutes. The solution then was heated at 60° for 1.5 hr while 2 ml of acetic anhydride was added slowly. Finally, the mixture was cooled, the zinc formate was removed by filtration, and the clear yellow filtrate was dried under reduced pressure at 60°, leaving a solid residue with a yellow-white color. The residue was stored in a desiccator until completely dry.

[4-^{14}C]Theophylline (VII) (10)—Compound VI was dissolved in 4.5 ml of boiling water, and 0.36 ml of 40% NaOH was added dropwise with

[1] Sodium [^{14}C]cyanide, International Chemical and Nuclear Corp.

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vigorous stirring. The solution was heated for 10 min, at which time another 0.36 ml of 40% NaOH was added. Finally, 50% acetic acid was added in sufficient quantity to give a weak acid reaction. Pale-yellow crystals of theophylline precipitated on cooling. The yield was 0.370 g or 49% with respect to sodium cyanide. The crude theophylline was dissolved in a 10-fold quantity of boiling water, charcoal was added, and the hot solution was filtered. On cooling, white crystals of theophylline were formed.

RESULTS AND DISCUSSION

A solution of [4-^{14}C]theophylline was prepared by dissolving 15.45 mg in sufficient deuterated dimethyl sulfoxide to make 1 ml, and an NMR[2] spectrum was taken with tetramethylsilane as an internal standard. It was identical to the spectrum of a commercial theophylline powder[3]. Furthermore, a methanolic solution of the synthesized compound was spotted on a silica gel plate and developed in chloroform–methanol (9:1) (15) along with a sample of commercial theophylline powder on the same plate. UV spectrodensitometry[4] at 274 nm showed the synthesized and commercial products to have identical R_f values of 0.47; no other UV-absorbing compounds were detected. Scanning the plate with a radiochromatogram scanner[5] showed no measurable radioactivity except that associated with the theophylline peak. Two 0.02-ml samples of the radiolabeled product solution in deuterated dimethyl sulfoxide were counted by scintillation spectrometry[6], and the specific activity was calculated to be 0.69 mCi/mmole.

The introduction of a radiolabel within the purine ring of theophylline is desirable and advantageous in metabolism studies. The described synthesis gives a high yield based on the isotope and is stoichiometric as shown by the calculated and resultant specific activities of the product.

[2] Jeolco C-60HL.
[3] Sigma Chemical Co.
[4] Vis-UV-2, Farrand Optical Co.
[5] Model 7201, Packard Instrument Co.
[6] Model 3320, Packard Instrument Co.

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