THE SYNTHESIS AND PURIFICATION OF 8-^{14}C-THEOPHYLLINE

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SUMMARY

The enzymes responsible for the metabolism of methylated xanthines such as theophylline, theobromine, and caffeine have until now escaped identification (1, 2). However, a sensitive radiometric method of analysis of theophylline metabolism (3) was made possible by the synthesis and purification of 8-^{14}C-theophylline (^{14}C-Theo). ^{14}C-Theo was synthesized by the incorporation of ^{14}C from ^{14}C-formic acid into the 8-position of the purine ring. Purification of the ^{14}C-Theo was accomplished by subjecting the crude product to cation-exchange column chromatography with AG50W-X12, 50-100 mesh resin of the hydrogen ion form.

Previous attempts to determine the identity of the enzymes involved in theophylline metabolism have been made using unlabeled theophylline (4, 5, 6). Radioactive [^{3}H(G)]theophylline has been available in the past, but is not the isotope of choice for drug metabolism studies. Tritium from labile positions, i.e., attached to oxygen, nitrogen or sulfur atoms, can be removed, but another problem associated with tritiated drugs has been observed when their metabolism is studied. Reports have been made of tritium being found in tissue water, not associated with the parent tritiated compound or its metabolites (7). Burg and Stein, 1972, (8) noticed that when they administered both ^{14}C-1-methyl

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caffeine and [^{3}H(G)]caffeine to mice, the urinary metabolites labeled with tritium alone were of minor significance.

8-^{14}C-Theo was considered to be the best choice of radioactive theophylline for use in metabolism studies since the metabolites (1,3-dimethyl uric acid, 1-methyl uric acid, and 3-methyl xanthine) obtained in the urine of subjects administered theophylline indicated that the purine ring is not degraded during metabolism (9).

METHODS AND RESULTS

Source of chemicals. The following compounds were obtained as follows: ^{14}C-formic acid from Amersham, Searle (Arlington Heights, Ill.); 1,3-dimethyl, 4,5-diaminouracil hydrate from Aldrich Chemical Co. (Milwaukee, Wisc.); cation-exchange resin AG50W-X12, 50-100 mesh of the hydrogen ion form from Bio-Rad Laboratories (Rockville Centre, N.Y.); theophylline from K & K Laboratories (Plainview, N.Y.).

Procedure for synthesis. The original Traube synthesis, 1900, (10) was modified for the radioactive synthesis of theophylline in order to obtain a product of relatively high specific radioactivity in reasonable yield. A solution containing 0.42 ml water, 100 mg uracil hydrate (0.59 mmoles), 0.03 ml formic acid (0.53 mmole) and 3 mCi ^{14}C-formic acid containing 0.057 mmole was refluxed at 85°C for 4.5 hr. The unreacted formic acid was removed at 50°C under reduced pressure. The residue was freed of unreacted formic acid by three times redissolving it in 3 ml of water and removing the water each time by distillation under reduced pressure at 50°C.

To the residue was added 0.7 ml of 1 N NaOH and the mixture was evaporated to dryness at 50°C under reduced pressure. The residue was then heated gradually to 220°C in an oil bath and the temperature was maintained at 220-240°C for one hour. A yellow product was obtained and dissolved in 30 ml glass distilled water.

Purification. The cation exchange resin column (1 x 19 cm) was prepared from

an aqueous slurry of the resin. The resin was conditioned with 175 ml 1 N HCl and 450 ml 5 N HCl and finally rinsed with 600 ml glass distilled water. The product dissolved in 30 ml glass distilled water was slowly applied to the column over a 60 min period. Then the sides of the glass column were rinsed six times with water. A flow rate of about 1 ml/min was established with water as the eluent and 10 ml fractions were collected from the column.

The elution of chromogenic material from the column was monitored spectrophotometrically at 270 nm (Figure 1). Spectra of the collected fractions were also made and the ratio of the absorbance at 245 nm to that at 270 nm was calculated each time since that represented the Amin/Amax ratio (∿ 0.27 for theophylline). Large amounts of impurities with a spectrum (and a A_{245}/A_{270} ratio) clearly distinguishable from that of theophylline eluted in the first 20 fractions. ^{14}C-Theo did not elute until the 130th fraction. Collection of ^{14}C-Theo was stopped after the 229th fraction eluted because the UV spectrum of the eluate began to change from that of theophylline and the Amin/Amax ratio began to increase. Fractions 130–229 were pooled, flash evaporated at 50°C under reduced pressure, and redissolved in 30 ml glass distilled water.

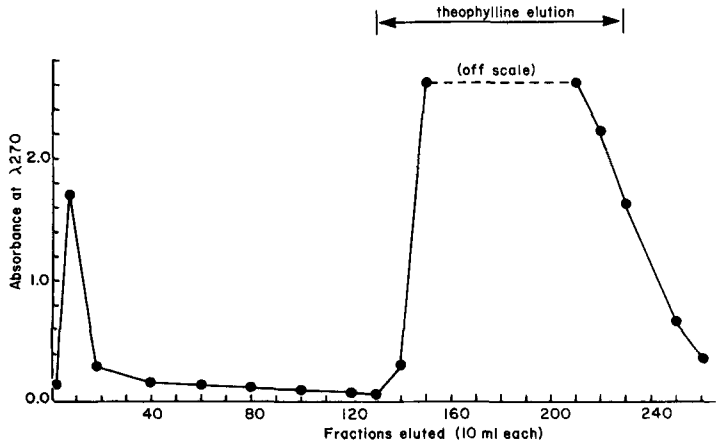

Figure 1. Purification of the synthesized 8-^{14}C-theophylline by eluting it with water from a cation-exchange column filled with AG50W-X12, 50–100 mesh resin of the hydrogen ion form. Fractions 130–229 contained theophylline alone.

Characterization of the 8-^{14}C-Theophylline. The chemical purity of the 8-^{14}C-theophylline was determined spectrophotometrically by comparison of the absorbance spectrum of the radioactive compound to that of unlabeled theophylline. The spectra of the two compounds were identical when recorded with a Perkin-Elmer Recording Spectrophotometer (Model 402) (Figure 2). The Emax (10.5×10^3 in water at A_{270}) compared well with that which has been reported [Emax = 10.7×10^3 in water at A_{270} (11)]. The synthesized ^{14}C-Theo was also identified and shown to be chemically pure by anion-exchange high pressure liquid chromatography performed with ammonium acetate-acetic acid as the eluting buffer. Radiochemical purity was demonstrated to be greater than 99% by paper chromatography in three solvent systems: A) tert.butyl alcohol-methyl ethyl ketone-formic acid-water

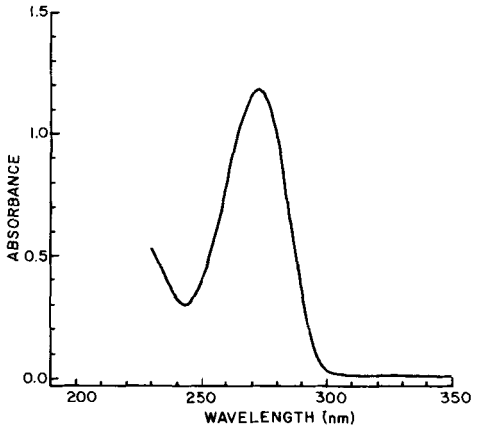

Figure 2. The absorbance of reference theophylline and the synthesized 8-^{14}C-theophylline in water were identical.

(40:30:15:15), B) n-butyl alcohol-glacial acetic acid-water (50:25:25), (12), and C) isopropyl alcohol-phosphoric acid-water (73:17:10). The R_f values for the ^{14}C-Theo corresponded to those for the unlabeled reference theophylline: 0.72, 0.76 and 0.81 in Solvents A, B, and C, respectively.

The chemical yield was 56% and the specific radioactivity was calculated to be 4.5 mCi/mmole.

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