SYNTHESIS OF THEOPHYLLINE-8-^{13}C

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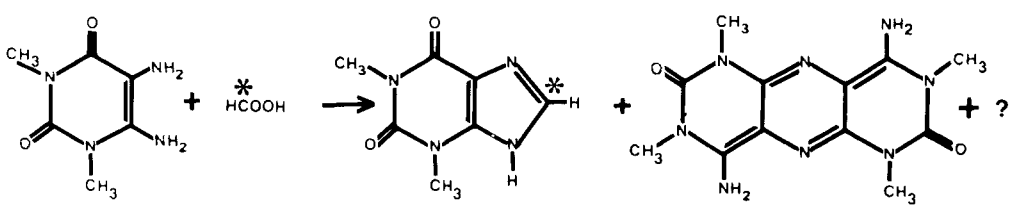
Theophylline is a very effective bronchodilator which has found widespread use in patients with asthma and other obstructive airways diseases (1-5). Because this drug is used widely and often chronically (some patients for life), an accurate and complete knowledge of its pharmacokinetics and metabolic disposition is imperative. Because some of the known (and perhaps unknown) metabolites of theophylline are also endogeneous metabolites in man, it is necessary to use isotopically labeled theophylline for an accurate study of its metabolism. Quantitation of drugs in biological fluids is best done with internal standards which mimic as closely as possible the physical and chemical properties of the target drugs and the isotopically labeled compounds are therefore excellent internal standards. Because we have been interested in the metabolic disposition of theophylline as well as quantitating it in phamacokinetic studies we have synthesized theophylline-8-^{13}C.

The synthesis is essentailly that of Traube (6) as modified by Khmelevskii and Abramova (7) and Klingler (8). The synthesis involved condensation of 5,6-diamino-1-3-dimethyluracil (1) with formic-^{13}C acid under basic conditions to produce theophylline-8-^{13}C (2). Thin-layer chromatography was then used to purify the final product which contains 1, caffeine, the dimeric compound 3, and other unidentified contaminants.



EXPERIMENTAL

To a 50 ml flask with magnetic stirrer was added 15 ml distilled water, 2.0 g (11.8 mmol) 5,6-diamino-1,3-dimethyluracil (Aldrich Chem. Co., Inc., Milwaukee, Wis.), and 0.5 g (10.6 mmol) formic-^{13}C acid (90% ^{13}C, Stohler Isotope Chemicals, Rutherford, N. J.). The mixture was stirred until the starting material dissolved. The pale yellow solution was allowed to stand

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overnight when scraping the walls of the flask resulted in the formation of white crystals. The mixture was allowed to stand for three more days during which time additional crystals formed until a solid network of crystals was spaced throughout the liquid phase. To this was added 1 g sodium hydroxide previously dissolved in 5 ml water. The mixture was heated at 90°C for 15 min., cooled with an ice bath, and finally neutralized with concentrated HCl. A small amount of gas was liberated during neutralization. The reaction mixture was cooled (4°C) overnight, and pale yellow crystals (theophylline) formed. The crystals were filtered, dissolved in 30 ml hot water, and treated with decolorizing carbon. The carbon was removed by filtration, and the water layer cooled to yield 250 mg theophylline-8-^{13}C (13% yield). To increase the yield, the theophylline-8-^{13}C present in the filtrate of the initial reaction mixture was isolated by preparative silica gel TLC (20 cm x 20 cm x 2 mm "Uniplates", Analtech, Inc., Newark, N. J.). The developing solvents in successive separations were ether:methanol 9:1 (R_f = 0.58), ether:methanol 7:3 (R_f = 0.62), and water:methanol 2:8 (R_f = 0.81). In each case the theophylline-8-^{13}C was eluted from the silica gel with warm methanol:water 1:1. The material obtained after these separations was purified by sublimation under vacuum (less than 0.1 torr). The pale yellow material which sublimed was treated with decolorizing carbon and was combined with the crystallized 250 mg theophylline-8-^{13}C. The combined material was purified by preparative silica gel TLC using water as the developing solvent (R_f = 0.67). The final product was eluted from the silica gel with water to yield 514 mg theophylline-8-^{13}C (26.7% yield).

RESULTS AND DISCUSSION

The synthetic procedure described was used to prepare theophylline-8-^{13}C suitable for administration to humans and therefore was highly purified. The yield of final product (26.7%) could be increased by avoiding the use of decolorizing carbon and should still be suitable for use as an internal standard or other applications where high purity is not essential.

The chemical purity of the final product was established by GC, NMR, GC-MS, and MS using probe introduction. The isotopic abundance was established by NMR and MS. The NMR spectrum of theophylline-8-^{13}C run in dimethylsulfoxide-d_6

exhibited siglets at 3.22 and 4.22 ppm due to the methyl groups on N-3 and N-1, respectively. The singlet which appears at 8.05 ppm due to the proton on C-8 in theophylline is split in the spectrum of theophylline-8-^{13}C into a doublet at 6.83 and 9.17 ppm. The coupling constant between the proton and ^{13}C atom is 185 Hz. The proton on N-7 appears as a broad signal beyond 10 ppm. The mass spectrum of theophylline-8-^{13}C exhibited a molecular ion at m/e = 181. Like the molecular ion, the fragment ions at m/e = 151, m/e = 124, and m/e = 96 appear one mass unit higher than in the spectrum of the unlabeled compound. All of these shifts are in agreement with known fragmentation pathways (9). The ions at m/e = 53 and m/e = 68 are not shifted. The ratio of 180 to 181 established the isotopic abundance as 90% ^{13}C in agreement with the isotopic purity of the formic-^{13}C acid precursor.

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