A SIMPLE, RAPID SYNTHESIS OF CAFFEINE-1,7-$^{13}CH_3$

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SUMMARY

This paper describes a simple, rapid synthesis of caffeine-1,7-$^{13}CH_3$ produced by the reaction of iodomethane-^{13}C with 3-methylxanthine. The synthesis is complete in one day, requires only the most rudimentary laboratory equipment, and is amenable to large scale synthesis. Overall yield after product isolation is 82%, and the product is of 99% chemical purity and 99% isotopic purity.

Key Words: caffeine-1,7-$^{13}CH_3$, iodomethane-^{13}C, methylxanthines, synthesis, mass spectra

INTRODUCTION

Caffeine (3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6-dione; 1,3,7-trimethylxanthine) is a plant alkaloid which enjoys virtually ubiquitous use as a central nervous system stimulant. The pharmacodynamics of caffeine have been extensively investigated (1). Present efforts include pharmacokinetic studies, particularly with regard to metabolic conversion to other bioactive xantnines. Other studies focus on the possible role of caffeine as a co-carcinogen (2) or a purinergic antagonist (3). Isotopically labelled caffeine is often used as a tracer in this work. Alkylation of methylxanthines with iodoalkanes and alkyl sulfates is well known (4,5) and syntheses of caffeine nave been accomplished incorporating ^{2}H, ^{3}H, ^{11}C, ^{13}C, ^{14}C, or ^{15}N (6-9).

The purpose of this paper is not to describe a synthesis based on novel chemistry. Rather, this scheme is a simple, high yield procedure which can be accomplished in any laboratory without any special techniques or apparatus.

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The synthesis requires one day, followed by simple extractive purification. It is useful for incorporation of ^{2}H, ^{3}H, ^{13}C or ^{14}C.

EXPERIMENTAL

Synthesis - The reaction vessel used was a two-necked, 250 ml round bottom flask suspended over a magnetic stir plate. The flask was foil wrapped to exclude light. Into 50-100 ml CH_3OH, 0.2 - 2.0 mmol 3-methylxanthine (3 - MX, Aldrich Chemical, Milwaukee, WI) was added and solubilized by the addition of KOH-saturated CH_3OH. At time zero, iodomethane-^{13}C($^{13}CH_3I$, 99 atom %, MSD Isotopes, St. Louis, MO) was added such that the mol ratio $^{13}CH_3I$:3-MX = 20. The mixture was sealed and stirred at ambient temperature (20-24°). The pH of the solution was checked periodically using pH-indicator paper and adjusted to pH = 12-14 by the addition of methanolic KOH. Rates of product formation were assessed by reverse phase HPLC as described below.

Upon completion of the reaction, the flask was fitted with a short path distillation head and an ice-cooled receiver was attached. Using gentle warming of the flask, unreacted $^{13}CH_3I$ was collected and retained for future synthetic work. CH_3OH was then distilled under reduced pressure and collected.

The resultant white solid was redissolved in 100 mL H_2O, and extracted with 3 x 30 mL fractions of $CHCl_3$. After back-extraction against dilute aqueous KOH (pH 14), the organic phase was gently evaporated using a rotary evaporator. The resultant white flakes were collected and assessed as to chemical and isotopic purity by comparison to USP caffeine (US Pharmacopeial Convention, Rockville, MD).

Chemical Purity - Chemical purity was assessed by melting range determination, gradient-elution reverse phase HPLC (20 cm C_{18} column,CH_3OH,H_2O,40°, flow rate 2 mL x min^{-1}), and cation exchange chromatography [1m Zipax^{R} SCX column

(Dupont, Wilmington, DE), 1% aqueous acetic acid, 25°, flow 2 mL x min^{-1}]. Spectrophotometric detection (λ=254 nm) was used in both chromatographic modes.

Isotopic purity was assessed by direct probe electron impact (70 eV) mas spectrometry using a VG 7035 mass spectrometer (VG Instruments, Stamford, CT).

RESULTS AND DISCUSSION

The conversion of 3-MX to caffeine proceeds through intermediate methylation to either 1,3-dimethylxanthine (theophylline) or 3,7-dimethylxanthine (theobromine) as illustrated in Figure 1.

Under these conditions, methylation of N^7 proceeded more rapidly than N^1-methylation, and theobromine accumulated early in the reaction. Theophylline

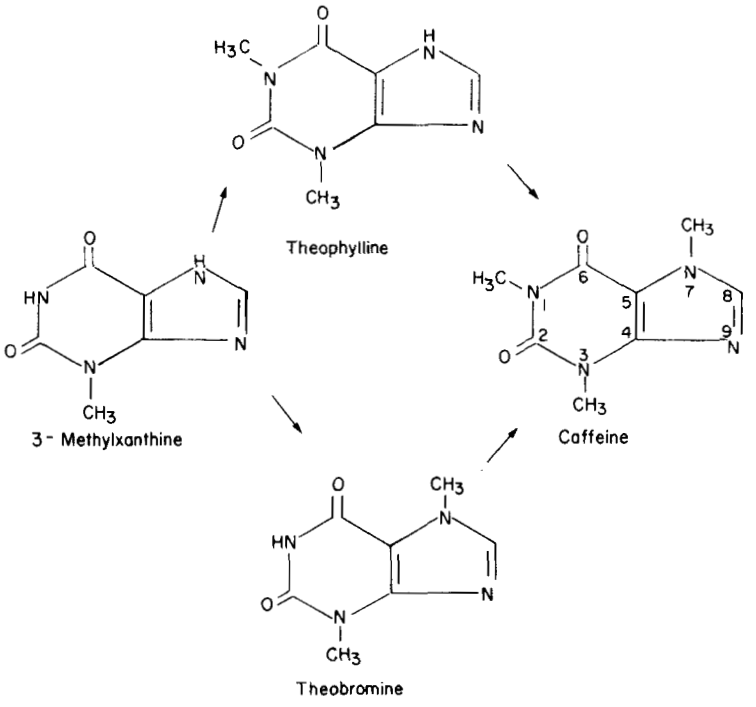

Figure 1. Methylation pathways for caffeine. Reaction proceeds through two intermediate dimethylxanthines, theobromine or theophylline.

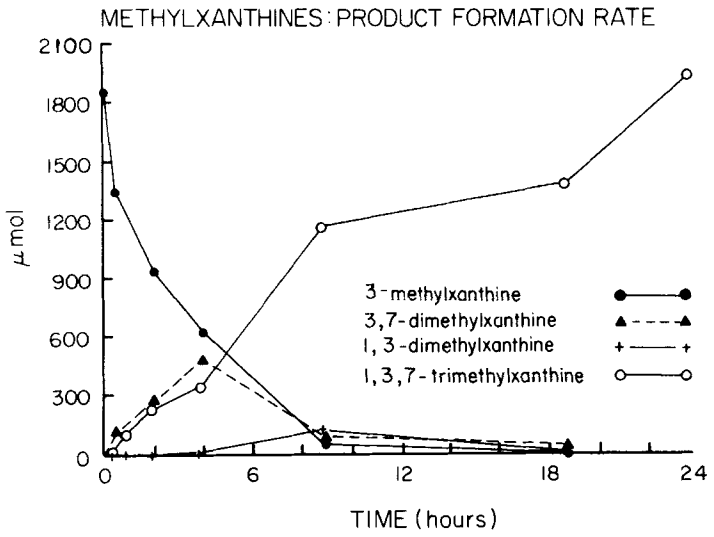

Figure 2. Reaction progress was assessed by reverse phase HPLC. Theobromine (3,7-dimethylxanthine) is formed more rapidly and consumed more slowly than is theophylline (1,3-dimethylxanthine).

produced was rapidly 7-methylated to caffeine. After 24 hours the reaction was complete (Figure 2). Overall yield was 82%, with loss occurring primarily during extraction.

After extraction and extensive drying under reduced pressure over P_2O_5, chemical purity was assessed. The melting range for caffeine-1,7-$^{13}CH_3$ was 224-225° as compared to 223-225° for USP caffeine. Both began to sublime at 175°. Samples were then subjected to liquid chromatographic analysis. In both LC modes caffeine-1,7-$^{13}CH_3$ co-eluted with authentic caffeine. The xanthines in Figure 1 are completely resolved by these methods and no traces of unreacted methyl- or dimethylxanthines were detected. The limits of detection are such that less than 1% contamination would have been detected, thus chemical purity was greater than 99%.

Electron impact mass spectra of both caffeine isotopes were obtained, and are shown in Figure 3. Table 1 is a listing of fragmentation patterns previously described in accurate mass studies (9, 10) along with the expected

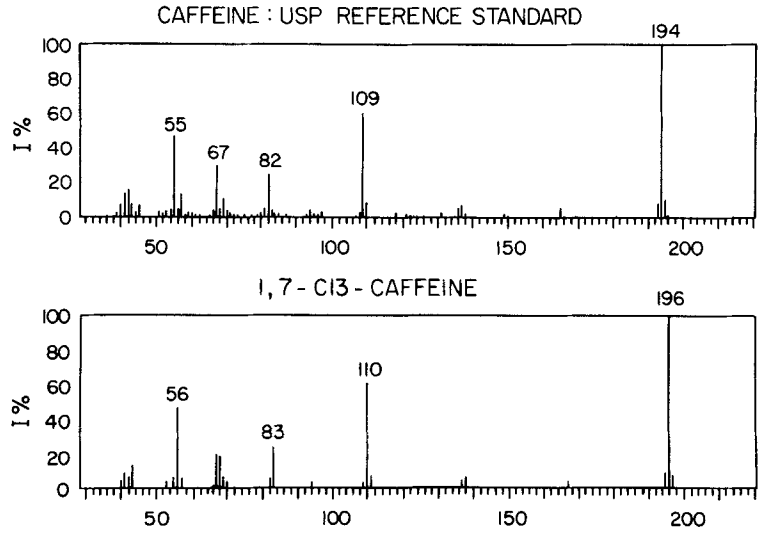

Figure 3. Direct probe EI (70eV) mass spectra of USP standard caffeine and caffeine-1,7-$^{13}CH_3$.

and observed data for caffeine-1,7-$^{13}CH_3$. These data agree with respect to fragmentation pattern and relative ion abundances.

If $^{13}CH_3I$ of somewhat less than 99% isotopic purity had been used in this synthesis, the most likely isotopic impurities in the product would have been caffeine-1-$^{13}CH_3$ or caffeine-7-$^{13}CH_3$. Either of these incompletely labelled

Table 1: Fragmentation pattern for caffeine-1,7-$^{13}CH_3$

Reported m/z for Caffeine (Ref 7)	Predicted m/z for Caffeine-1,7-$^{13}CH_3$	Observed m/z	Fragment
194	196	196	$C_6{}^{13}C_2H_{10}O_2N_4{}^+$
137	138	138	$C_5{}^{13}CH_7ON_3{}^+$
109	110	110	$C_4{}^{13}CH_7N_3{}^+$
82	83	83	$C_3{}^{13}CH_6N_2{}^+$
67	67 = 68[a]	67 ~ 68	$C_3H_3N_2{}^+$, $C_2{}^{13}CH_3N_2{}^+$
55	56	56	$C_2{}^{13}CH_5N^+$

a) loss of CH_3 from N^3 or N^7 equally likely

caffeine structures would exhibit a molecular ion and base peak of m/z = 195. The relative abundance of m/z = 195 [$(M - 1)^+$] for caffeine-1,7-$^{13}CH_3$ was 9%, while the relative abundance of th $(M - 1)^+$, m/z = 193 ion for USP caffeine was 8%. This difference in abundance of $(M - 1)^+$ ions indicate that the isotopic impurity of the labelled product was no greater than 1%. Thus the isotopic purity of caffeine-1,7-$^{13}CH_3$ produced in this synthesis was 99%, which is in agreement with the stated purity of the $^{13}CH_3I$.

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