Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXIV, No. 4

SYNTHESIS OF DEUTERIUM LABELED 1,3-DIMETHYLURIC ACID

Kumiko Mamada, Takashi Furuta, and Yasuji Kasuya
Tokyo College of Pharmacy
1432-1 Horinouchi, Hachioji, Tokyo 192-03, Japan

SUMMARY

A synthetic procedure for 1,3-di(trideuteromethyl)uric acid is described. Deuterium labeling of the N-1 and N-3 positions was achieved by methylation of 4-amino-5-carbethoxyuracil with di(trideuteromethyl)-sulfate followed by cyclization of the dimethylated intermediate by heating. The synthesis resulted in a product with 99.4% d_6 isotopic purity, which is selectively deuterated in the methyl groups at the N-1 and N-3 positions and chemically pure.

Key Words: 1,3-di(trideuteromethyl)uric acid, Deuterium, Mass fragmentography, IR spectrum, NMR spectrum.

INTRODUCTION

Traditional techniques for the study of absolute or relative bioavailability need to perform two individual studies in each subject, where an intravenous or a reference and an oral test doses are administered on different occasions, separated by a suitable "wash-out" period. Because of intrasubject variability in the drug disposition, however, it is usually the case that a large number of subjects are required to assess the bioavailability.

Variability in an individual's clearance of theophylline has been known and this is an important consideration when estimating bioavailability[1,2]. We have proposed a method for compensating for this problem, using the serum concentration of theophylline and the urinary excretion data on its major metabolites to make

0362-4803/87/040361–08$05.00

Received May 21, 1986
Revised August 11, 1986

an estimation of the clearance after oral administration using the intravenous dose as reference[3]. Direct evidence for the validity of the proposed method will be provided by a stable-isotope coadministration technique. To apply this technique we are in the process of developing an assay method for the quantitation of theophylline and its major metabolite, 1,3-dimethyluric acid, in biological fluids using gas chromatography-mass spectrometry-selected ion monitoring (GC-MS-SIM). This paper describes the synthesis of 1,3-di(trideuteromethyl)uric acid (DMU-d_6) for use as an analytical internal standard for the GC-MS-SIM.

EXPERIMENTAL

Di(trideuteromethyl)sulfate (dimethylsulfate-d_6, $(CD_3)_2SO_4$, 99.5 atom%d) and 4,5-diamino-2,6-dihydroxypyrimidine hemi-sulfate were obtained from Merk and Sigma, respectively. TLC was performed on Kieselgel 60 F_{254} (0.25 mm in thickness, Merk) using a solvent system of acetonitrile:3.5% ammonium hydroxide 3:1. IR spectra were recorded with a Hitachi 260-30 spectrometer for KBr tablets. ^{1}H-NMR spectra were determined with a Bruker AM-400 spectrometer for solutions in deuterodimethylsulfoxide. The mass spectra were recorded with a Hitachi M-80. The direct EI mass spectometer conditions were: ionization source temperature, 200°; and ionization energy, 20 eV.

4-Amino-5-carbethoxyuracil (2)

To a suspension of 10.003 (53.32 mmole) of 4,5-diamino-2,6-dihydroxypyrimidine hemi-sulfate in 100 mL of 2N sodium hydroxide was added 10 mL (105.05 mmole) of ethyl chloroformate. The mixture was heated under reflux at 80° for 2 hr and then stirred for a further hour at room temperature. Afterwards, acetic acid was added to the reaction mixture (pH 6) and the produced pale

yellow amorphous precipitate (2) was filtered out, washed with ice-cold water and dried in vacuum. The yield was 8.991 g (42.01 mmole, 80.3%): ^{1}H-NMR 1.16-1.20 (t, 3H, J=7.0 Hz, 5'-CH_3), 3.95-4.00 (q, 2H, J=7.0 Hz, 4'-CH_2), 6.03 (s, 2H, 1'-NH_2), 7.40 (s, 1H, 2'-NH), 10.02 (s, 1H, 3-NH), and 10.27 (s, 1H, 1-NH).

1,3-Di(trideuteromethyl)-4-amino-5-carbethoxyaminouracil (3)

To a stirred suspension of 0.402 g (1.88 mmole) of (2) in 2 mL of water was added 0.5 mL (5.26 mmole) of dimethylsulfate-d_6 in one portion. The pH of the reaction mixture was maintained at 8.5-9.0 by adding 6N sodium hydroxide solution dropwise at an appropriate rate until completion of the reaction (4 hr). After centrifugating the reaction mixture, the supernatant was lyophilized in vacuum at -40° to give 1.097 g of the crude product (3). A portion (0.235 g) of the crude product (3) was dissolved in about 20 mL of chloroform. The chloroform solution was washed with a saturated solution of sodium chloride. The chloroform layer was separated and the sodium chloride solution was extracted with 20 mL of chloroform five times. After centrifugation, the combined chloroform layers were dried over anhydrous sodium sulfate and evaporated to about 1 mL. The resulting colorless precipitate (74 mg, 0.30 mmole, 74% yield) of (3) gave a single spot on TLC (acetonitrile:3.5% ammonium hydroxide 3:1, Rf 0.46): ^{1}H-NMR 1.18-1.21 (t, 3H, J=7.0 Hz, 5'-CH_3), 3.97-4.02 (q, 2H, J=7.0 Hz, 4'-CH_2), 6.67 (s, 2H, 1'-NH_2), and 7.54 (s, 1H, 2'-NH).

1,3-Di(trideuteromethyl)uric acid (4)

A 20-mL centrifuge tube containing 50 mg (0.20 mmole) of (3) under nitrogen atmosphere was placed in an alloy bath preheated to 160°. The temperature of the heating bath was then gradually raised to 240° over a period of 1 hr and maintained at 240° for

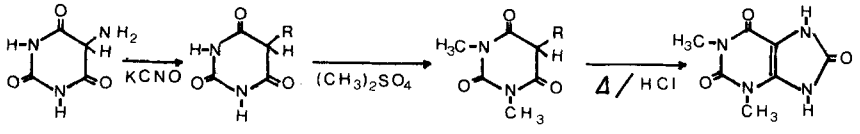
another 1 hr. After cooling, recrystallization from ethanol and water (5:1) followed by drying in vacuum gave 27 mg (0.12 mmole, 60.0% in yield) of colorless needle crystals as the monohydrated form of (4): IR ν_{C-D} 2172 cm^{-1} and 2106 cm^{-1}; ^1H-NMR 10.73 (s, 1H) and 11.60 (s, 1H); MS m/z 258 (M^+, diethyl derivative); Anal. Calcd. for $C_7H_2D_6N_4O_3$ H_2O: C, 38.18; N, 25.44; H, 4.58. Found: C, 38.19; N, 25.28; H, 4.66.

RESULTS AND DISCUSSION

In the synthesis of deuterium labeled 1,3-dimethyluric acid (DMU) for use as an analytical internal standard, special attention was paid to introduce more than three deuterium atoms in the molecule for the accurate and selective mass spectrometric analysis. The stability of label and the product with high isotopic purity were also of our primary concern.

Methods for the direct methylation of uric acid did not seem promising for the purpose of obtaining the desired deuterium labeled product with high yield. The direct methylation approach would result in a variety of methylated products depending on the methylating agents and the reaction conditions employed[4,5].

Bilz and Heyn[6] synthesized 1,3-dimethyluric acid (DMU) by treatment of pseudouric acid with dimethylsulfate followed by cyclization of the dimethylated intermediate by heating in the presence of hydrochloric acid as shown in Scheme 1.



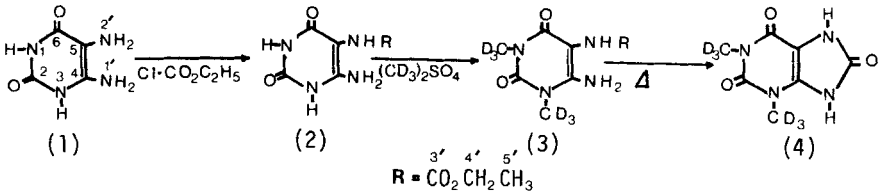
R = $NHCONH_2$

Scheme 1

Application of this method for DMU-d_6 seemed, however, not to be appropriate because the use of concentrated hydrochloric acid

during the cyclization step would cause the exchange reaction of deuterium with hydrogen. The isotopic yield would not then be satisfactorily high.

The method employed here for the synthesis of 1,3-di(trideuteromethyl)uric acid (DMU-d_6) was a modification of the procedures of Khmelevskii and Abramona[7], and Taylor and Sowinski[8]. The synthetic sequence is shown in Scheme 2. Introduction of six deuterium atoms to the N-1 and N-3 positions of the uracil molecule was achieved by treatment of 4-amino-5-carbethoxyaminouracil (2) with dimethylsulfate-d_6. The ring closure of 1,3-di(trideuteromethyl)-4-amino-5-carbethoxyaminouacil (3) provided upon heating the desired DMU-d_6 (4) in a relatively high yield (70%).



Scheme 2

Treatment of (2) with dimethylsulfate-d_6 gave the desired deuterium labeled intermediate (3) in a good yield (74%). According to Bredereck and Edenhofer[9], 5-sulfaminouracil or 5-nitrouracil is monomethylated at the N-3 position using an equimolar dimethylsulfate. Ukai, et al.[10] pointed out the predominant formation of the N-3 methylated product upon methylation of 4-aminouracil with excess dimethylsulfate. It seems therefore that methylation at the N-3 position of (2) is favored and that the 1,3-dimethylated product (3) is formed through the intermediary formation of the N-3 monomethylated compound, 3-methyl-4-amino-5-carbethoxyaminouracil.

As is evident from Fig. 1, the IR spectrum of DMU-d_6 shows

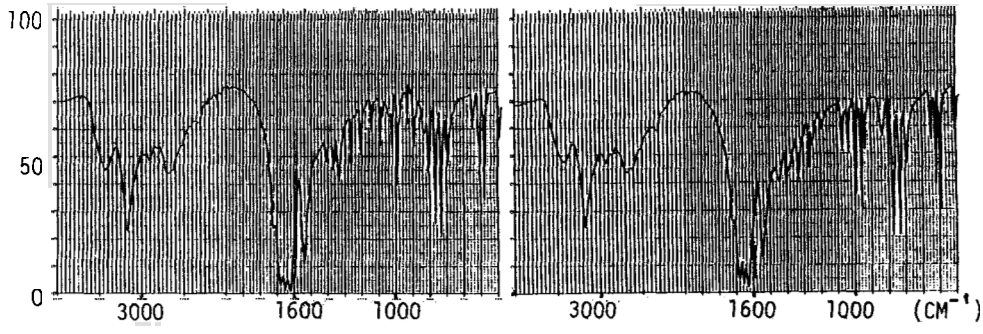
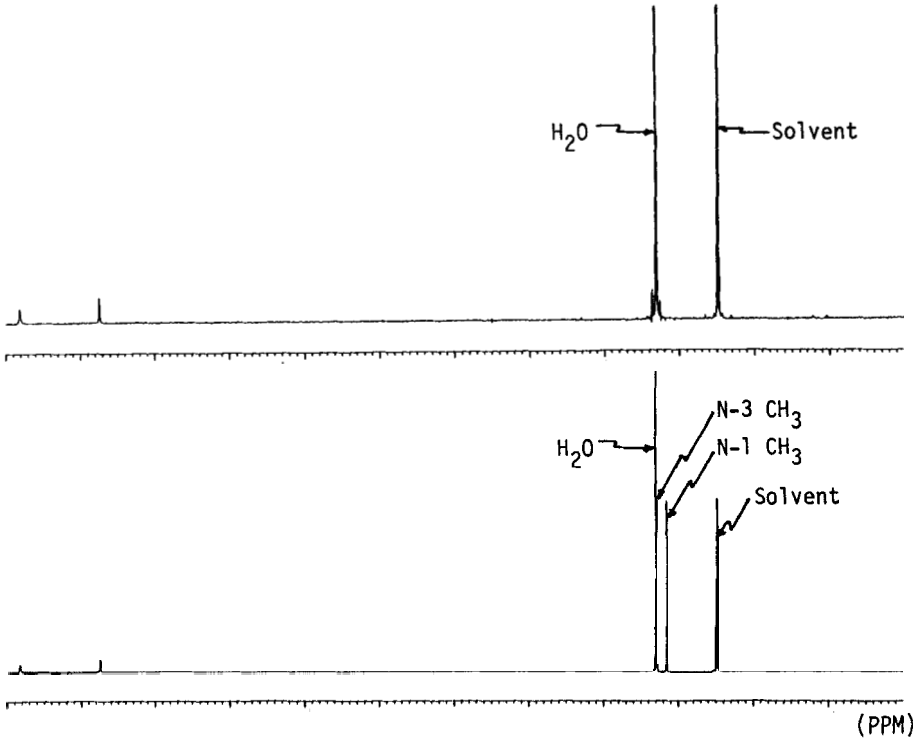


Fig. 1 IR Spectra of DMU-d_6 (Left) and DMU (Right)

C-D stretching vibration bands at 2172 cm^{-1} and 2106 cm^{-1}. A C-H bending vibration at 1450 cm^{-1} observed in the spectrum of unlabeled reference DMU disappears in the spectrum of DMU-d_6.

In the ^{1}H-NMR spectrum of unlabeled DMU (Fig. 2), the signals at 3.1 ppm and 3.3 ppm are assigned as the N-1 and the N-3 methyl protons, respectively[11]. The corresponding signals did of course disappear in the spectrum of DMU-d_6.



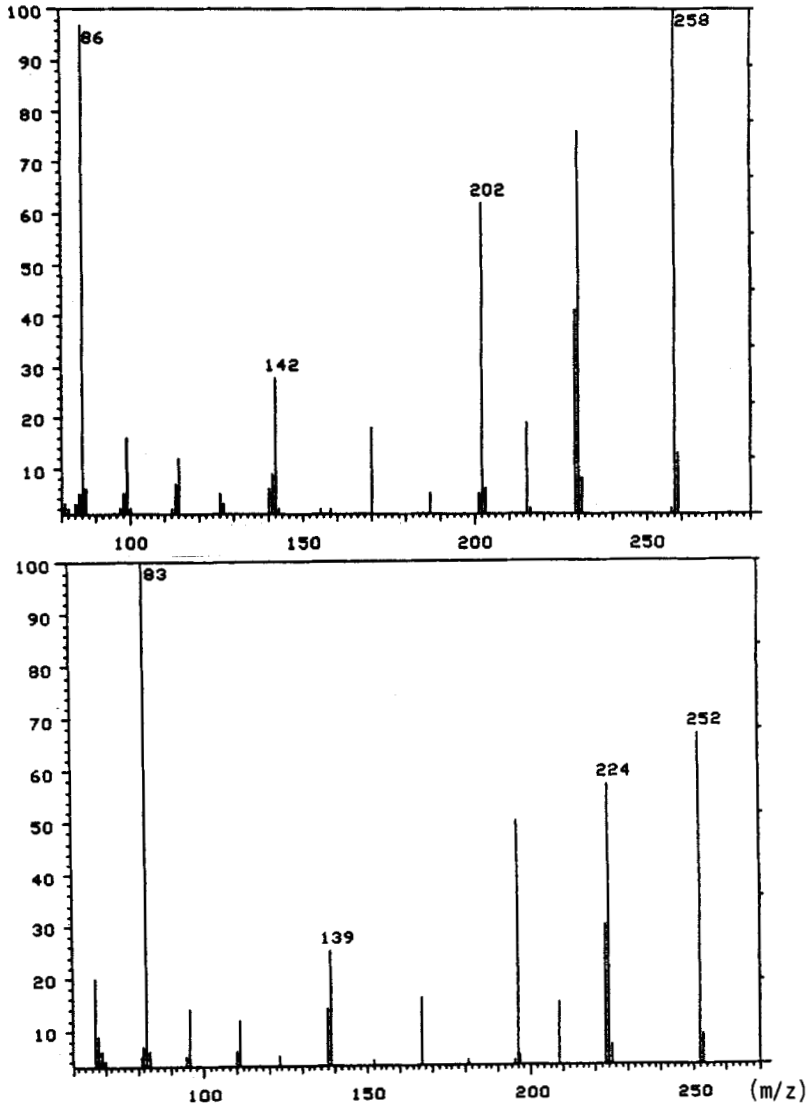


Fig.3 Mass Spectra of The Diethyl Derivatives of DMU-d_6(Upper) and DMU(Lower)

Derivatization of DMU for the GC-MS analysis was performed using ethyliodide and tetramethylammonium hydroxide. The diethyl derivative of DMU was separated by TLC before GC-MS. The mass spectrum of the diethylderivative of DMU-d_6 (Fig. 3, upper) showed a prominent molecular ion at m/z 258 (the unlabeled reference compound; m/z 252), with two successive losses of 28 mass units (CH_2=CH_2) giving peaks at m/z 230 (m/z 224) and m/z

202 (m/z 196). A fragment ion at m/z 170 corresponded to a loss of N-CD_3 (m/z 167, N-CH_3) from the ion at m/z 202 (m/z 196). Three further successive losses of 28 mass units (C=O) gave peaks at m/z 142 (m/z 139), m/z 114 (m/z 111), and m/z 86 (m/z 83). These results confirmed that six deuterium atoms were incorporated into the N-1 and N-3 methyl groups of DMU. Mass spectrometric determination of DMU-d_6 obtained in this experiment revealed very high isotopic purity of 99.9 atom% d (d_6; 99.4%, d_5; 0.6%).

In mass spectrometry, an increase of six atomic mass units will enable us to decrease the blank value, leading to a significant increase in sensitivity when DMU-d_6 is used as an internal standard for mass fragmentographic assays. A study on the quantitative determination of DMU in human blood and urine by mass fragmentography after administration of theophylline is now in progress and will be described in the following paper.

REFERENCES

1. Walson P.D., Strunk R.C., and Taussig L.M. - J. Pediatr., 91: 321(1977).

2. Upton R.A., Thiercelin J.F., Guentert L.N., Wallace S.M., Powell J.R., Sansom L., and Riegelman S. - J. Pharmacokin. Biopharm., 10: 123(1982).

3. Kasuya Y., Ohno T., Kubota N., Takahashi H., and Hirayama H. - J. Pharmacokin. Biopharm., 13: 571(1986).

4. Biltz H. and Herrmann L. - Chem. Ber., 54: 1676(1921).

5. Fischer E. - Chem. Ber., 32: 435(1899).

6. Bilz H. and Heyn M. - Liebigs Ann. Chem., 423: 185(1921).

7. Khmelevskii V.I. and Abramova E.I. - Zhur. Obshchei. Khim., 28: 2021(1958).

8. Taylor E.C. and Sowinski F. - J. Org. Chem., 39: 907(1974).

9. Bredereck H. and Edenhofer A. - Chem. Ber., 88: 1306(1955).

10. Ukai T., Yamamoto Y., and Kanetomo S. - Yakugaku Zasshi, 74: 674(1954).

11. Pfleider W. - Liebigs Ann. Chem., (12): 2030(1974).