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A New Synthesis of Double Labeled [7, 9 -$^{13}C_2$] Folic Acid

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Summary

A convenient small scale chemical synthesis of double labeled folic acid with ^{13}C at positions C-7 and C-9 is reported. [1,3-$^{13}C_2$] acetone was converted into folic acid in two steps, with [1, 1, 3]-trichloroacetone as the labeled intermediate.

Key Words: Folic acid, ^{13}C labeling.

INTRODUCTION

Dihydrofolate reductase is an enzyme required for the growth and maintenance of cells as it produces the important cofactor, tetrahydrofolate. Rapidly growing tumor cells may require more of this enzyme; therefore, the competitive inhibition of this enzyme *in vivo* with methotrexate and other antifolates has led to an accepted clinical method for the treatment of certain cancers. In conjunction with our ongoing interest in this enzyme, we are involved in a project to determine the solution conformation of pteroylglutamic acid (**2**, folic acid) in the active site of dihydrofolate reductase from *E. coli* using two-dimensional NMR methods. Heteronuclear 2D-NMR techniques using a ^{13}C label in folate would provide a sensitive method to determine the bound conformation of this substrate and minor bound species would also be more easily detected. The natural abundance of ^{13}C in folic acid is too low to allow facile ^{13}C NMR measurements so the synthesis of ^{13}C enriched folic acid was undertaken.

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EXPERIMENTAL

Materials. [1,3-$^{13}C_2$] acetone (99%) was purchased from Cambridge Isotopes. 4-Aminobenzoyl-L-glutamic acid, 2,4,5-triamino-6-hydroxypyrimidine, and sodium bisulfite were acquired from Sigma Chemical Co and used without further purification.

Instrumentation. Ultraviolet absorption spectra were recorded on a Perkin-Elmer diode array spectrophotometer. ^1H and ^{13}C NMR spectra were acquired on Bruker AM-500 or Bruker WM-360 spectrometers operating in quadrature mode.

[1, 1, 3-$^{13}C_2$] Trichloroacetone (**1**). To vigorously stirred [1, 3-$^{13}C_2$] acetone (30 g, 50 mMol) in a 50 mL round bottom flask at 31 °C was added freshly distilled sulfuryl chloride (20.9g, 155 mMol) at a steady rate over 60 minutes such that the reaction temperature never exceeded 31 °C. After the addition was completed, the reaction was heated at 65 °C for 16 hrs. After cooling to room temperature, SO_2Cl_2 (bp 69 °C), 1,1-dichloroacetone (bp 118 °C), and monochloroacetone (bp 120 °C) were removed by distillation through a 6 cm vigreux column at atmospheric pressure. The remaining residue was transferred to a 25 mL round bottom flask and distilled through a 6 cm vigreux column under reduced pressure to give 1.3 g (bp 70-75 °C, 40 mm Hg). The [1,3]-dichloroacetone and the [1,1,3]-trichloroacetone have the same boiling point and therefore, cannot be separated by distillation. ^1H NMR indicated that the products were present as a 50 mole percent mixture, each obtained in 9% yield. ^1H NMR (360 MHz, $CDCl_3$) of 1,1,3-trichloroacetone: δ=6.25 ppm (d, $^{13}CH_2$, J_{CH}= 180 Hz); δ=4.63 ppm (d, ^{13}CH, J_{CH}= 150 Hz). ^1H NMR (360 MHz, $CDCl_3$) of 1,3-dichloroacetone: δ=4.36 ppm (apparent doublet of triplets, $^{13}CH_2$, J_{CH}= 150 Hz, J_{HH} = 19 Hz.).

[7,9,-$^{13}C_2$] Folic acid (2). A mixture of [2,4,5]-triamino-6-hydroxypyrimidine (1.02 g, 4.16 mMol), 4-aminobenzoyl-L-glutamic acid (1.15 g, 4.30 mMol), and $NaHSO_3$ (1.26 g, 12.2 mMol) was finely powdered and dissolved in 176 mL of water. The pH was adjusted to 4.0 with saturated $NaHCO_3$ and the flask was gently swirled to dissolve the solids. To this was added a 2 mL ethanol solution of the mixture of [1,1,3]-trichloroacetone and 1,3-dichloroacetone (700 mg for the combined weight of the tri- and dichloroacetones, 2.2 mMol for the [1,1,3]-trichloracetone). The reaction was kept in the dark for three days with frequent addition of

saturated $NaHCO_3$ to maintain the pH at 4.0. A fine red precipitate appeared over this time, and this was collected by filtration and washed with 100 mL each of water, ethanol, and ether to give about 2 g of an orange powder. The powder was dissolved in 400 mL of water brought to pH 11 with NaOH and centrifuged at 8000 G for 20 minutes to pellet a gelatinous red mass which was discarded. The supernatant was brought to pH 7 with acetic acid, and the gelatinous red mass was again pelleted using previous centrifugation conditions. The supernatant was then brought to pH 3.2 with acetic acid and the flask stored overnight at 4 °C to precipitate the crude folic acid which was collected by centrifugation, washed with 100 mL each of water, ethanol, and ether. This material was dried to give 760 mg of a red powder which was recrystallized by suspending the solid in 400 mL of boiling water, filtered (hot) to remove particulates, and allowed to slowly cool to room temperature. The yellow crystals of folic acid (89 mg, 10%) were extremely pure as determined by NMR. The product coeluted with authentic commercial folic acid on an HPLC column (C_{18} Whatman Partisil reverse phase) eluting with a gradient of 0-30% ethanol in 50 mM sodium phosphate-7 mM tetrabutylammonium phosphate. The ultraviolet spectrum was identical to that of authentic folic acid, (pH 13, H_2O) λ_{max} at 368, 283, and 256 nm. The two labeled carbons yielded resonances identical to those of authentic folic acid (^{13}C NMR 125.76 MHz, proton coupled, 100 mM sodium phosphate in D_2O, pH 7) δ=149.1 ppm (d, J_{CH}= 182 Hz); δ=46.0 ppm (t, J_{CH} = 133 Hz), relative to methanol as an internal standard (δ 49.9 ppm relative to TMS). The proton NMR spectrum was identical to that for authentic folic acid except for the presence of ^{13}C-^{1}H splitting (^{1}H NMR 500.13 MHz, 100 mM sodium phosphate in D_2O, pH 7); δ=8.50 ppm (d, 1H J_{CH} = 182 Hz; H7); δ=7.64 ppm (d, 2H, J = 6.8 Hz; CH3'-CH5'); δ=6.75 ppm (d, 2H, J = 6.8 Hz; CH2'-CH6'); δ=4.54 ppm (d, 2H, J_{CH} = 133 Hz; CH_2-9); δ=4.26 ppm (m, 1H; $C^{\alpha}H$); δ=2.26 ppm (m, 2H; $C^{\gamma}H_2$); δ=2.10 ppm (m, 1H; $C^{\beta}H$) δ=1.98 ppm (m, 1H; $C^{\beta}H$)). The exchangeable NH and NH_2 protons are not detected in the D_2O solution; the carboxylate groups are apparently not protonated at this pH.

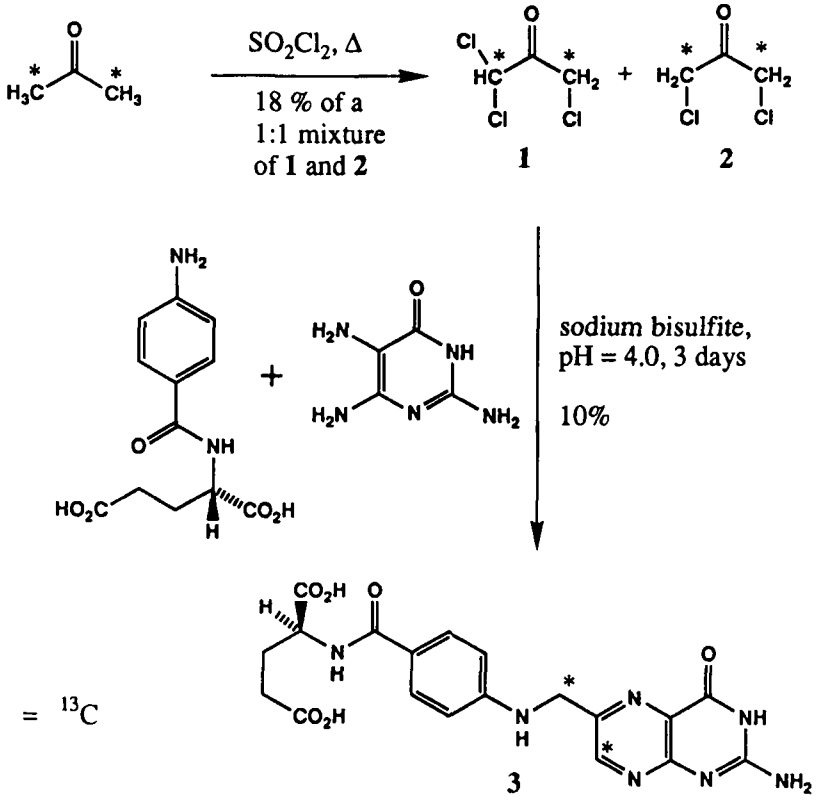
RESULTS and DISCUSSION

The [8-^{13}C] folic acid has been synthesized (1) from [2-^{13}C] glucose in seven steps. This method is quite tedious and expensive; therefore, a more direct and convenient route was sought. Also, the synthesis of [7,9-^{14}C] folic acid has been reported (2), but the overall yield for this three

step route from [2-^{14}C] acetic acid was only 0.4%, so this method was rejected. However, folic acid has been synthesized (3) in one step from 1,1,3-trichloroacetone (**1**). This last method was selected as likely to produce an efficient and convenient route to labeled folic acid, provided a ready source of [1,1,3]-trichloroacetone could be devised.

Scheme 1 depicts the route used to prepare [7,9-$^{13}C_2$] folic acid from [1,3-$^{13}C_2$] acetone. The conditions reported by Wyman and Kaufman (4) for the chlorination of acetone to give [1,1,3]-trichloroacetone were modified by increasing the ratio of SO_2Cl_2 to acetone, and by running the reaction at elevated temperature. The chlorination of [1,3-$^{13}C_2$] acetone with sulfuryl chloride gave a mixture of monochloracetone, [1,1]- and [1,3]-dichloracetone, and trichloroacetone. Careful distillation of this mixture afforded an inseparable mixture of labeled [1,3]-dichloroacetone (bp 173 °C) and [1,1,3]-trichloroacetone (bp 172 °C) in a 1:1 ratio

It was found that separation of these two compounds was not possible nor was it required for the successful synthesis of folic acid. The [1,3]-dichloroacetone/[1,1,3]-trichloroacetone



SCHEME 1

mixture was combined with [2,4,5]-triamino-6-hydroxypyrimidine, and 4-aminobenzoyl-L-glutamic acid at pH 4 in the presence of bisulfite for three days. After purification of the reaction mixture by precipitation of impurities at pH 11 and pH 7, the crude folic acid was further purified by recrystallation from water to yield [7,9-$^{13}C_2$] labeled folic acid in 10% overall yield. Analysis by HPLC on a C_{18}-reverse phase column demonstrated that folic acid was obtained in a high state of purity using this relatively simple and direct synthesis of ^{13}C labeled folic acid.

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REFERENCES

1. Selinsky B. S., Perlman M. E., London R. E., Unkefer C. J., Mitchell J., and Blakley R. L. -Biochemistry 29: 1290 (1990).

2. Weygand, F. and Swoboda O. P. -Chem. Ber. 89: 18 (1956).

3. Uyeo S., Mizukami S., Kubota T. Takagi S. -J. Am. Chem. Soc. 72: 5339 (1950).

4. Wyman D. P., and Kaufman P. R. -J Org. Chem. 29: 1956 (1964).