A New One-Step Synthesis of Leucovorin from Folic Acid and of 5-Formyl-5,6,7,8-tetrahydrohomofolic Acid from Homofolic Acid Using Dimethylamine–Borane in Formic Acid

Ronald A. Forsch and Andre Rosowsky*

Dana-Farber Cancer Institute and the Department of Pharmacology, Harvard Medical School, Boston, Massachusetts 02115

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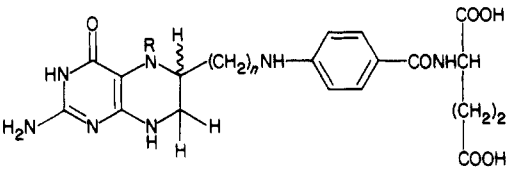
Leucovorin or folinic acid (**1**), a mixture of the 6*R* and 6*S* diastereomers of 5-formyl-5,6,7,8-tetrahydrofolic acid, is an important clinical agent which may be used as a remedy for megaloblastic anemia and as an antidote for inadvertent overdosages of antifolates in patients with cancer, parasitic diseases, or autoimmune disorders such as psoriasis and rheumatoid arthritis.[1] The most extensive use of **1** has been as an adjuvant in high-dose methotrexate therapy of patients with tumors that are resistant to conventional methotrexate regimens.[2]

In view of the above it is surprising that few significant improvements in the manufacture of **1** have occurred over the past 30 years. A commercial process for obtaining **1** from folic acid consists of catalytic hydrogenation (PtO_2) in formic acid followed by several days of storage at room temperature in formic acid, during which time the initially formed 5,6,7,8-tetrahydrofolic acid undergoes N^5-formylation and cyclodehydration to N^5,N^{10}-methenyl-5,6,7,8-tetrahydrofolic acid. Cleavage of the N^5,N^{10}-methenyl compound to the desired product, **1**, is performed in a separate step involving treatment with hot alkali.[3-5] Recent variants of this approach involve a two-step chemical reduction ($Na_2S_2O_4$, then $NaBH_4$) of folic acid followed by reaction with methyl formate in Me_2SO–pyridine[6] and a one-step reduction ($NaBH_4$ alone) followed by reaction with ethyl orthoformate.[5] In the present paper we report a new method of synthesis of **1** from folic acid that can be performed in a single operation, using dimethylamine–borane (BH_3·$HNMe_2$) in formic acid at 0–5 °C to reduce the pyrazine ring and directly introduce the N^5-formyl group in 60–70% isolated yield. Advantages of this process over earlier ones are that it avoids platinum-catalyzed hydrogenation, which can be both costly and hazardous on a large scale, and that it produces N^5-formylation without concomitant ring closure to the N^5,N^{10}-methenyl derivative, thus obviating the need for strong base treatment as a separate step. Also described here is the use of this method to prepare 5-formyl-5,6,7,8-tetrahydrohomofolic acid (**2**), a new antifolate with specific biochemical effects directed against the de novo biosynthetic pathway to purines.[7] Compound **2** is of interest because of its structural relatedness to N^5,N^{11}-methenyl-5,6,7,8-tetrahydrohomofolic acid, which has been proposed to be the active purine antimetabolite generated from homofolic acid in living cells.[8,9] Furthermore, **2** is an analogue of 5-methyl-5,6,7,8-tetrahydrohomofolic acid (**3**),[10] which recently underwent preclinical[11] and early clinical[12] trial as an anticancer drug. A significant difference between **3** and **2** is that the change of N^5 from an amine nitrogen to an amide nitrogen markedly enhances chemical stability.



1, n = 1, R = CHO
2, n = 2, R = CHO
3, n = 2, R = Me

Use of BH_3·$HNMe_2$ in AcOH at 60 °C has been made to prepare 5,6,7,8-tetrahydrofolic acid from folic acid,[13] and it was therefore envisaged that this reagent could be similarly used to reduce homofolic acid, with the N^5-formyl group being introduced in a separate step via reaction with formic acid and 1-ethyl-3-(3-(dimethylamino)propyl)-carbodiimide (EDC) as described by Moran and Colman.[14] Preliminary small-scale synthesis of **2** by this approach was successful,[7] but on a larger scale it was found that removal of the last traces of AcOH following reduction in this solvent was troublesome. Since residual AcOH during EDC-mediated N^5-formylation would be likely to produce N^5-acetylation as a side reaction, we considered the possibility of conducting the BH_3·$HNMe_2$ reaction in formic acid. When the reaction was carried out at low temperature (0–5 °C) we observed that formation of the N^5,N^{11}-methenyl compound (λ_{max} 315 nm)[8,9] did not occur and that **2** (λ_{max} 283 nm) was the sole major product. At room temperature or above, ring closure was observed. Compound **2** was isolated in analytically pure form in 60% yield as a calcium salt. Similarly, reaction of folic acid with BH_3·$HNMe_2$ in formic acid at 0–5 °C gave analytically pure calcium leucovorin (**1**, Ca salt) in 64% yield.

In considering possible explanations for the unusually facile in situ N^5-formylation of tetrahydrofolic and tetrahydrohomofolic acid we were led to search the literature for examples of the use of formic acid as a solvent in BH_3·$HNMe_2$ reductions, but found none. However two references to the use of boron triformate, $(HCOO)_3B$, as a formylating agent were found.[15] These reports led us to speculate that BH_3·$HNMe_2$ reacts with formic acid to generate boron triformate in situ, and that it is this species (probably a better formylating agent than formic acid itself) which gives rise to **1** and **2** from folic acid and homofolic acid, respectively. We propose that the reason for the success of this method is that the putative active

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species boron triformate can N^5-formylate at a temperature which is low enough to preclude cyclization to an N^5,N^{10}- or N^5,N^{11}-methenyl derivative.

Experimental Section

IR spectra were obtained on a Perkin-Elmer Model 781 double-beam recording spectrophotometer, UV spectra were obtained on a Varian Model 210 UV/visible instrument, and NMR spectra were obtained on a Varian Model T60A instrument with chemical shifts (δ) reported relative to Me_4Si. TLC was performed on Eastman 13181 silica gel or Eastman 13254 cellulose sheets containing a fluorescent indicator. Spots were visualized under 254-nm illumination. Microanalyses were performed by Galbraith Laboratories, Knoxville, TN, and MCL Laboratories, Lowell, MA. Folic acid was purchased from Sigma Chemical Company, St. Louis, MO. Homofolic acid was provided by Dr. J. A. R. Mead, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

Leucovorin, Calcium Salt (1, Ca Salt). A solution of folic acid (477 mg, 1 mmol) in 97–100% formic acid (10 mL) was cooled in an ice bath and treated with $BH_3{\cdot}HNMe_2$ (858 mg, 15 mmol) in small portions over 1 h with vigorous stirring. When addition was complete the light-orange solution was left to stand in the refrigerator at 4 °C for 24 h. The solution was then poured with stirring into ether (50 mL), the ether was decanted, and the residue was triturated with fresh ether until a powder was obtained. After decantation of the ether, water (10 mL) was added and calcium hydroxide was added to the suspension, in small portions with stirring, until the pH was strongly alkaline. Final solubilization was achieved with the aid of an ultrasonic bath. The pH was adjusted to 7.5 with dilute formic acid, a small amount of fine insoluble material was filtered off, and the filtrate was diluted with EtOH (120 mL). After several hours of refrigeration the crude product was collected, reprecipitated once more from aqueous EtOH, and dried in vacuo at 65 °C over P_2O_5 to obtain a white solid (396 mg, 64% yield); R_f 0.7 (cellulose, 3% NH_4Cl); IR ν (KBr) 3400, 1610–1640 cm^{-1}; NMR δ (D_2O) 1.5–3.4 (m, alkyl), 6.60 (d, J = 8 Hz, aryl protons ortho to N^{10}), 7.57 (d, J = 8 Hz, aryl protons ortho to CONH); UV λ_{max} (H_2O) 285 nm (ϵ 25 700). Anal. Calcd for $C_{20}H_{21}N_7O_7Ca{\cdot}0.25Ca(OOCH)_2{\cdot}4.5H_2O$: C, 39.39; H, 4.92; N, 15.68; Ca, 8.01. Found: C, 39.52; H, 5.26; N, 15.78; Ca, 8.01.

5-Formyl-5,6,7,8-tetrahydrohomofolic Acid, Calcium Salt (2, Ca Salt). A solution of homofolic acid (491 mg, 1 mmol) in 97–100% formic acid was cooled in an ice bath. Then $BH_3{\cdot}HNMe_2$ (118 mg, 2 mmol) was added all at once with vigorous stirring at 15-min intervals for a total of 8 times (16 mmol). When all additions were complete, the solution was placed in the refrigerator (4 °C) overnight before being poured into ether (100 mL) with stirring. The ether was decanted and the residue was washed repeatedly with ether and dissolved in water (5 mL). A minimum amount of NH_4OH was added to dissolve the compound, and $CaCl_2{\cdot}2H_2O$ (1 g) was added, followed by EtOH (100 mL). After overnight refrigeration, the collected product was dissolved in water (15 mL) with the help of a sonicating bath. A small amount of insoluble material was removed by filtration, the filtrate was decolorized with charcoal, and the product was reprecipitated with EtOH (100 mL), collected, and dried in vacuo at 60–65 °C over P_2O_5 to give a colorless powder (375 mg, 60% yield); R_f 0.7 (cellulose, 3% NH_4Cl); IR (KBr) ν 3390, 1610–1640, 1560 cm^{-1}; NMR δ (D_2O) 1.5–2.7 and 2.9–3.7 (m, alkyl), 6.87 (d, J = 8 Hz, aryl protons ortho to N^{10}), 7.87 (d, J = 8 Hz, aryl protons ortho to CONH); UV λ_{max} (H_2O) 285 nm (ϵ 26 000). Anal. Calcd for $C_{21}H_{23}N_7O_7Ca{\cdot}5.5H_2O$: C, 40.38; H, 5.49; N, 15.70; Ca, 6.42. Found: C, 40.38; H, 5.38; N, 15.45; Ca, 6.44.

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Registry No. 1, 58-05-9; 1·Ca, 1492-18-8; 2, 96482-98-3; 2·Ca, 96482-99-4; $BH_3{\cdot}NHMe_2$, 74-94-2; HCO_2H, 64-18-6; folic acid, 59-30-3; homofolic acid, 3566-25-4.

Acid-Catalyzed Photochemical Generation of Benzyl Cations as a Probe of the Electron-Donating Abilities of Benzene Substituents in the Singlet Excited State

Peter Wan

Department of Chemistry, University of Victoria, Victoria, British Columbia, Canada V8W 2Y2

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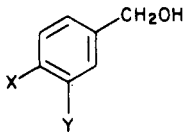
In a recent study[1] we showed that certain methoxybenzyl alcohols undergo efficient photochemical dehydroxylation (solvolysis) in aqueous solution to give the corresponding benzyl cations, which could be subsequently trapped by external nucleophiles (eq 1). *o*- and *m*-methoxy-substituted benzyl alcohols showed this reactivity; the para isomer was unreactive under similar conditions. This study was the first, to our knowledge, which characterized in quantitative terms the long-postulated paradigm of different relative reactivities of methoxy-substituted benzenes in organic photochemistry,[2] initially postulated by Zimmerman and Sandel.[3] Additionally, we showed that novel catalytic effects[4] due to the hydronium ion was observable at sufficiently low pH's, the overall effect being to increase the rate of photochemical dehydroxylation.

$$ArCH_2OH \underset{H_2O}{\overset{h\nu}{\rightleftharpoons}} [ArCH_2^+] \xrightarrow{ROH} ArCH_2OR \quad (1)$$

An important application of the above results is in the use of the reaction as a probe of the relative electron-donating abilities of substituents other than methoxy, assuming that photochemical dehydroxylation occurs to measurable extent for other substituted benzyl alcohols. We report here such an application to the fluoro and methyl substituents and provide evidence to suggest that *m*-fluoro *m*-methyl groups have significantly better electron-donating effects compared to their para analogues in S_1.

Substituted benzyl alcohols 1–4 were chosen for study because they showed relatively strong fluorescence ($\phi_f \sim 0.1$), thus enabling emission spectroscopy to be used to probe their excited-state behavior. Fluorescence emission from these alcohols was quenched on going from pH 7 to lower pH, as shown in Figure 1 for *m*-fluorobenzyl alcohol (**2**). The proportion of fluorescence quenching, as measured by ϕ_f/ϕ_f^0, where ϕ_f^0 represents the fluorescence yield in pH 7, plotted against pH (or H_0) is shown in Figure 2 for all these compounds. Both of the meta isomers are quenched more efficiently with increasing acidity than



1, X = F; Y = H
2, X = H; Y = F
3, X = CH_3; Y = H
4 X = H; Y = CH_3

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