Chemical Conversion of Folic Acid to Pteroic Acid

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Treatment of folic acid with acetic anhydride in acetic acid gave a mixture of acetylated azlactones, which are cleaved with mild base at room temperature to give mainly acetylated pteroic acids. Removal of the acetyl groups from the components of this mixture with hot base gave pteroic acid in 55–60% yield contaminated with folic acid. Although treatment of folic acid with base gave about a 21% yield of pteroic acid as determined by high-pressure liquid chromatography (HPLC), the purified yield of pteroic acid was only 10%. One of the byproducts was shown to result from hydrolysis of the glutamic acid moiety and opening of the pyrimidine ring to give a pyrazine derivative.

Pteroic acid (**5**) is a valuable intermediate in the synthesis of analogues and derivatives of folic acid (**1**).[1,2] Although numerous methods have been reported for the synthesis of **5**, these methods involve either difficult purification procedures or long reaction sequences.[3-5] In recent years some investigators have found it more convenient to prepare small amounts of crude **5** by bacterial degradation of **1**.[2,6] This method is attractive because of the low cost of **1** but is unsuitable for most laboratories. In this paper we report a procedure for the chemical conversion of **1** to **5**.

Treatment of **1** with 3–6 N HCl gave rapid degradation and resulted in the formation of black resinous tars. In 1 N HCl a complex mixture containing **5** was obtained, but HPLC and mass spectral data indicated that **5** was also decarboxylated under these conditions to give **6** (see Scheme I).

In the treatment of **1** with hot 0.1–0.2 N NaOH under N_2, hydrolysis was slow. In contrast, **5** was formed in hot 1–2 N NaOH, but high-pressure liquid chromatography indicated that the maximum yield of **5** was about 21%.[7] This limit in yield was shown to be caused by concurrent reactions. One byproduct was the pyrazine **4**, which was isolated and characterized after prolonged treatment of **1** with base.

Reaction of **1** with acetic anhydride in HOAc gave a product that was shown by HPLC and the ^1H NMR spectrum to be a mixture of two components. The field-desorption mass spectrum suggested that these components were the azlactones **2** [m/e 456 $(M + 1)^+$] and **3** [m/e 507 (M^+)], but the spectrum was unusual in that the latter occurred in a cluster of peaks (m/e 504–509). The azlactone structures are consistent with the reported conversion of *N*-[4-(acylamino)benzoyl]glutamic acids to the corresponding azlactones under similar conditions.[9]

Treatment of the mixture of **2** and **3** with hot HOAc gave a 1:1 mixture of the mono and diacetyl derivatives of **1** and **5**. The derivatives of **1** resulted from hydrolysis of the azlactone ring at a C–O bond, whereas the derivatives of **5** resulted from hydrolysis at both C–O and C=N bonds. A similar pattern of azlactone cleavage was observed on treatment of the mixture of **2** and **3** with hot 0.5 N NaOH. In this reaction the azlactone rings were hydrolyzed, and, in addition, the acetyl groups were removed to give a 1:1 mixture of **1** and **5**. In contrast, mild base (pH 8–9) at room temperature favored the formation of the products resulting from C=N bond cleavage. Under these conditions the mixture of **2** and **3** was hydrolyzed to give a mixture of **7** and **8** (83%) containing mono- and diacetyl **1** (12%). The increase in C=N bond cleavage under mild conditions was attributed to a greater participation of the carboxyalkyl substituent of the azlactone ring. The participation of a carboxy group in azlactone cleavage has also been observed on treatment of 4-[2-(alkoxycarbonyl)-benzylidene]oxazol-5-ones with base to give isoquinoline-3-carboxylic acid.[10] The removal of the acetyl groups from the mixture of **7**, **8**, and acetylated **1** was effected under N_2 with hot 0.5 N KOH to give **5** (~60% from **1**) contaminated with **1**.

Although **5** prepared by these methods contained **1**, mixtures of **1** and **5** were readily separated by column chromatography. In addition, extraction with hot water of samples containing less than 5% of **1** preferentially removed the more soluble **1**. In the absence of an antioxidant, both of these methods generated trace amounts of byproducts. However, procedures have been reported for the purification of **5** either by chromatography in the presence of ascorbic acid[2] or by recrystallization of the stable and more soluble 10-trifluoroacetyl derivative.[1]

Experimental Section

HPLC chromatograms were determined on a Waters Associates ALC-242 liquid chromatograph equipped with a reversed-phase μ-Bondapak C_{18} column with an eluting solvent of 0.1 M acetate (pH 3.6)–acetonitrile (9:1). The UV absorption spectra were determined with a Cary 17 spectrophotometer, the mass spectra with a Varian MAT 311A spectrometer, and the proton NMR spectra with a Varian XL-100-15 spectrometer with tetramethylsilane as an internal reference.

Azlactones of Mono- and Diacetylfolic Acid (2 and 3). A mixture of $1{\cdot}2H_2O$ (10.0 g, 20.9 mmol) in acetic anhydride (175 mL) and acetic acid (25 mL) was refluxed with stirring under N_2 for 1 h. The resulting red solution was filtered into a flask containing ether (3 L). The hygroscopic, off-white precipitate was collected under N_2, washed with ether, and dried in vacuo over P_2O_5 and NaOH pellets: yield 9.21 g (81%); mp, indefinite with decomposition above 170 °C; HPLC, **2** (27%), **3** (73%); UV (0.1 N NaOH, unstable) λ_{max}, nm ($\epsilon \times 10^{-3}$) 256 (35.2), 354 (8.26); FD mass spectrum for **2**, m/e 466 $(M + 1)^+$; for **3**, m/e 507 (M^+), 508 $(M + 1)^+$, 509 $(M + 2)^+$; ^1H NMR (CF_3CO_2D, 8% w/v) δ 2.20 (br s, CH_3CO_2H), 2.26 (s), 2.55 (s) (CH_3CO), 9.17 (s), 9.22 (s) (7-CH).

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(7) Refluxing **1** with 2 N NaOH followed by DEAE cellulose chromatography in the presence of ascorbic acid gave a 10% yield of **5**.[8]
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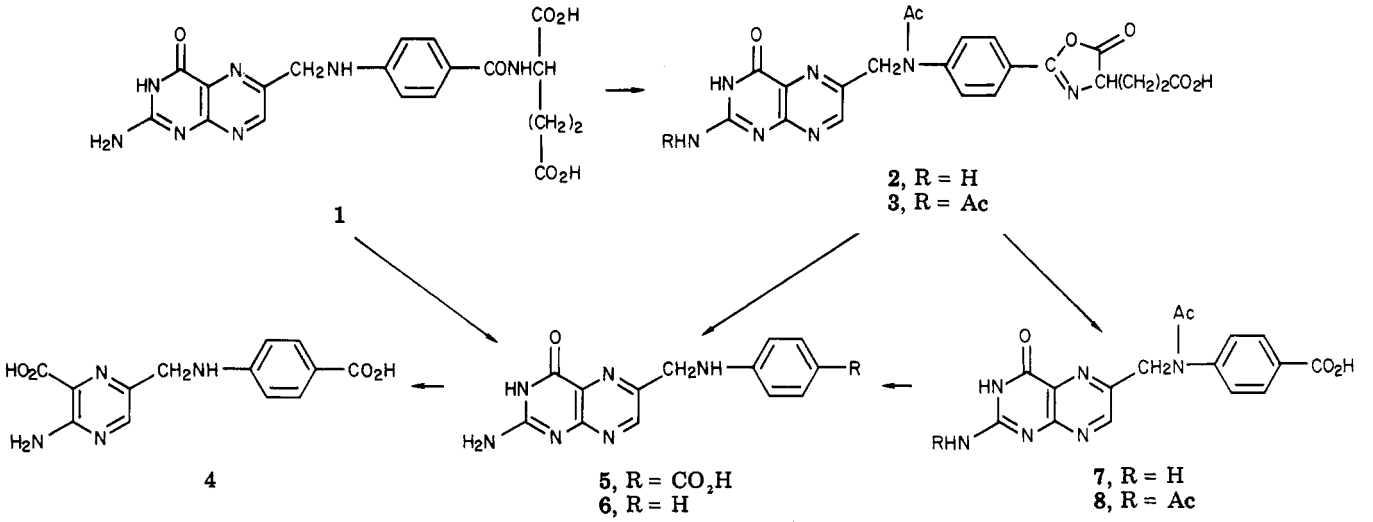
Scheme I



Anal. Calcd for $C_{21}H_{19}N_7O_6 \cdot C_{23}H_{21}N_7O_7(1:3) \cdot 0.75CH_3CO_2H$: C, 53.18; H, 4.38; N, 18.09. Found: C, 53.67; H, 4.43; N, 18.05.

Azlactone Hydrolysis. A suspension of the mixture of azlactones (850 mg) in deoxygenated water (85 mL) was dissolved by the dropwise addition of 50% and then 1 N NaOH while the pH (meter) was maintained below 11. The resulting solution (pH 9) was stirred at room temperature under N_2 with protection from light. The pH dropped with time and was readjusted to 9 after 5 and 22 h. After 44 h, the solution (pH 8.2) was adjusted to pH 3.5 with HCl to precipitate 8 as a tan solid: yield 225 mg; HPLC, 7 (7%), 8 (83%), 10-Ac-1 (~1%) and 2,10-Ac_2-1 (~1%); FD mass spectrum for 7, *m/e* 354 (M^+); for 8, *m/e* 396 (M^+); for 2,10-Ac_2-1, *m/e* 525 (M^+).

The filtrate was adjusted to pH 7.5 with a slurry of $Ca(OH)_2$ and diluted with ethanol (1.5 volumes) to deposit the calcium salts: yield 86 mg; HPLC, 7 (77%), 8 (~5%), 1 (3%), 10-Ac-1 (13%), 2,10-Ac_2-1 (2%). Further dilution of the filtrate with ethanol (6 volumes) gave an additional amount of the calcium salts: yield 283 mg; HPLC, 7 (69%), 8 (8%), 10-Ac-1 (6%), and 2,10-Ac_2-1 (13%). The total yield (594 mg) indicated essentially complete recovery of the products [7 (47%), 8 (36%), and acetylated 1 (12%)]. These results show that hydrolysis of the glutamate linkages of 2 and 3 was 84% complete.

In an earlier experiment, the azlactone mixture resulting from $1 \cdot 2H_2O$ (25.0 g, 52.4 mmol) was recrystallized twice from H_2O (1.5 L). The product (7.7 g) was dissolved in aqueous NaOH (pH 8.8), and the solution was stirred at room temperature for 18 h. After treatment with charcoal, the solution was acidified to pH 3.5 to deposit a mixture of the free acids: yield 5.0 g (24% of 7 and 8 from 1); HPLC, 7 (53%), 8 (39%), and acetylated 1 (~5%). A sample (1.0 g) of this material was dissolved in 0.5 N KOH (100 mL), and the solution was refluxed under N_2 with stirring for 20 h and adjusted to pH 10.5 with HCl. After filtration of the turbid mixture, the filtrate was adjusted to pH 2 with HCl. The yellow precipitate was collected by filtration, washed successively with HCl (pH 3) and water, and dried in vacuo over P_2O_5 at 78 °C: yield 0.74 g; HPLC, 5 (90%), 1 (8%). These results indicated that 5 was obtained in 86% yield.

Separation of Folic (1) and Pteroic (5) Acids. A mixture (2.0 g) of 1 (44%) and 5 (55%) resulting from treatment of 2 and 3 with 0.5 N NaOH was separated by elution of 1 from a cellulose (Whatman CC-31, 300 g) column with 0.1 M KH_2PO_4 (pH 7) followed by extrusion of the packing and extraction of the band containing 5 with KOH (pH 12): yield 0.76 g; HPLC, 5 (87%) and trace impurities resulting from oxidation of 5.

Although recrystallization of 5 (HOAc, pyridine) was unsuccessful, extraction of a sample (0.33 g) of 5 containing a small amount of 1 with H_2O in a hot Soxhlet apparatus for 24 h gave 5 containing <1% 1: yield 0.19 g; HPLC, 5 (87%) and trace impurities resulting from oxidation of 5; UV (0.1 N NaOH) λ_{max}, nm ($\epsilon \times 10^{-3}$) 256 (28.0), 276 (25.7), 365 (8.80); ^1H NMR (CF_3CO_2D, 7% w/v) δ 5.3 (CH_2), 8.2 (m, C_6H_4), 9.0 (7-CH).

Anal. Calcd for $C_{14}H_{12}N_6O_3 \cdot 0.5H_2O$: C, 52.34; H, 4.08; N, 26.16. Found: C, 52.44; H, 4.06; N, 26.14.

The cooled extract gave an additional amount of solid: yield 0.14 g; HPLC, 5 (76%), 1 (6%).

3-Amino-6-[[(4-carboxyphenyl)amino]methyl]-2-pyrazinecarboxylic Acid (4). A solution of $1 \cdot 2H_2O$ (10.0 g, 20.9 mmol) in deoxygenated 2.5 N KOH (500 mL) was refluxed with stirring under N_2 for 96 h, treated with charcoal and filtered (Celite). The filtrate was neutralized to pH 9 with concentrated HCl, and the residue that precipitated with removed by filtration. The filtrate was adjusted to pH 2.5 with HCl, and the yellow precipitate was collected by filtration, washed with cold water, and dried in vacuo over P_2O_5 at 78 °C: yield 3.66 g; HPLC, 4 (87%), 5 (10%), 1 (2%).

Further treatment of the above product (3.66 g) with 2 N KOH (150 mL) under the same conditions for 20 h gave 4 contaminated with impurities; yield 2.78 g. This sample was purified by elution from cellulose (Whatman CC31) with 0.1 M KH_2PO_4 (pH 7). The fractions containing homogeneous product (TLC) were pooled and acidified to pH 2 with HCl, and the solid that deposited was collected by filtration, washed with warm HCl (500 mL, pH 3), and dried in vacuo over P_2O_5 at 78 °C: yield 2.09 g; mp 227–228 °C dec with prior charring. A portion of this material was recrystallized from a large volume of acidified H_2O (pH 3) to give the analytical sample: mp 233–236° dec with charring and foaming; UV (0.1 N NaOH) λ_{max}, nm ($\epsilon \times 10^{-3}$) 254 (15.5), 278 (18.7), 342 (7.16); ^1H NMR (Me_2SO-d_6, 7% w/v) δ 4.36 (s, CH_2), 7.23 (dd, C_6H_4), 8.30 (s, 5-CH).

Anal. Calcd for $C_{13}H_{12}N_4O_4 \cdot 0.3H_2O$: C, 53.17; H, 4.32; N, 19.08. Found: C, 53.22; H, 4.17; N, 19.08.

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Registry No. 1, 59-30-3; 1 calcium salt, 78168-16-8; 10-Ac-1 calcium salt, 78168-17-9; 2,10-Ac-1 calcium salt, 78168-18-0; 2, 78168-19-1; 3, 78168-20-4; 4, 78168-21-5; 5, 119-24-4; 7, 78168-22-6; 7 calcium salt, 78168-23-7; 8, 70844-36-9; 8 calcium salt, 78168-24-8.