Synthesis of *N*-([2-([(2-Amino-3,4-dihydro-4-oxo-6-pteridinyl)methyl]amino)-5-pyrazinyl]carbonyl)-L-glutamic Acid (2′,5′-Diazafolic Acid)

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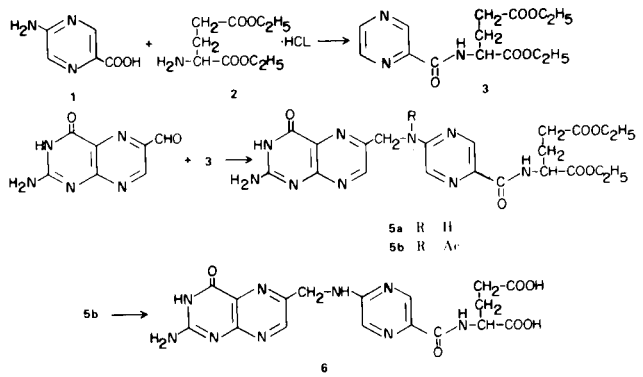
Received April 21, 1975

In the biosynthesis of purines, pyrimidines and some amino acids, tetrahydrofolate derivatives serve as agents in the transfer of one-carbon units. In these cases, one-carbon units are combined with nitrogen of position 10 and transferred to each substrate. Therefore, a decrease of electron density at N^{10} may be considered to lead to the diminished formation of one-carbon transfer agents. An increase in the electron density at N^{10}, conversely, may stabilize the agent and diminish the transfer of one-carbon unit from the folic acid type cofactor to the substrate. In order to decrease the electron density at N^{10}, Roberts and Shealy (1) synthesized some folic acid analogs which had pyridine, thiazole, or pyrimidine rings substituted for the benzene ring in the *p*-aminobenzoyl moiety of the molecule. But these compounds were less effective than the well-known antagonists, aminopterin and amethopterin. For similar purposes, we synthesized a folic acid analog in which the benzene ring was replaced by the pyrazine ring.

Scheme



The folic acid analog **6** was obtained *via* the reductive condensation of **3** with **4** in the presence of *p*-toluenethiol. Intermediate **3** was obtained by condensation of 2-amino-5-pyrazinecarboxylic acid **1** (2) and diethyl glutamate hydrochloride **2**. Initially, dicyclohexylcarbodiimide was used in this condensation reaction, but there was no product except for condensed product of **1** and dicyclohexylcarbodiimide. It was identified by nmr data δ 0.50-2.00 ppm (m, 22H, cyclohexyl protons). So, **1** and **2** were stirred in dimethylformamide with triethylamine and ethyl chloroformate, and **3** could be obtained. The crude product was purified by silica gel chromatography and recrystallization from benzene.

Compound **3** and **4**, which was obtained by the method of Sletzinger *et al.* (3), were refluxed in 2-methoxyethanol containing *p*-toluenethiol to give **5a**. Acetylation of **5a** gave **5b**, and it was purified by alumina chromatography and recrystallization from acetone.

Hydrolysis of purified compound **5b** in 0.1 *N* sodium hydroxide gave the desired 2′,5′-diazafolic acid **6**. The biological activities of compound **3**, **5** and **6** are now under investigation.

EXPERIMENTAL

Melting points are uncorrected. Proton magnetic resonance spectra were determined with a Hitachi R-20B spectrometer using tetramethylsilane as an internal standard. Infrared spectra as nujol mulls were determined with a Shimazu IR-27G spectrometer.

Diethyl *N*-[(2-Amino-5-pyrazinyl)carbonyl]glutamate (**3**).

Compound **1** (6.0 g., 43 mmoles) was suspended in 150 ml. of anhydrous dimethylformamide, the suspension was cooled in an ice bath, and triethylamine (13.1 g., 130 mmoles) and ethyl chloroformate (4.7 g., 43 mmoles) were added. After 1 hour, diethyl glutamate hydrochloride (10.3 g., 43 mmoles) was added, and the reaction mixture was stirred at room temperature for 3 days. The precipitate was removed by filtration and washed with dimethylformamide, and the filtrate was evaporated. The residue from the evaporated filtrate was dissolved in 130 ml. of ethyl acetate, and the solution was washed first with 1 *M* sodium carbonate solution, then water and finally dried. This solid was applied to a chromatographic column (Merck silica gel 60, 30-70 mesh, 200 g.) and developed with chloroform-methanol (96:4). Fractions containing the product were combined and evaporated. The crude product was recrystallized from benzene to yield 3.4 g. (25%) of **3**, m.p. 114.5°; ir ν (cm^{-1}): 1740 (ester), 1650, 1540 (amide); nmr δ (deuteriochloroform): 1.22 (t, 3H, CH_3), 1.29 (t, 3H, CH_3), 2.00-2.70 (m, 4H, CH_2CH_2), 4.11 (q, 2H, OCH_2), 4.23 (q, 2H, OCH_2), 4.52-5.05 (m, 1H, NCH), 5.20-5.90 (broad s, 2H, NH_2), 7.83 (broad s, 1H, C_3-H), 7.99 (d, 1H, CONH), 8.65 ppm (broad s, 1H, C_6-H).

Anal. Calcd. for $C_{14}H_{20}N_4O_5$: C, 51.84; H, 6.22; N, 17.28. Found: C, 51.66; H, 6.17; N, 17.23.

Diethyl *N*-([2-[(2-Acetylamino-3,4-dihydro-4-oxo-6-pteridinyl)-methyl]acetylamino)-5-pyrazinyl]carbonyl)-L-glutamate (**5b**).

A solution of **3** (2.6 g., 8 mmoles) and *p*-toluenethiol (5.95 g., 48 mmoles) in 140 ml. of 2-methoxyethanol were heated to reflux. Then the flask was flushed with argon gas and **4** (1.86 g., 8 mmoles) was added. The reaction mixture was refluxed under an argon atmosphere for 24 hours. After cooling, the dark grey gelatinous precipitate was filtered, washed with ether, and dried. The filtrate was evaporated to an oily residue which gave more the same product on treatment with ether.

The combined solids were heated in refluxing acetic anhydride for 5 hours. The hot mixture was filtered, and the filtrate was evaporated. Addition of ether to the residue gave a solid, which was filtered, washed with ether and dried. This solid was applied to a chromatographic column (Merck aluminium oxide active, neutral, 80 g.) and developed with chloroform-methanol (95:5). Fractions containing the product were combined and evaporated. The crude product was recrystallized from acetone to yield 227 mg. (4.9%) of **5b**, m.p. 200-201.5°; ir ν (cm^{-1}): 1730 (ester), 1690, 1665 (amide); nmr δ (deuteriochloroform): 1.18 (t, 3H, CH_3), 1.27 (t, 3H, CH_3), 2.20-2.55 (m, 4H, CH_2CH_2), 2.37 (s, 3H, $COCH_3$), 2.41 (s, 3H, $COCH_3$), 4.08 (q, 2H, OCH_2), 4.21 (q, 2H, OCH_2), 4.60-4.95 (m, 1H, NCH), 5.41 (broad s, 2H, NCH_2), 8.25 (d, 1H, NH), 8.96 (s, 1H, C_3'-H), 9.11 (s, 2H, C_6'-H, C_7-H), 10.60-11.70 ppm (broad band, 2H, OH, NH).

Anal. Calcd. for $C_{25}H_{29}N_9O_8$: C, 51.45; H, 5.00; N, 21.60. Found: C, 51.43; H, 4.93; N, 21.54.

N-([2-([(2-Amino-3,4-dihydro-4-oxo-6-pteridinyl)methyl]amino)-5-pyrazinyl]carbonyl)-L-glutamic Acid (**6**).

Compound **5b** (45 mg., 0.077 mmoles) was dissolved in 30 ml. of deaerated 0.1 *N* sodium hydroxide, and the solution was stirred at room temperature under argon atmosphere. After 16 hours, the solution was acidified with 1*N* hydrochloric acid to *p*H 3.5 and isolated and washed five times with 0.005 *N* hydrochloric acid by centrifugation. The product was dried *in vacuo* to yield 32 mg. (94%), m.p. 206° dec.; ir ν (cm^{-1}): 1715 (acid carbonyl), 1675, 1505 (amide); nmr δ (0.5 *N* sodium deuteroxide) 1.95-2.60 (m, 4H, CH_2CH_2), 4.20-4.65 (broad band, 2H, C_9-H), 7.75-8.04 (broad band, 1H, C_3'-H), 8.20-8.35 (broad band, 1H, C_6'-H), 8.48 ppm (s, 1H, C_7-H).

Anal. Calcd. for $C_{17}H_{17}N_9O_6$ 1.5 H_2O: C, 43.41; H, 4.29; N, 26.80. Found: C, 43.21; H, 4.13; N, 26.44.

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