solution. Adjustment of quantities was necessary for a specific hydrazide. Time of incubation at 40° was generally 24 hr, with the exception of 2-thiophenecarboxylic hydrazide, for which the time was reduced to 1.5 hr. Removal of products by suction filtration was followed by washing with water, drying, and weighing. For 2-pyrrolecarboxylic hydrazide, only the median optimum pH for the other five reactions, pH 4.00, was used to obtain the product from Z-glycine. pH optima are summarized in Table I. For nitrogen analyses and melting point determinations, products were dissolved in hot methanol, treated with decolorizing carbon, and suction filtered four or five times with thorough washing of any solid on the filter paper with fresh methanol into the filtrate each time to remove soluble solid. The final filtration involved a glass funnel with a fritted disk. The solvent was then removed by evaporation under the hood, with subsequent drying of solid.

Reactions between Hydrazides of Heterocyclic Carboxylic Acids and Z-L-Alanine and Z-DL-Alanine. Buffer, 0.50 *M*, at the pH optimum for the reaction between Z-glycine and a given hydrazide was used for these reactions. For 2-pyrrolecarboxylic hydrazide, pH 4.00 was again used. Quantities of solutes are all relative to a total of 100 ml of resultant buffered solution. For reactions or attempted reactions involving Z-DL-alanine and a hydrazide, 5 ml of hexamethylphosphortriamide was added as a solubilizing agent, with the exception of isonicotinic *N*-oxide hydrazide and 2-furoic hydrazide. In recording results, first an abbreviation of a reaction product from Table I is given. Second, the weight of L-cysteine·$HCl·H_2O$ and therefore active papain is recorded. Third, the moles of hydrazide are immediately followed by the moles of *N*-acylamino acid. Fourth, the periods of incubation at 40° are given. Fifth, the weights of products obtained for each incubation period are indicated. Sixth, weights of recrystallized products from combined incubation periods that were dissolved in sufficient Eastman Spectrograde pyridine to produce 5.00 ml of solution at 25° precede the observed optical rotation at 25° in a Rudolph Model 80 high precision polarimeter, in a 2-dm polarimeter tube.

Recrystallized products, by means of essentially the same method as for products from Z-glycine, were used for nitrogen analyses, melting points, and mixture melting points, as well as optical rotations. Details are given in Table I.

FLH: 0.500 g; 0.0100 mol, 0.0100 mol; 0–24, 24–48 hr; 0.33 g, 0.075 g; 0.0901 g for α_{obsd} −1.834°.

FAH: 0.417 g; 0.0133 mol, 0.0133 mol; 0–24, 24–48 hr; 1.05 g, 0.100 g; 0.1090 g for α_{obsd} −2.194°.

TLH: 0.400 g; 0.0100 mol, 0.0100 mol; 0–24 hr; 1.31 g; 0.1691 g for α_{obsd} −3.750°.

TAH: 0.400 g; 0.0100 mol, 0.0200 mol; 0–24 hr; 1.29 g; 0.1000 g for α_{obsd} −2.092°.

PyLH: 0.500 g; 0.0100 mol, 0.0200 mol; 0–24 hr; 0.26 g; 0.1000 g for α_{obsd} −2.045°.

PyAH: 0.500 g; 0.0100 mol, 0.0200 mol; 0–24 hr; 0.25 g; 0.1000 g for α_{obsd} −1.962°.

ILH: 0.600 g; 0.0100 mol, 0.0100 mol; 0–72 hr; 0.35 g; 0.1103 g for α_{obsd} −2.250°.

IAH: 0.461 g; 0.0123 mol, 0.0123 mol; 0–72 hr; 0.31 g; 0.1126 g for α_{obsd} −1.386°.

Acknowledgments. The Squibb Institute for Medical Research supplied a sample of isonicotinic *N*-oxide hydrazide for preliminary experimentation. The Wallerstein Co., Deerfield, Ill., donated the dried papaya latex. This research was supported by grants from the Society of the Sigma Xi and a Federick Gardner Cottrell grant from the Research Corporation. Mr. C. F. Geiger, Ontario, Calif., ran the nitrogen analyses.

Registry No.—Papain, 9001-73-4; picolinic acid *N*-oxide, 824-40-8; picolinic acid, 98-98-6; peracetic acid, 79-21-0; methyl picolinate *N*-oxide, 38195-81-2; methyl nicotinate, 93-60-7; methyl isonicotinate, 2459-09-8; methyl nicotinate *N*-oxide, 15905-18-7; methyl isonicotinate *N*-oxide, 3783-38-8; hydrazine, 302-01-2; picolinic *N*-oxide hydrazide, 54633-17-9; nicotinic *N*-oxide hydrazide, 23597-85-5; isonicotinic *N*-oxide hydrazide, 6975-73-1; 2-thiophenecarboxylic hydrazide, 2361-27-5; ethyl 2-thiophenecarboxylate, 2810-04-0; 2-pyrrolecarboxylic hydrazide, 50269-95-9; 2-pyrrolecarboxylic acid, 634-97-9; Z-glycine, 1138-80-3; furoic hydrazide, 3326-71-4; Z-L-alanine, 1142-20-7; Z-DL-alanine, 4132-86-9; FGH, 56587-73-6; FLH, 56587-74-7; TGH, 56587-75-8; TLH, 56587-76-9; PyGH, 56587-77-0; PyLH, 56587-78-1; PiGH, 56587-79-2; NGH, 56587-80-5; IGH, 56587-81-6; ILH, 56587-82-7.

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The Circular Dichroism Spectra of Folic Acid and 10-Thiafolic Acid and the Problem of Racemization in the Synthesis of Analogs of Folic Acid through the Cyclization of Substituted 2-Amino-3-cyanopyrazines[1]

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In view of the central role of derivatives of folic acid in cellular metabolism[2] and the usefulness of analogs of folic acid in the treatment of neoplastic disease,[3,4] a great deal of effort has been expended on searching for improved syntheses of molecules related to folic acid.

A relatively simple synthesis of 6-substituted pteridines was introduced by Taylor and his coworkers.[5-7] The reaction of aminomalononitrile with α-ketoaldoximes yields 2-amino-3-cyano-5-substituted pyrazine 1-oxides, deoxygenation and guanidine cyclization of which yields pteridines. This procedure, like some older but more cumbersome syntheses,[8,9] has the advantage of avoiding ambiguity in the positioning of the side chain and, in addition, delays the problems introduced by the extreme insolubility of pteridines until the final stages of the synthetic sequence. Recently, this synthesis was applied to the preparation of analogs of the antineoplastic agent methotrexate by Chaykovsky and his coworkers,[10] while our laboratory explored the usefulness of this approach in synthesizing 10-thiafolic acid, 10-thiapteroic acid, and related compounds.[11]

To prepare the latter group of compounds two synthetic routes were followed (Figure 1). In the first approach, reaction of 2-amino-3-cyano-5-chloromethylpyrazine[6] with ethyl 4-thiobenzoate, followed by cyclization with guanidine, yielded the ethyl ester of the 4-amino derivative of 10-thiapteroic acid. Mild hydrolysis led to the formation of 10-thiapteroic acid from which 10-thiafolic acid could be prepared using condensation with diethyl L-glutamate via the mixed anhydride method.

Alternatively, the complete side chain could be formed before addition to the pyrazine ring. In this approach, diethyl 4-thio-*N*-benzoyl-L-glutamate[11] was permitted to react with 2-amino-3-cyano-5-chloromethylpyrazine. Cyclization with guanidine, followed by mild hydrolysis, led to the formation of 4-amino-4-deoxy-10-thia-10-deazafolic acid or 10-thiaaminopterin.

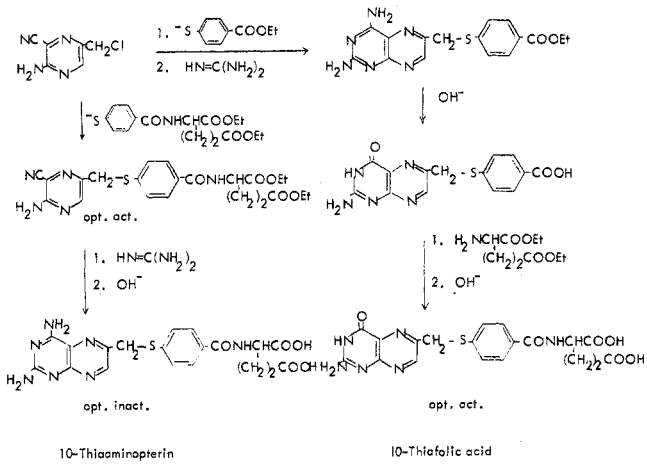

Figure 1.

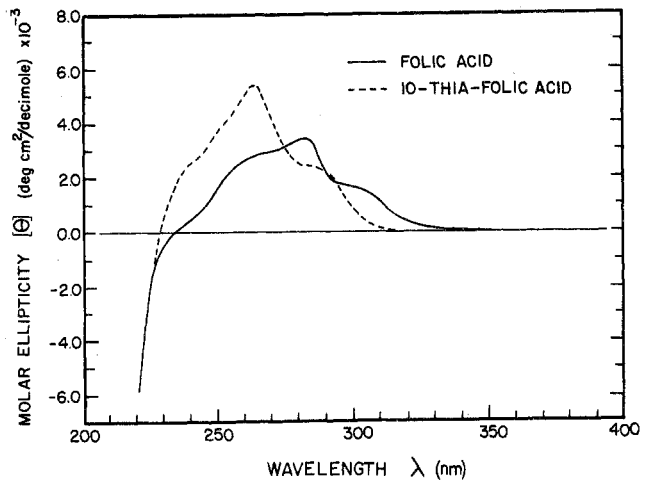

Figure 2. CD spectrum (0.01 *N* NaOH) of 10-thiafolic acid compared with that of folic acid.

In comparing the CD spectra of folic acid and of its 10-thia analog, it was noted that while the shapes of the absorption curves were similar, a considerable hypsochromic shift is seen in the 10-thia as compared to the 10-amino compound (Figure 2). Presumably, resonance involving the interaction of the heteroatom with the benzene ring is favored to a greater extent in the aminophenyl than in the thiophenyl compound. It should be noted that, while the CD spectra of methylenetetrahydrofolates have been measured,[12] the CD spectrum of folic acid has not been reported previously.

In contrast to the compounds discussed above, 10-thiaaminopterin proved to be optically inactive. This indicated that the *p*-aminobenzoyl-L-glutamyl side chain had racemized during the very basic conditions of the guanidine cyclization. An attempt was made to minimize racemization during the guanidine cyclization by heating the reaction mixture for 3.5 hr at 80° instead of refluxing it overnight. Analysis using TLC showed that cyclization was less than half complete, while the product has racemized totally, indicating that racemization is faster than cyclization.

Aminopterin synthesized by the addition of *p*-aminobenzoyl-L-glutamic acid to 2-amino-3-cyano-5-chloromethylpyrazine, followed by guanidine cyclization, also proved to be racemic. Both in the synthesis of aminopterin and the synthesis of 10-thiaaminopterin the intermediate 5-substituted 2-amino-3-cyanopyrazines retained full optical activity.

The problem of side-chain racemization during the guanidine cyclization cannot be ignored since it affects the biological interactions of the products. In the analog of methotrexate carrying a D-glutamic acid rather than an L-glutamic acid residue, ability to inhibit the growth of L-1210 leukemia cells is lowered considerably.[13] In addition, it was noted, using NMR spectroscopy, that the orientation of the aromatic rings of *p*-aminobenzoyl-L-glutamate and *p*-aminobenzoyl-D-glutamate on being bound to dihydrofolic acid reductase is quite different.[14]

The racemization problem in synthesizing folic acid analogs by the use of the Taylor synthesis can be avoided by carrying out the cyclization at the pteroic acid level and then forming the amide carrying the optically active substituent. No racemization takes place during the relatively mild conditions used to hydrolize esters of folic or related compounds.

Experimental Section

Materials. 2-Amino-3-cyano-5-chloromethylpyrazine was synthesized by the procedure of Taylor and Kobayashi.[6] 10-Thia-10-deazafolic acid and 10-thia-10-deaza-4-amino-4-deoxyfolic acid were prepared by a synthesis described elsewhere.[10]

CD Spectra. The spectra shown in Figure 2 were obtained with a Jasco J-20 automatic recording spectropolarimeter.

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Registry No.—Folic acid, 59-30-3; 10-thiafolic acid, 54931-98-5.

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Reaction of *n*-Butyllithium and 2,2,6,6-Tetramethylpiperidine Nitroxyl[1]

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Stable nitroxyl radicals[2-4] are widely used as radical scavengers[4] and as probes for certain types of molecular motion.[5] In the course of other problems that utilized ni-