Sato, *Bull. Chem. Soc. Jpn.*, **42**, 1322 (1969); C. Demuynck and A. Thuillier, *Bull. Soc. Chim. Fr.*, 2434 (1969).
(16) J. Wieland and H. Koppe, *Justus Liebigs Ann. Chem.*, **588**, 15 (1954); E. Zbiral and E. Werner, *Monatsh Chem.*, **97**, 1797 (1966); J. Knappe and U. Herzog-Weigand, *Justus Liebigs Ann. Chem.*, **701**, 217 (1967); G. A. Russell and L. A. Ochrymowycz, *J. Org. Chem.*, **34**, 3618 (1969).
(17) A. J. Speziale and H. W. Frazier, *J. Org. Chem.*, **26**, 3176 (1961).
(18) In the case of rearrangement of α,β-epoxy ketones it is not known at present whether or not carbonyl group migration is concerted with epoxide ring opening,[2d] and the same point may be made with respect to the BF_3-induced rearrangement of glycidic thiol esters.
(19) H. O. House, J. W. Blaker, and D. A. Madden, *J. Am. Chem. Soc.*, **80**, 6386 (1958).
(20) F. F. Blicke and J. A. Faust, *J. Am. Chem. Soc.*, **76**, 3156 (1954).
(21) S. P. Singh and J. Kagan, *J. Org. Chem.*, **35**, 3839 (1970).
(22) H. O. House and J. W. Blaker, *J. Am. Chem. Soc.*, **80**, 6389 (1958). The salt we isolated had a decomposition point of 268–270° and the following NMR (D_2O, Me_4Si external standard): δ 7.33 (s, 5 H), 3.48 (s, 1 H), 1.63 (s, 3 H). We believe that it is the trans (*E*) isomer.
(23) S. P. Singh and J. Kagan, *J. Org. Chem.*, **35**, 2203 (1970).
(24) F. M. Fiordalisi, U.S. Patent 3,166,475 (1965); *Chem. Abstr.*, **62**, 9141 (1965).
(25) B. J. Nicolaus, L. Mariam, E. Bellasto, and E. J. Lopetit, *Gazz. Chim. Ital.*, **94**, 652 (1964).
(26) M. W. Grittos, J. W. James, and L. F. Wiggins, British Patent 1,088,846 (1967); *Chem. Abstr.*, **68**, 105193x (1968).
(27) H. A. Offe, W. Siefken, and G. Domagk, *Z. Naturforsch. B*, **7**, 446 (1952).
(28) C. Sabate-Alduy and J. Lematre, *Bull. Soc. Chim. Fr.*, 4159 (1969).
(29) D. F. Thompson, P. L. Bayless, and C. R. Hauser, *J. Org. Chem.*, **19**, 1490 (1954).
(30) L. Claisen, *Ber.*, **38**, 693 (1905).

Synthesis of 10-Thiofolic Acid. A Potential Antibacterial and Antitumor Agent[1a]

M. G. Nair,* Patricia T. Campbell, and C. M. Baugh

Department of Biochemistry, University of South Alabama, Mobile, Alabama 36688

Received January 27, 1975

An unambiguous synthesis of 10-thiofolic acid has been carried out in good yield starting from *p*-carbomethoxy thiophenol. Methods have been developed for the quantitative conversion of **6** to the bromo ketone **8**. *p*-Carbomethoxy thiophenol (**3**) and the acid (**2**) were found to be unstable in organic solvents in the presence of oxygen and were converted to the corresponding disulfides (**4** and **5**). A reduction procedure has been developed for the rapid and clean conversion of **14** to **15**, and a procedure for the simultaneous cyclization–oxidation of **15** to **16**. 10-Thiofolic acid (**1**) has been tested for its ability to inhibit the growth of two folate-requiring organisms and showed good antifolate activity. It has also shown moderate activity in preliminary screening against L-1210 leukemia in mice.[23]

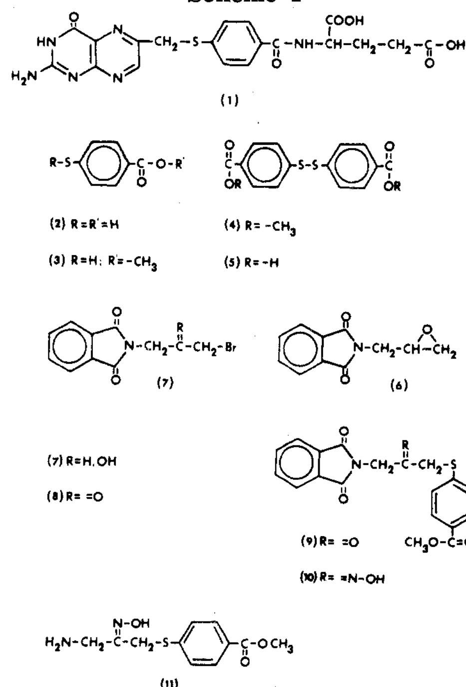
The synthesis and biological evaluation of homofolic acid[1b] and its reduced derivatives (**20**)[2,3] have given impetus to the search for folic acid analogs which are altered in the region corresponding to the C^9–N^{10} bridge in folic acid. A number of analogs in this class have been reported recently.[4–10] It appeared to us that the replacement of the 10-amino group of folic acid by a heteroatom would result in folate analogs whose tetrahydro forms[2,3] could contribute interference to the thymidylate synthesis reaction owing to their inability to form cyclic one-carbon intermediates through positions 5 and 10. This paper details the synthesis and preliminary antifolate activity of such a compound, 10-thiofolic acid (**1**).

At the outset, a convenient procedure for the preparation of *p*-carbomethoxythiophenol (**3**) was required. This was accomplished by a route previously described by Wiley,[11] and the disulfide **4** was also isolated from the reaction mixture. Hydrolysis of **3** gave *p*-carboxythiophenol (**2**), which was converted to the disulfide **5** during crystallization. The quantitative conversion of *N*-(2,3-epoxypropyl)phthalimide to the bromo ketone **8** was carried out by modifications of the original literature procedures.[12–14] Reaction of **3** with **8** in pyridine produced **9** in 75% yield, which was subsequently converted to the oxime **10**. This compound was isolated as a 1:1 mixture of the syn and anti isomers as evidenced by NMR spectroscopy and thin layer chromatography. Since the eventual removal of this carbonyl protective group was required at a later step, no attempt was made to separate and identify the individual isomers.

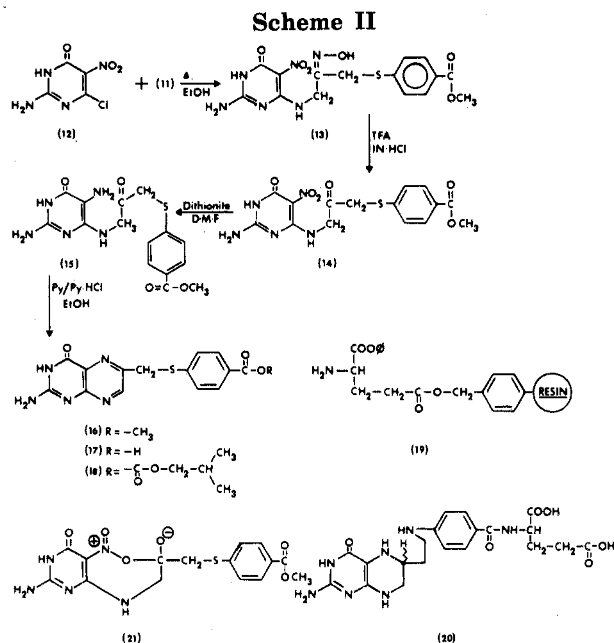
The NMR spectral observations also excluded a possibility that the product might be a thioketal formed by the reaction of 2 mol of thio ester with the carbonyl moiety of **8** in preference to the nucleophilic displacement of the bromide by **3**.

Although several methods are described in the literature for the cleavage of the phthalimide function for compounds similar to **10**, including treatment with strong acids and bases, the use of hydrazine[15] was preferred because of its ability to cleave such systems under mild conditions. This was accomplished smoothly and in high yield. These reactions are summarized in Scheme I. Several attempts were

Scheme I



Scheme II



made at this stage to cleave the oxime back to the ketone, but always resulted in the recovery of the starting material or tarred products unsuitable for further investigations. An attempt was made, however, to hydrolyze the ester function of **11** to the carboxylic acid by standard procedures. None of these experiments gave the desired products, and consequently compound **11** was condensed directly with 2-amino-6-chloro-4-hydroxy-5-nitropyrimidine (**12**) in ethanol to compound **13** in excellent yield (Scheme II). Reductive cyclization of **13** by Raney nickel[16-19] catalysts was ruled out by the presence of sulfur and the compound's insolubility in solvents commonly used for hydrogenations.

The carbonyl function in **13** was deprotected easily by the use of a 1:1 mixture of trifluoroacetic acid and 1 *N* HCl at 60° for 20 min to the corresponding ketone (**14**). Treatment of ketone **14** with several reducing agents, including stannous chloride, and catalytic hydrogenation in DMF with Pt and Pd did not accomplish the desired reduction of the nitro group as evidenced by the persistence of the absorption due to the 5-nitro group of **14.** The resistance of **14** toward reducing agents is not immediately apparent, but a neighboring group participation by the nitro group and the carbonyl group could presumably result in an ionic species (**21**). Therefore, the use of reagents such as sodium dithionite in highly polar solvents like DMF was considered for this reaction. In fact, an excellent reduction procedure was worked out which reduces compound **14** to **15** at 50° in 15 min. This behavior appears to be general to such systems, and we are presently investigating this phenomenon in detail. The reduction product was subsequently cyclized by the use of a pyridine–pyridine hydrochloride buffer in ethanol for several hours, which also resulted in the spontaneous oxidation of the cyclization product to **16** in 90% yield. Hydrolysis of **16** in the usual manner and subsequent workup gave compound **17** in ~50% yield after purification by ion exchange chromatography. The material showed a uv spectrum similar to that of pteroic acid, and the structure was confirmed by examination of the NMR spectrum of **17** in TFA.

The problem of attaching the glutamate moiety to 10-thiopteroic acid remained. This was accomplished in the following way. Briefly, 10-thiopteroic acid was converted to the mixed anhydride **18** by dissolving the compound in 50:50 DMSO–dioxane and treating with 1 molar equiv of isobutyl chloroformate at 0° in the presence of *N*-methylmorpholine as a proton acceptor. *t*-Boc-L-glutamic acid α-benzyl ester was attached to the Merrifield chloromethyl resin by standard procedure[19] and the amino group was deprotected as described earlier[20] to **19.** The coupling of the active anhydride **18** to **19** was done overnight at room temperature. Cleavage from the resin and final purification was accomplished as described previously by this laboratory[5,20] for similar compounds.[21]

10-Thiofolic acid showed all the spectral characteristics expected of this compound and the structure was conclusively established by ultraviolet and NMR spectroscopy. Relevant among the spectral data are the NMR signals in D_2O due to the C_7 proton of the pteridine moiety, which appeared as a singlet at 8.54 ppm, and the characteristic AB pattern of the resonances due to the aromatic protons as two clean doublets at 7.8 and 7.4 ppm. The rest of the anticipated signals due to the C_9 methylene protons and the glutamate protons appeared in the usual pattern. The uv spectrum of 10-thiofolic acid was as expected and was very similar to that of folic acid, showing λ_{max} at 369, 285, and 261 nm when run in 0.1 *N* NaOH. These spectral observations are in perfect agreement with the required structure (**1**).

Both 10-thiofolic acid and 10-thiopteroic acid were tested for their ability to inhibit the growth of two standard folic acid requiring bacteria, *Streptococcus faecium* (ATCC 8043) and *Lactobacillus casei* (ATCC 7469). These studies employed Difco folic acid assay media for the specific organism and were carried out in duplicate. For *S. faecium,* 9×10^{-10} g/ml of 10-thiofolic acid and 1.5×10^{-9} g/ml of 10-thiopteroic acid were required for 50% inhibition of growth as monitored turbidimetrically at 650 nm. For *L. casei,* 8×10^{-9} g/ml of 10-thiofolic acid gave 50% inhibition. No inhibition of *L. casei* was seen with 10-thiopteroic acid at 10^{-5} g/ml. In the *L. casei* inhibition studies, the folic acid concentration was 5×10^{-11} g/ml and in the *S. faecium* it was 2.5×10^{-10} g/ml.

Experimental Section

Melting points are uncorrected and were determined on a Fisher-Johns apparatus. NMR spectra were run in $CDCl_3$ on a 90-MHz Perkin-Elmer R-32 spectrometer with Me_4Si or TSP as internal lock signal. Field strengths of the various proton resonances are expressed in parts per million and coupling constants as hertz. Peak multiplicity is depicted as usual: s, singlet; d, doublet; t, triplet; q, quartet; br, broadened singlet or unresolved multiplet; and c, complex signal whose center is given. Uv spectra were determined in a Beckman Model 25 spectrophotometer. Chromatography was carried out on DEAE cellulose in the chloride form with 1.2 × 22 cm packing unless otherwise specified. A linear NaCl gradient in 0.005 *M* phosphate buffer pH 7, 1 l. each from zero to 0.5 *M* NaCl, was used to elute the column. Mass spectra were run at Research Triangle Institute in North Carolina. Elemental analyses were by Galbraith Laboratories, Inc., Knoxville, Tenn.[22] Yields represent the actual amount of pure compound isolated, assuming 100% reaction.

Preparation of *p*-Carbomethoxythiophenol (3) and Disulfide 4. The synthetic sequence starting with 17.1 g of *p*-aminobenzoic acid to the esterification step (as described by Wiley[11]) was repeated. Crystals were formed while cooling this reaction mixture containing **3.** These were removed by filtration. This compound was identified as disulfide **4** by observing the molecular ion in the high-resolution mass spectrum at 334.0336 (calcd, 334.0333): yield 2.0 g; mp 124°; NMR 8.3 d, 7.84 d (J = 9 Hz, aromatic), and 4.2 ppm (methoxyl). Anal. Calcd for $C_{16}H_{14}O_4S_2$: C, 57.48; H, 4.19; S, 19.16. Found: C, 57.34; H, 4.12; S, 19.36.

Vacuum distillation of the filtrate at 11 mm gave **3** boiling at 134–140°: yield 9.0 g; NMR 7.85 d, 7.35 d (J = 9 Hz, aromatic), 3.85 (methoxyl), and 3.75 ppm (thiol). Anal. Calcd for $C_8H_8O_2S$: C, 57.4; H, 4.76; O, 19.04. Found: C, 57.28; H, 4.59; O, 19.21.

Preparation of *N*-(3-Bromo-2-hydroxypropyl)phthalimide (7). In a typical experiment, 10 g of *N*-(2,3-epoxypropyl)phthalimide was dissolved in 75 ml of dry dichloromethane and gaseous hy-

drogen bromide was bubbled through until saturated, requiring approximately 20 min. The reaction mixture was evaporated to dryness under vacuum, and the crude product melted at 110–112°: NMR 7.82, c (aromatic protons), 4.2, c (C_2H), 3.95 and 3.55, dd (J = 8 Hz, C_3 and C_1 methylene protons), and 3.02 ppm s (hydroxyl, completely exchangeable with D_2O).

Preparation of *N*-(3-Bromo-2-oxopropyl)phthalimide (8). In an erlenmeyer flask, 10 g of the hydroxy bromide 7 was stirred with 400 ml of acetone at room temperature. When all of the material was in solution, the solution was cooled to 15° and 100 ml of Jones reagent was added portionwise with stirring in such a manner that the temperature did not rise above 30°. After the addition, which took 10 min, the mixture was stirred at room temperature for 15 min, transferred to a round-bottomed flask, and evaporated to ~150 ml under vacuum. The outside bath temperature was kept at 35°. Treatment of this reaction mixture with 1 l. of ice-cold water gave crystals which were removed by filtration and washed several times with distilled water until the filtrate was colorless: yield 9.5 g; mp 151–152°; NMR 7.85, c (aromatic protons), 4.80, s and 4.06 ppm s (C_3 and C_1 methylene protons). Anal. Calcd for $C_{11}H_8BrNO_3$: C, 46.81; H, 2.84; Br, 28.37; O, 17.02. Found: C, 46.92; H, 2.97; Br, 28.21; O, 16.92.

Displacement of Bromine in 8 by *p*-Carbomethoxythiophenol. Preparation of 9. In an oven-dried three-necked flask, fitted with a reflux condenser and nitrogen inlet, were placed 2 mmol each of bromo ketone 8 and thio ester 3. The reactants were slowly heated in a stream of nitrogen to 90° with the aid of an oil bath, and pyridine was added slowly from a dropping funnel until the mixture went into solution (~3 ml). The reaction mixture was then heated to reflux in nitrogen for 45 min, when crystals began to appear. The reaction product was cooled and poured over 100 g of crushed ice, then stirred. The cream-colored solid thus obtained was collected by filtration and recrystallized from methanol: yield 75%; mp 153–154°; NMR 8.05, d (J = 8 Hz, two aromatic protons adjacent to carbomethoxy), 7.38, d (J = 8 Hz, two aromatic protons adjacent to sulfur), 7.85, c (four aromatic protons of the phthalimide moiety), 4.78, s and 3.92, s (methylene protons), and 3.95 ppm (carbomethoxy). Anal. Calcd for $C_{19}H_{15}NO_5S$: C, 61.79; H, 4.07; O, 21.68; S, 8.67. Found: C, 61.76; H, 4.13; O, 21.54; S, 8.50.

Preparation of the Oxime 10. Compound 9 (4.3 g) and 1.21 g of hydroxylamine hydrochloride were suspended in a three-necked, round-bottom flask and the flask was swept with a slow stream of nitrogen. To this was added 48 ml of 1:1 pyridine–ethanol mixture and the mixture was refluxed for 2 hr. The reaction product was evaporated to dryness under vacuum and the resulting pale yellow, gummy material was treated with 25 ml of ethyl acetate and 24 ml of distilled water. After shaking vigorously, the contents were poured into a separatory funnel and the aqueous layer was discarded. The ethyl acetate layer was washed twice with 100 ml of distilled water. The ethyl acetate layer was then dried over anhydrous sodium sulfate and filtered, and the filtrate was evaporated to dryness. The viscous gum thus obtained was recrystallized from methanol, yield 4.0 g, mp 135–136°. Anal. Calcd for $C_{19}H_{16}N_2O_5S$: C, 59.38; H, 4.17; N, 7.29; S, 8.33. Found: C, 59.71; H, 4.27; N, 7.35; S, 8.32.

Cleavage of 10 by Hydrazine to the Amino Ester 11. The oxime (3.8 g, 9.9 mmol) was suspended in a three-necked round-bottom flask with 100 ml of absolute alcohol and stirred under reflux in a slow stream of nitrogen with repeated addition of four 100-ml portions of alcohol when all the material went into solution. The solution was cooled to room temperature and 9.9 mmol of 95% hydrazine in 10 ml of absolute alcohol was added. This mixture was stirred for 72 hr in nitrogen at room temperature, then refluxed for 30 min. After the reaction mixture was cooled to 0°, it was filtered. The filtrate was evaporated to dryness and the residue was treated with 9.9 ml of 1 N HCl in 20 ml of water and stirred vigorously for 1 hr. The pH was adjusted to 3 by the addition of more HCl and the mixture was filtered. The clear filtrate thus obtained was treated with 10% ammonium hydroxide so that the pH became 10. A white precipitate was formed in the solution. After cooling to 0°, the precipitate was separated by filtration, washed with water, and recrystallized from methanol: yield 1.6 g; mp 154–156°; relevant NMR signals in polysol-D at 7.86, d (J = 9 Hz, aromatic), 7.45, dd (J = 9 Hz, aromatic), 4.0, s and 3.92, s (methylene protons attached to sulfur), 3.85, s (carbomethoxy), 3.51, s and 3.39 ppm, s (methylene protons attached to amino group). Anal. Calcd for $C_{11}H_{14}N_2O_3S$: C, 51.97; H, 5.51; N, 11.02; S, 12.60. Found: C, 51.93; H, 5.39; N, 10.95; S, 12.51.

Reaction of 2-Amino-6-chloro-4-hydroxy-5-nitropyrimidine with Amino Ester 11. Preparation of Intermediate 13. In an atmosphere of nitrogen, 560 mg of 12 was dissolved in 140 ml of absolute alcohol and refluxed with 700 mg of 11 for 30 min. After this period, 0.7 ml of *N*-methylmorpholine was added and the mixture was again refluxed for 30 min. The crystals which appeared at this point were collected by filtration, washed with water and a small amount of alcohol, and recrystallized from absolute alcohol: mp 185°; yield 1.05 g; $\lambda_{292}/\lambda_{347}$ 1.08. Anal. Calcd for $C_{15}H_{16}N_6O_6S$: C, 44.12; H, 3.92; N, 20.59; S, 7.84. Found: C, 43.9; H, 3.86; N, 20.46; S, 7.74.

Deprotection of the Carbonyl Group of 13. Preparation of 14. In an erlenmeyer flask, 1 g of oxime 13 was treated with 50 ml of trifluoroacetic acid with stirring and slowly heated to 50° until all the material was dissolved. An equal volume of 1 N HCl was added and the flask was kept in a water bath at 60°. Crystals began to appear slowly. After 20 min, the reaction mixture was cooled to 0°, filtered, and washed several times with water and finally with absolute alcohol: mp 247–250°; yield 900 mg; λ_{max} (0.1 N NaOH) 294 nm (ϵ 16,011) and 332 (14,673). Anal. Calcd for $C_{15}H_{15}N_5O_6S$: C, 45.80; H, 3.92; N, 17.81; O, 24.43. Found: C, 45.71; H, 3.77; N, 17.91; O, 24.37.

Dithionite Reduction of 14. The deprotected nitro compound 14 was suspended in an erlenmeyer flask (350 mg) and dissolved in 100 ml of purified DMF. The solution was heated to 50° and 5 g of solid purified sodium dithionite was added. Water was added to this stirring suspension, portionwise, while maintaining the temperature until all the dithionite went into solution. The mixture was allowed to stir for an additional 15 min, then diluted to 700 ml with ice-cold water. A white, fluffy precipitate was formed, which was collected by filtration, washed several times with water and finally with absolute alcohol, and then dried under vacuum. The compound is unstable on exposure to air and rapidly degraded to products unsuitable for further work: yield 300 mg; mp >300°; λ_{max} (0.1 N NaOH) 335 and 280 nm; $\lambda_{280}/\lambda_{335}$ 1.65.

Simultaneous Cyclization–Oxidation of 15 to 16 and Hydrolysis to 10-Thiopteroic Acid (17). The dithionite reduction product (15, 300 mg) was added to a deaerated mixture of 25 ml of pyridine, 25 ml of absolute alcohol, and 5 drops of concentrated HCl and refluxed in a nitrogen atmosphere for 2.5 hr. The solution was cooled to room temperature and allowed to stir in the presence of air for 48 hr. The contents in the flask were evaporated under vacuum to dryness. Distilled water (25 ml) was added, and the contents were evaporated again. Ice (50 g) was then added and the mixture was triturated with a spatula followed by filtration and several water washes. The uv spectrum of this compound in 0.1 N NaOH showed λ_{max} at 365, 280, and 260 nm, which is typical of a 6-substituted, fully oxidized form of 2-amino-4-hydroxypteridine.

The product thus obtained was transferred to a three-necked flask fitted with a nitrogen inlet and reflux condenser and 25 ml of 0.75 N NaOH was added. It was refluxed in nitrogen for 2 hr. The solution was cooled to room temperature and adjusted to pH 7.2 with 1 N HCl. The solution was then diluted to 1 l. with distilled water and applied to a 27 × 2.5 cm DEAE Cl^- column. The column was washed with distilled water and eluted with a linear NaCl gradient from zero to 0.5 M, in 0.005 M phosphate buffer, pH 7.0. 10-Thiopteroic acid eluted at 0.375 M NaCl concentration. All the tubes corresponding to this peak were pooled and concentrated to 100 ml, cooled to 0°, and acidified to pH 3.5 with 1 N HCl. The precipitate thus obtained was washed several times with water and dried under vacuum over P_2O_5 for 48 hr: yield 140 mg; λ_{max} (0.1 N NaOH) 369 nm (ϵ 9638), 285 (21,068), and 261 (32,850); NMR (TFA with Me_4Si as internal standard) 9.05, s (C_7), 8.1, d (J = 9 Hz, $H_{2'6'}$); 7.55, d (J = 9 Hz, $H_{3'5'}$), and 4.62 ppm, s (C_9 methylene protons). Anal. Calcd for $C_{14}H_{11}N_5O_3S$: C, 51.06; H, 3.34; N, 21.28; O, 14.59; S, 9.73. Found: C, 50.97; H, 3.40; N, 21.27; O, 14.63; S, 9.58.

Solid-Phase Coupling of 10-Thiopteroic Acid with L-Glutamic Acid. Preparation of 10-Thiofolic Acid (1). *t*-Boc-L-glutamic acid α-benzyl ester was esterified to the chloromethylated Merrifield resin as usual. The resin ester, corresponding to 2 mmol of glutamic acid, was deprotected by the use of 20% trifluoroacetic acid in methylene chloride for 20 min at room temperature, washed, neutralized, and kept ready for coupling (19).

10-Thiopteroic acid (1 mmol) was dissolved in 20 ml of dry DMSO by heating. The solution was cooled to room temperature, an equal volume of dry THF was added, and the solution was chilled to 0°. Then 1.25 mmol of *N*-methylmorpholine was added, allowed to mix well by shaking, and kept at 0° for an additional 15 min. To this solution was added exactly 1 equiv (1 mmol) of freshly distilled isobutyl chloroformate and the reaction was allowed to proceed for 15 min at 0° to form the mixed anhydride (18). This

mixed anhydride (18) was then poured into the reaction vessel containing 19, and the coupling reaction was carried out by rocking the reaction vessel at room temperature for 18 hr. After this period, the reaction mixture was filtered and the resin-bound product was washed successively with DMSO, DMSO–THF mixture, and *p*-dioxane.

The resin was then suspended in 20 ml of 1:1 dioxane–2 *N* NaOH mixture, which was deaerated previously, and mixed well in a closed vessel for 1 hr at room temperature and for 20 min at 50°. The filtered solution was diluted to 100 ml and slowly acidified with 1 *N* HCl at 0° to pH 3.5. The yellow precipitate of 1 thus formed was collected by filtration, washed several times with distilled water, and dried under vacuum over P_2O_5: yield 80%; mp >300°; λ_{max} (0.1 *N* NaOH) 369 mm (ϵ 9638), 285 s (21,068), and 261 (32,850); NMR (D_2O–NaOD with SDSS as internal standard) 8.54, s (C_7 H), 7.8, d (J = 9 Hz, $H_{2'6'}$), 7.40, d (J = 9 Hz, $H_{3'5'}$), 4.42, t (α proton of glutamate moiety), and 1.0–2.5 ppm, c (four protons of glutamic acid). Anal. Calcd for $C_{19}H_{18}N_6O_6S$: C, 49.78; H, 3.93; N, 18.34; O, 20.96; S, 6.99. Found: C, 49.69; H, 3.96; N, 18.46; O, 21.08; S, 6.99.

10-Thioaminopterin, the 4-amino-4-deoxy analog of 1, has also been synthesized in this laboratory by a similar procedure and will be discussed in a later communication in this series.

Acknowledgments. This work was supported by Grant CI-86N of the American Cancer Society and by NIH Grant 1-R01-CA-16048. We are grateful to Miss Eleanor Braverman for her interest and assistance in this work and to Mr. D. J. Underwood for his technical assistance.

Registry No.—1, 54931-98-5; 3, 6302-65-4; 4, 35190-68-2; 6, 5455-98-1; 7, 6284-27-1; 8, 6284-26-0; 9, 54931-99-6; 10, 54932-00-2; 11, 54932-01-3; 12, 1007-99-4; 13, 54932-02-4; 14, 54932-03-5; 15, 54932-04-6; 16, 54932-05-7; 17, 54932-06-8; L-glutamic acid, 56-86-0.

References and Notes

(1) (a) Trivial names in general usage will be used for these compounds: 10-thiofolic acid = *N*-[*p*-[[(2-amino-4-hydroxy-6-pteridinyl)methyl]thio]benzoyl]glutamic acid; 10-thiopteroic acid = 2-amino-4-hydroxy-6-(*p*-carboxythiophenoxymethyl)pteridine; homofolic acid = *N*-[*p*-[[(2-amino-4-hydroxy-6-pteridinyl)ethyl]amino]benzoyl]glutamic acid. Other abbreviations include: DHFR, dihydrofolate reductase; DEAE, diethylaminoethyl; *t*-Boc, *tert*-butyloxycarbonyl; SDSS, sodium 2,2-dimethyl-2-silapentane-5-sulfonate. (b) L. Goodman, J. DeGraw, R. L. Kisliuk, M. Friedkin, E. J. Pastore, E. J. Crawford, L. T. Plante, A. A. Nahas, J. F. Morningstar, G. Kwok, L. Wilson, E. F. Donovan, and J. Ratzan, *J. Am. Chem. Soc.*, **86**, 308 (1964).
(2) T. C. Crusberg, R. Leary, and R. L. Kisliuk, *J. Biol. Chem.*, **245**, 5292 (1970).
(3) R. L. Kisliuk and Y. Gaumont, *Proc. Int. Symp. Pteridines, Chem. Biol., 4th, 1969*, 357 (1970).
(4) M. G. Nair and C. M. Baugh, *J. Org. Chem.*, **38**, 2185 (1973).
(5) M. G. Nair and C. M. Baugh, *J. Med. Chem.*, **17**, 223 (1974).
(6) J. I. DeGraw, R. L. Kisliuk, Y. Gaumont, and C. M. Baugh, *J. Med. Chem.*, **17**, 470 (1974).
(7) M. G. Nair, L. P. Mercer, and C. M. Baugh, *J. Med. Chem.*, **17**, 1268 (1974).
(8) J. I. DeGraw, R. L. Kisliuk, Y. Gaumont, C. M. Baugh, and M. G. Nair, *J. Med. Chem.*, **17**, 552 (1974).
(9) R. F. Struck, Y. F. Shealy, and J. A. Montgomery, *J. Med. Chem.*, **14**, 693 (1971).
(10) J. I. DeGraw, P. Tsakotellis, R. L. Kisliuk, and Y. Gaumont, *J. Heterocycl. Chem.*, **8**, 105 (1971).
(11) P. F. Wiley, *J. Org. Chem.*, **16**, 810 (1951).
(12) M. Weizmann and S. Malkova, *Bull. Soc. Chim. Fr.*, **47**, 356 (1930).
(13) D. P. Tschudy and A. Collins, *J. Org. Chem.*, **24**, 556 (1959).
(14) R. D. Elliot, C. Temple, Jr., and J. A. Montgomery, *J. Org. Chem.*, **35**, 1676 (1970).
(15) R. A. Boissonnas, *Adv. Org. Chem.*, **13**, 182 (1963).
(16) R. D. Elliot, C. Temple, Jr., and J. A. Montgomery, *J. Med. Chem.*, **17**, 553 (1974).
(17) J. A. Montgomery, R. D. Elliot, S. L. Straight, and C. Temple, Jr., *Ann. N.Y. Acad. Sci.*, **186**, 227 (1971).
(18) R. Mozingo, D. E. Wolf, S. A. Harris, and F. Folkers, *J. Am. Chem. Soc.*, **65**, 1013 (1943).
(19) G. M. Badger, H. J. Rodda, and W. H. F. Sasse, *J. Chem. Soc.*, 4162 (1954).
(20) C. L. Krumdieck and C. M. Baugh, *Biochemistry*, **8**, 1568 (1969).
(21) M. G. Nair and C. M. Baugh, *Biochemistry*, **12**, 3923 (1973).
(22) Where analyses are indicated only by symbols of elements, analytical results obtained for these elements or functions were within ±0.4% of the theoretical values.
(23) Increase in mean survival time of 27% at 8 mg/kg. We are thankful to Dr. H. B. Wood of NCI for the antitumor screening data.

Prostaglandins. VII. A Stereoselective Total Synthesis of Prostaglandin E_1[1]

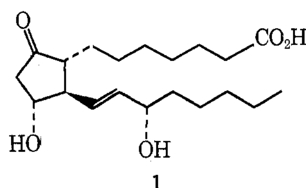
Masateru Miyano* and Michael A. Stealey

Searle Laboratories, Division of G. D. Searle & Co., Chicago, Illinois 60680

Received January 24, 1975

The stereoselective total synthesis of (±)- and (−)-prostaglandin E_1 (1) is described. Chromous sulfate reduction of 7-(2-formyl-3-tetrahydropyranyloxy-5-oxocyclopent-1-enyl)heptanoic acid (3b) afforded the saturated aldehyde 10b, which was condensed with *n*-hexanoylmethylenetriphenylphosphorane (7) to form 11-*O*-tetrahydropyranyl-15-dehydroprostaglandin E_1 (11). Reduction of 11 with thexyl tetrahydrolimonyllithium borohydride followed by hydrolysis gave 1. The mechanism of stereochemical control is discussed in detail. The total synthesis was extended to the preparation of (±)-ω-homoprostaglandin E_1 (32c) and (±)-15-methyl-ω-homoprostaglandin E_1 (32d).

The prostaglandins,[2a,b] a family of oxygenated C_{20} fatty acids of widespread occurrence in animal tissues, exhibit a broad range of biological activities[2c] and presumably play an important role in several physiological processes. Prostaglandin E_1 (PGE_1, 1), one of the most active and ubiquitous hormones of this species, has been synthesized[3-10] chemically by several different groups. Some[4,7,8b,9b] of these total syntheses of PGE_1 were stereochemically controlled.



1

The primary objective of our study was to develop an efficient general route to new prostaglandin analogs which might possess more selective biological activities. This target was partly achieved with a facile seven-step total synthesis[8a,11] of racemic PGE_1 (1) and $PGF_{1\alpha}$ (8) along with their stereoisomers as outlined in Scheme I. This scheme was not stereoselective in some steps. Thus, almost equal amounts of 5 and 6 were obtained after the Wittig condensation. Also, comparable amounts of (±)-PGE_1 (1) and its 15 epimer (28) were formed in the reduction step. Some of the stereoisomers[11] exhibited interesting biological activities.[12]

Later, large quantities of PGE_1 were required for biological study. Owing to the inevitable formation of the stereo-