An Improved Synthesis of Homopteroic and Homofolic Acids (1-3)

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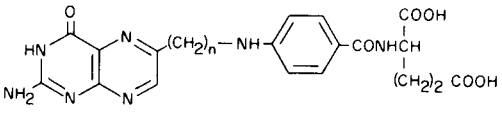
A more practical synthesis of homopteroic and homofolic acids involving condensation of 2,4,5-triamino-6(1*H*)pyrimidinone (**3**) with 1-acetoxy-4-[*N*-acetyl-(*p*-carbethoxyphenyl)amino]-2-butanone (**7**) is described. The biological activities of homofolic (**1-b**) and homopteroic (**2-b**) acids were compared and found to be identical with the activities of these products prepared by the unambiguous route.

Homofolic and homopteroic acids (**1-b** and **2-b**) are analogs of folic and pteroic acids (**1-a** and **2-a**) containing an additional methylene group between the pteridine and *p*-aminobenzoic acid moieties. These compounds are currently of great interest in the chemical treatment of cancer (4) and malaria (5). The former is a potent inhibitor of bacteria and tumors, and the latter has been found to be effective against *Plasmodium cynomolgi* and a pyrimethamine-resistant variant of this organism. These



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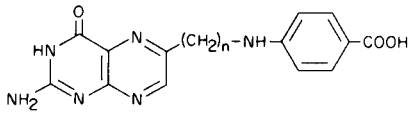

2 a: n = 1
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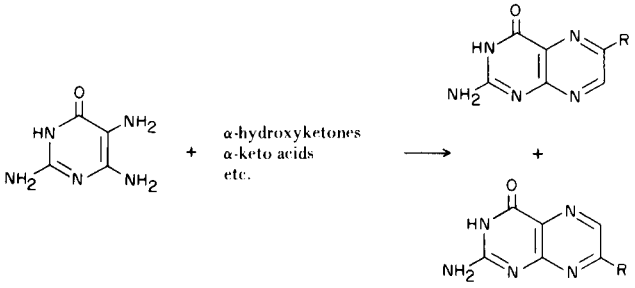
two compounds have been synthesized by L. Goodman and his associates (4-a,6) by an elegant method involving multiple steps. Overall yields of this synthesis were naturally very small and several of the intermediate steps were not suitable for larger scale preparation.

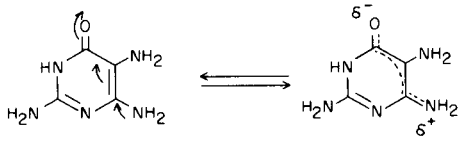
A new synthetic method is reported here simplifying the route to homofolic acid. This new method is based on the condensation of 2,4,5-triamino-6(1*H*)pyrimidinone (**3**) with 1-acetoxy-4-[*N*-acetyl-(*p*-carbethoxyphenyl)amino]-2-butanone (**7**).

Condensations of the triaminopyrimidine (**3**) with hydroxyacetone (7,8,9), α-keto acids (10) or their esters (11) and similar compounds (12) to form pteridines have already been reported. However, a mixture of 6- and 7-substituted isomers were invariably formed, often with a



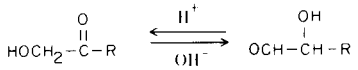
predominance of the unwanted 7-substituted isomer. Our initial attempts to condense 1-bromo-4-[*N*-acetyl-(*p*-carbethoxyphenyl)amino]-2-butanone (**6**) with triaminopyrimidine (**3**) by an adaptation of the procedure of Baugh and Shaw (9) led to a low yield of an intractable mixture of the 6- and 7-substituted isomers (based on spectroscopic data).

In principle, condensations of the pyrimidine (**3**) with suitable intermediates are *p*H-dependent, due to the unequivalent location of the amino groups on the pyrimidine ring. In reactions with α-keto acids or their esters,



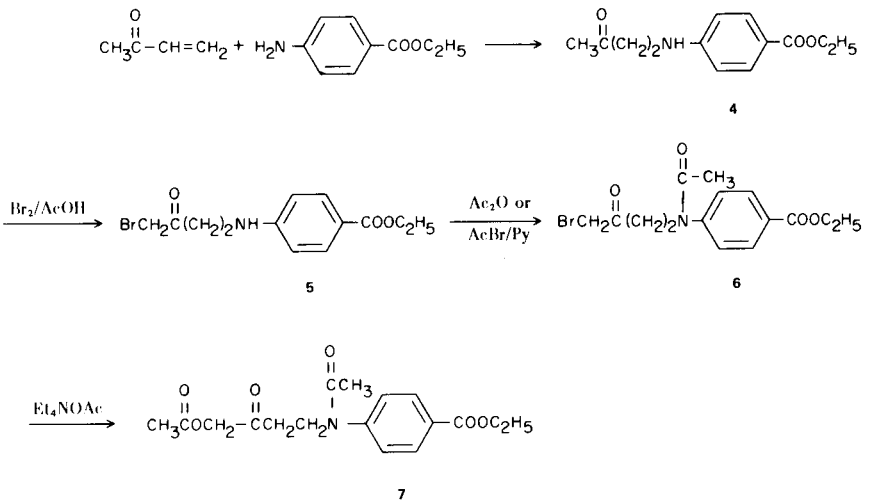
6-substituted 7(8*H*)pteridinones predominate in neutral or weakly acidic medium (10a-i) due to the favored con-

densation of the more basic 5-amino group with the more reactive keto group, while 7-substituted 6(5*H*)pteridinones predominate in strongly acidic media (10j-l) due to the protonation of the more basic 5-amino group. The *p*H also influences the equilibrium of a Lobry De Bruyn-Van Ekenstein transformation (13) on α-hydroxyketone during the condensation reaction to produce 6- and 7-isomers.



In order to obtain the desired 6-isomer with exclusion of the 7-substituted isomer, we undertook a study of the condensing ketone, as well as the influence of *p*H on the reaction mixture. As a result of these studies, we found that the reaction occurs in the desired direction when the triaminopyrimidine (**3**) is condensed with 1-acetoxy-4-[*N*-acetyl-(*p*-carbethoxyphenyl)amino]-2-butanone (**7**). Intermediate **4** was prepared from methyl vinyl ketone and ethyl *p*-aminobenzoate in excellent yield (84%), m.p. 100-101°. Bromination of **4** to form **5** (m.p. 80-83°) was carried out by a modification of the method of DeGraw (6) followed by *N*-acetylation either with acetic anhydride (75% yield) or acetyl bromide in the presence of pyridine to form **6**. The latter reagents gave the cleaner product in higher yield (84 ~ 90%). The *N*-acetyl α-bromoketone (**6**) was converted to **7** with tetraethylammonium acetate in almost quantitative yield (95 ~ 98%).

After conversion of commercially available triaminopyrimidine sulfate to its hydrochloride (**3**), cysteine hydrochloride was added to prevent autooxidation before diluting with sodium acetate buffer. The mixture thus obtained was treated with a solution of the α-acetoxyketone (**7**) in dioxane and adjusted to *p*H 9-9.4 with triethylamine. The mixture was stirred under nitrogen for 12-16 hours at 60°. A number of the reactions were run in open air or bubbling air into the mixture, but these methods gave a lower yield with low purities. Concentration of the mixture by evaporation under reduced pressure, neutralization to *p*H 7 with hydrochloric acid followed by refrigeration gave the completely aromatized homopteroate (**9**) as a yellow powder. An absence of uv λ max at 330 mμ indicated that the sensitive dihydro product (**8**) (14) is oxidized during the isolation period. However, oxidation of the crude mixture with hydrogen peroxide increased the yield of **2-b** remarkably because completely aromatized pteridines are far less soluble than dihydropteridines. Thus, oxidation of the crude product with hydrogen peroxide together with subsequent saponification gave 40 ~ 50% of homopteroic acid. A sequential reaction mechanism was tentatively proposed as Figure 1. All available physical data including ir, uv spectra and Rf value of paper chromatogram agreed well with those of an authentic sample which was prepared by the method of DeGraw (6). In addition to chemical degradation to 2-amino-4(3*H*)oxopteridine-6-carboxylic acid (12e,15), the homopteroic acid (**2-b**) prepared by our new method was converted into homofolic acid by the procedure of DeGraw *et al.* (6). Reaction of **2-b** with trifluoroacetic anhydride followed by treating with hot acetic anhydride yielded a tan-colored crystalline product (**10**). Treatment of **10** with isobutyl chloroformate and condensation with diethyl L-glutamate produced a good yield (70-80%) of the completely blocked homofolic acid (11). Saponification of **11** with 0.2 *N* sodium hydroxide followed by acidification yielded homofolic acid as a gelatinous precipitate which was collected by centrifugation (95%).



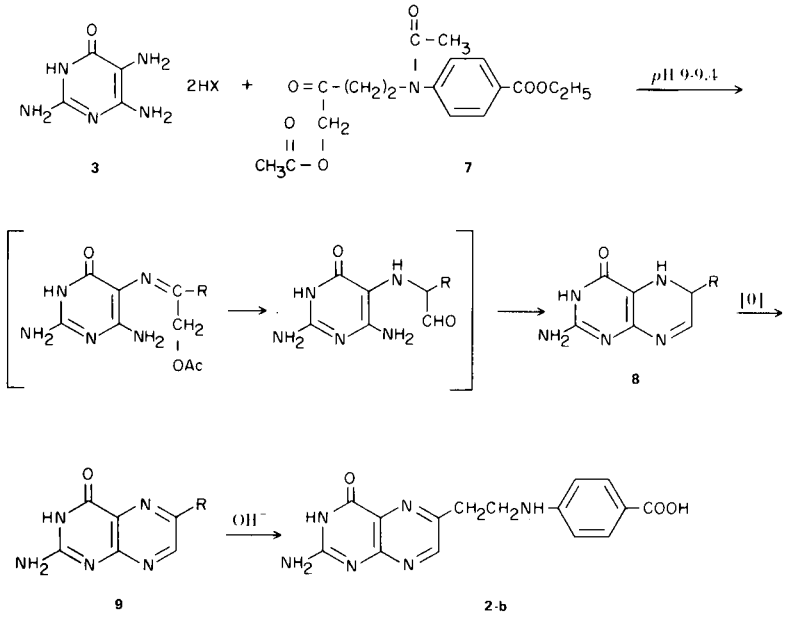
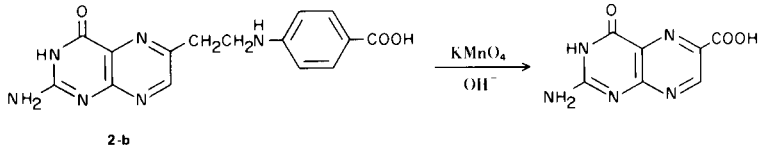
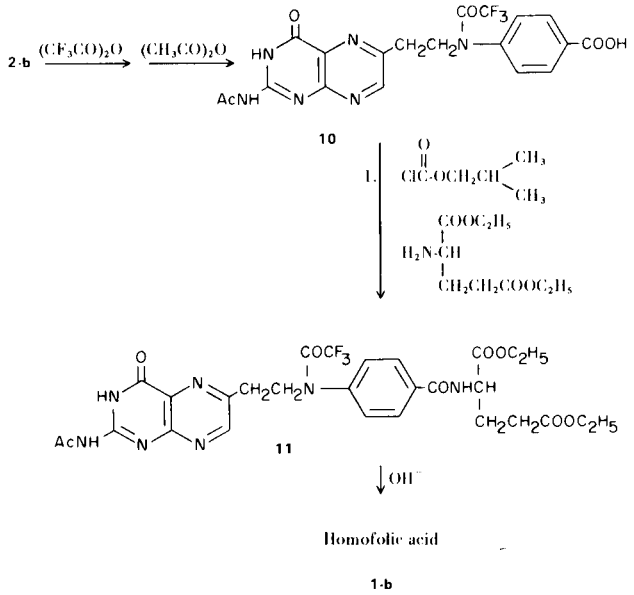


Figure 1





Homofolic acid

1-b

Biological Results (16).

Homofolic acid thus obtained, when tested for biological activities, gave the expected results: when reduced by dithionite to the dihydro form, it inhibited *Escherichia coli* thymidylate synthetase, and when reduced catalytically to tetrahydro form, it inhibited the growth of *Streptococcus faecum.*

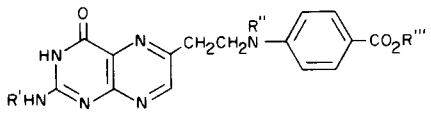
These results were directly comparable with the data published on the authentic product (4a). The results of tumor-inhibitory testing in the CCNSC screening program were indistinguishable from those obtained with the product produced by the original synthesis.

EXPERIMENTAL (17)

4-(*p*-Carbethoxyphenyl)amino-2-butanone (4).

To a vigorously stirred solution of ethyl *p*-aminobenzoate (41.2 g., 0.25 mole) in absolute ethanol (80 ml.), methyl vinylketone

Uv Spectra and R_f of



			Prepared by Our New Method				Prepared by Lit. Method (6)			Reported (Ref. 6)		
R′	R″	R‴	$\lambda_{max}^{pH\ 13}$	ϵ	λ_{max} / λ_{min}	R_f	ϵ	λ_{max} / λ_{min}	R_f	ϵ	λ_{max} / λ_{min}	R_f
					λ min = 325 mμ							
H	H	H	256	25,100	6.42		29,200	7.01		26,900		
			277	21,600	5.35	0.54	23,150	5.73	0.50	21,900		
			365	7,570	1.70		7,590	1.88		7,630		
H	CH_3CO-	-C_2H_5	253	27,800	8.45		26,900	8.13		24,750		
			366	7,790	2.23	0.83	6,250	2.16	0.85	7,180		
H	CF_3CO-	H	256	26,800	4.96		27,300	4.78		25,650		
			277	21,300	4.15	0.81	22,600	4.01	0.84	20,900	at $\lambda_{max}^{pH\ 11}$	
			365	7,880	1.48		7,850	1.47		7,560		
CH_3CO-	CF_3CO-		255		4.07			4.10				
			277		3.46	0.94		3.47	0.94			
			365		1.15			1.33				
H	H	-NHCH(COOH)$(CH_2)_2$COOH	255	24,400	4.13		25,000	4.01		24,600		
			281	20,100	3.69	0.81	20,700	3.41	0.81	19,450		
			365	7,430	1.29		7,380	1.24		7,880		

Biological Results of Homofolic Acid

Sample	Inhibition of *S. faecum* (τ/ml. for 50% inhibition)	Inhibition of *E. coli* Thymidylate Synthetase (*M* for 50% inhibition)	λ 295 mμ for Tetrahydrohomofolic Acid in 0.2 *M* HS$(CH_2)_2$OH (*p*H 7.4)
Lot 202-234	0.2	2.2×10^{-6}	X
205-242	0.27	2.3×10^{-6}	X
Reported (4a) (Authentic sample)	0.25	2.0×10^{-6}	X

(18) (17.5 g., 0.25 mole) was added. The reaction mixture was heated under reflux for 4.5 hours and the resultant mixture was cooled at 0°. The precipitated crystalline product was collected by filtration, washed with precooled alcohol, and dried *in vacuo* to give an analytically pure white crystalline product (49 g., 84%), m.p. 100-101°, lit. (6) m.p. 97-99°; a mixture melting point with that of the authentic sample (6) did not depress.

Anal. Calcd. for $C_{13}H_{17}NO_3$: C, 66.4; H, 7.3; N, 6.0. Found: C, 66.2; H, 7.3; N, 6.2.

1-Bromo-4-(*p*-carbethoxyphenyl)amino-2-butanone (**5**).

Conversion of **4** (201 g., 0.85 mole) to **5** was carried out by the procedure of DeGraw (6). The syrupy crude product in methylene chloride (1.8-1) was washed with water (3 x 300 ml.) and the organic solution was dried (sodium sulfate). Removal of the solvent under reduced pressure at room temperature gave a beige-colored semisolid. The crude product was thoroughly triturated with precooled ether and the solid product, for the first time, was collected by filtration to give a light colored product **5** (11.8 g., 44% (19)), m.p. 80-83°.

Anal. Calcd. for $C_{13}H_{16}BrNO_3$: C, 49.9; H, 5.1; Br, 25.5; N, 4.5. Found: C, 49.6; H, 5.2; Br, 25.6; N, 4.5.

The α-bromoketone (**5**) is slowly decomposed in air (or *in vacuo*) at room temperature. Recrystallization of **5** is difficult and it must be stored *in vacuo* under refrigeration. It is also recommended that the next step of reaction be carried out as soon as possible.

1-Bromo-4-[*N*-acetyl-(*p*-carbethoxyphenyl)amino]-2-butanone (**6**).

Method A.

A suspension of the α-bromoketone (**5**) (118.5 g., 0.38 mole) in acetic anhydride (590 ml.) was stirred under anhydrous condition at room temperature to give a clear solution within 30 minutes. The stirring was continued for an additional 2 hours and the solution was allowed to stand at room temperature overnight. After removal of the excess acetic anhydride under reduced pressure at 40-45°, the brownish oily residue was dissolved in methylene chloride (1350 ml.). The solution was washed with cold water (3 x 600 ml.), cold 2% hydrochloric acid (600 ml.), cold 5% sodium bicarbonate solution, and finally cold water (3 x 500 ml.).

The organic layer was treated with a mixture of Florisil-charcoal-sodium sulfate (590 g.:24 g.:20 g.) by stirring for 0.5 hour at room temperature. The mixture was filtered and the filter was washed thoroughly with methylene chloride. Combined filtrates were evaporated under reduced pressure at 25-30° to give a light colored oily product (**6**: 101.3 g., 75%), n_D^{25} 1.5485.

Anal. Calcd. for $C_{15}H_{18}BrNO_4$: C, 50.6; H, 5.1; Br, 22.4; N, 3.9. Found: C, 50.5; H, 5.1; Br, 22.1; N, 3.8.

Method B.

To a solution of pyridine (8 g., 0.1 mole) in methylene chloride (250 ml.) acetyl bromide (13.3 g., 0.104 mole) was added. An instant formation of pyridinium salt was stirred while **5** (31 g., 0.1 mole) in methylene chloride (250 ml.) was added in 5 minutes. The heterogeneous mixture was heated under reflux for 3 hours. The excess acetyl bromide and the solvent were removed by distillation under reduced pressure. The oily residue was suspended in water (500 ml.) and extracted with methylene chloride (3 x 300 ml.). The organic solution was treated with a mixture of sodium sulfate-charcoal (30 g.:5 g.) and was poured onto a column (d = 2.5 cm) which was packed with Florisil (150 g.). The column was eluted with methylene chloride and upon removal of the solvent gave a light colored oily product (6) (20) (30 g., 84%), n_D^{24} 1.5497.

Anal. Calcd. for $C_{15}H_{18}BrNO_4$: C, 50.6; H, 5.1; Br, 22.4; N, 3.9. Found: C, 50.5; H, 5.1; Br, 22.1; N, 3.8.

1-Acetoxy-4-[*N*-acetyl-(*p*-carbethoxyphenyl)amino]-2-butanone.

A solution of **6** (9 g., 0.025 mole) in absolute ethanol (50 ml.) was added to an excess amount of tetraethylammonium acetate (21) (10 g., 0.052 mole) in ethanol (30 ml.). After the reaction mixture was stirred at room temperature for 0.5 hour, the solvent was removed by distillation under reduced pressure at 30-35°. The residue was suspended in water (50 ml.) and the water insoluble material was extracted with methylene chloride (3 x 30 ml.), and dried (sodium sulfate). The solution was treated with charcoal (2 g.) and passed through a column packed with Florisil (50 g.), and after removal of the solvent, gave a light colored oily product (**7**) (8 g., 95%), n_D^{24} 1.5234.

Anal. Calcd. for $C_{17}H_{21}NO_6$: C, 60.8; N, 4.2. Found: C, 60.8; H, 6.4; N, 4.1.

Ethyl *N*-Acetylhomopteroate (**9**).

A suspension of 2,4,5-triamino-6(1*H*)pyrimidinone sulfate (42.9 g., 0.18 mole) in water (1 l.) containing barium chloride (43.8 g., 0.182 mole) was stirred at 100° for 0.5 hour. After the mixture was cooled to room temperature the precipitated barium sulfate was removed by filtration and thoroughly washed with water (500 ml.). To the triaminopyrimidine hydrochloride thus obtained cysteine hydrochloride (31.5 g., 0.19 mole) was added and diluted with 4 *M* sodium acetate buffer (1.5 l.). To the above mixture was added a solution of **7** (66 g., 0.185 mole) in *p*-dioxane (2 l.) and the solution was adjusted to *p*H 9.0 with triethylamine. The resultant mixture was heated at 60°, with stirring, under nitrogen atmosphere for 14 hours. The solution was concentrated to about 3 l. by evaporation *in vacuo* and was neutralized to *p*H 7 with 2 *N* hydrochloric acid. Refrigeration of the mixture for 72 hours gave a tan-colored solid which was collected by filtration and washed with water and ether to give a crude ethyl 5,6-dihydrohomopteroate (29.1 g.). An additional

2.8 g. of the product was collected by concentration of the mother liquor to about 2 l. at *p*H 9 followed by neutralization to *p*H 7, and then refrigeration overnight. Total yield was 31.9 g. (45%), m.p. did not melt $< 300°$, λ max (*p*H 13) 253 mμ (ε, 22,500), 278 (shoulder), 366 (5,365); R_f 0.85 (17).

These physical data indicated that the possible 5,6-dihydrohomopteroate (**8**) was completely aromatized to pteroate (**9**); however, oxidation with hydrogen peroxide raised the yield of **2-b**. Therefore, a suspension of the crude product of the above (5.3 g.) in 0.4 *N* hydrochloric acid (135 ml.) was stirred for 0.5 hour and clarified by filtration. To the stirred filtrate 30% hydrogen peroxide (2.5 ml.) in water (11.5 ml.) was added dropwise over a period of 1 minute and the stirring was continued for 1.5 hours. The mixture was adjusted to *p*H 7 with 29% ammonia and the deposited solid product was collected by filtration. The filter cake was washed with water (2 x 20 ml.), cold ethanol (10 ml.), and was dried *in vacuo* to afford a yellow solid product (4.75 g., 90%). The physical properties were not changed by the oxidation. Without further confirmation of the product hydrolysis to homopteroic acid (**2-b**) was carried out as follows.

Homopteroic Acid (**2-b**).

A solution of **9** (7.7 g., 0.0177 mole) in 10% sodium hydroxide (116 ml.) was stirred under nitrogen atmosphere at 100° for 2.5 hours. The resultant red-brown solution was chilled at -10° for 3 hours and the crystallized sodium salt was collected by centrifugation. The solid was dissolved in hot water (50 ml.) and adjusted to *p*H 3.2 with 1 *N* hydrochloric acid. The resultant gelatinous mass was diluted with water to a volume of 200 ml. and the mixture was stirred at 0° until it was evenly dispersed. The product was collected by centrifugation and washed with water, ethanol by repeating centrifugation. Yield of the yellow product (**2-b**) was 5.6 g. (87%), m.p. did not melt $<300°$; λ max (*p*H 13) 256 mμ (ε, 25,100), 277 (21,600) and 365 (7,570); lit. (6) λ max (*p*H 13) 256 mμ (26,900), 277 (21,900) and 365 (7,630); R_f 0.54 (17).

Anal. Calcd. for $C_{15}H_{14}N_6O_3$: C, 55.2; H, 4.3; N, 25.8. Found: C, 54.7; H, 4.3; N, 25.8.

Homofolic Acid (**1-b**).

Preparation of **1-b** from **2-b** was accomplished by the procedure described by DeGraw *et al.* (6) with minor modifications. An average yield for **2-b** to homofolic acid was about 70%; λ max (*p*H 13) 255 mμ (ε, 24,400), 281 (20,100), 365 (7,380); lit. (6) λ max (*p*H 15) 255 mμ (24,600), 281 (19,450), 365 (7,880: R_f 0.81.

Anal. Calcd. for $C_{20}H_{21}N_7O_6 \cdot \frac{1}{2}H_2O$: C, 51.7; H, 4.7; N, 21.1. Found: C, 51.3; H, 4.7; N, 20.7.

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(1) This work was carried out under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, U. S. Public Health Service, Contract No. PH43-66-506. Communications concerning this paper should be directed to Dr. Orrie M. Friedman, Collaborative Research, Inc., Waltham, Massachusetts 02154.

(2) Presented in part at the 154th Meeting of the American Chemical Society, Chicago, Illinois, Sept., 1967.

(3) The systematic names for the title compounds are: *p*-[2-(2-amino-4-(3*H*)-oxo-6-pteridinyl)ethyl]amino-benzoic acid and *N*-*p*-[2-(2-amino-4-(3*H*)-oxo-6-pteridinyl)ethyl]amino-benzoyl-L-glutamic acid.

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(16) The biological tests were carried out by Dr. R. Kisliuk at Tufts University School of Medicine, who is the co-author of the original report (4-a).

(17) Melting points were uncorrected, refractive indices were determined on a Bausch and Lomb refractometer, infrared spectra on a Perkin-Elmer Infracord, ultraviolet spectra on a Bausch and

Lomb spectrophotometer 505, analysis by Dr. Carol Fitz, Needham Heights, Mass. Paper chromatograms were run by the ascending technique on Whatman No. 1 paper with 0.1 *M* ammonium bicarbonate in water and the spots were located by uv light.

(18) Columbia Organic chemical product.

(19) Better yield (50 ~ 60%) was obtained when the reaction was carried out at half that size.

(20) The product is rather unstable and slowly decomposes to dark brownish oil. Purification of the product, whenever necessary is repeated as described to give an excellent purity.

(21) A solution of the commercially available tetraethyl-ammonium hydroxide (10% solution) was made slightly acidic (*p*H 6) with glacial acetic acid. The solution was evaporated to dryness *in vacuo* and the excess acetic acid was removed by co-evaporation with water.