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Mixed Thiolsulfonates and Sulfonamides from Polyfunctional Mercaptans Using Trifluoromethyl Thiosulfonates†

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Thiolsulfonates have shown biological interest both as antimicrobial agents and as protectants against ionizing radiations.[1,2] In a previous publication[3] we reported that the reaction of trifluoromethyl thiolsulfonates with mercaptans (eq 1) could be used to prepare S-polyfunctional

$$RSO_2SCF_3 + R'SH \longrightarrow RSO_2SR' + CF_3SH \quad (1)$$

thiolsulfonates which would be difficult to produce by the usual syntheses for mixed thiolsulfonates (eq 2 and 3)

$$RSO_2SK + R'Br \longrightarrow RSO_2SR' + KBr \quad (2)$$
$$RSO_2M + R'SCl \longrightarrow RSO_2SR' + MCl \quad (3)$$

where M is Na, Ag, etc.

Further, as will be demonstrated, these thiolsulfonates may be used for synthesis of asymmetrical disulfides (eq 4).

$$RSO_2SR' + R''SH \longrightarrow RSO_2H + R'SSR'' \quad (4)$$

This paper reports some results using these reactions to prepare compounds which were tested as radioprotectants and as bactericides.

The compounds fall into three groups: (1) asymmetrical thiolsulfonates from cysteine and related structures, (2) sulfonamides from mercaptopurines and similar compounds, (3) and asymmetrical disulfides.

The preparation of mixed thiolsulfonates RSO_2SR' (Table I) by any of the well-known methods is limited mainly by the reactivity of the SR' or R' moiety. If R' is an unsubstituted alkyl group in an alkyl halide one can generally alkylate the RSO_2SK (or Na) salt, eq 2. If R' is aryl and the SH group can be halogenated, one can prepare the sulfenyl halide and treat the salt RSO_2M (M = Na, Zn, etc.), eq 3. However, if the alkyl halide is unreactive or if the aryl mercaptan contains other groups that are sensitive to halogenation, this limits the scope of the method or prevents reaction altogether. Using trifluoromethyl thiolsulfonates, however, one can perform the reaction directly upon the SH group.

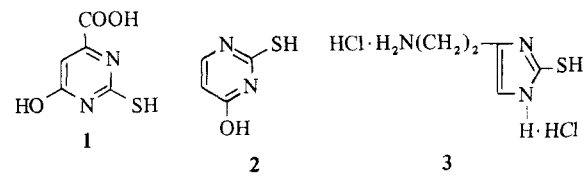
$$RSO_2SCF_3 + HSCH_2\underset{NH_2\cdot HCl}{CHCOOH} \longrightarrow RSO_2SCH_2\underset{NH_2\cdot HCl}{CHCOOH} + CF_3SH \quad (5)$$

It was discovered, during the several experiments preparing cysteine thiolsulfonates, that the main difficulty in using the RSO_2SCF_3 was prevention of the secondary reaction between the thiolsulfonate product and the starting mercaptan, eq 4. This not only reduced the yield but made purification of the product more difficult. By employing a 3–4 molar excess of the starting RSO_2SCF_3, disulfide formation was suppressed and a high yield of product was favored. Even under these conditions formation of some disulfide was noted and purification of the product sometimes was not easy. When the mercaptan was relatively unreactive, eq 1, as in thiosalicylic acid or *n*-dodecyl mercaptan, the mixed thiolsulfonate could be prepared in acid medium. This also helped to suppress disulfide formation and under these conditions a molar ratio of RSO_2SCF_3 and mercaptan was satisfactory.

The reaction of trifluoromethyl thiolsulfonates with mercaptopurines unexpectedly gave purinesulfonamides containing no -SH group. (This appears to be the first synthesis of this species as no previous report of purine sulfonamides has been found in the literature.) The reaction of RSO_2SCF_3 compounds with secondary amines was discussed earlier,[3] and was used to prepare sulfonamides. In mercaptopurines the thione structure exists in equilibrium with the mercapto form.[4] S is easily replaceable by H in purines and pyrimidines.[5] It would not be unusual, therefore, under the conditions of our reaction, to eliminate the mercapto S and obtain the sulfonamide. The nmr spectrum of the 6-mercaptopurine showed ring N but no NH peak, while in the ir there was strong SO absorption, and again no NH was observed. Both of the sulfonamides had strong ir absorption at 10.4 μ ($MeSO_2$).[6] Since mercaptopurines exist in tautomeric equilibrium between the thiol and thione forms, and this obviously involves the 7 and 9 N with the 8-SH position, we cannot say whether **12**, Table II, is the 7- or 9-methylsulfonamide. The compound derived from 6-mercaptopurine is likely to be the 7-methylsulfonamide since the 7-H would be nearer to the 6-SH group. Support for the 7 position was also obtained from nmr studies on the 6-mercaptopurine derivative and on purine itself. Comparison of the spectra shows a small shift in the values for hydrogens in the 4 and 6 positions and rather large shift for the 8-H. This suggests that the 7-sulfonamide is more likely since a 9-sulfonamide would probably cause a shift in only the 8-H. The hydrogens of the Me group absorb at δ 3.03 for aromatic systems and appear as a singlet. In the sulfonamide they are shifted only to δ 3.52, which is expected. The integration spectrum of the sulfonamide gave the ratio of H for the position 4, 6, 8, 10 as 1:1:1:3 which corresponds to the correct number and position.

The uv spectra were also obtained for the series of compounds, 6- and 8-mercaptopurines, and the sulfonamide derived from the 6 isomer.‡ The position of the $MeSO_2$ group cannot be distinguished by these spectra since corresponding values for other substituted purines are not available from the literature, and preparation of these compounds was felt to be beyond the scope of the present work.

Several pyrimidine and imidazole derivatives were treated with the RSO_2SCF_3 compounds. Repeated attempts were made to produce mixed thiolsulfonates using mercaptoorotic acid (**1**), thiouracil (**2**), and mercaptohistamine·2HCl (**3**). These reactions produced as the only identifiable products, sulfonyl sulfides, $RSO_2S_xSO_2R$ (where x = 1, 2, or 3), and high melting crystalline disulfides presumably from the



1 2 3

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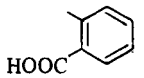
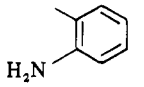
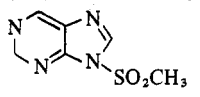
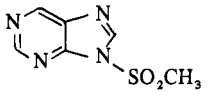
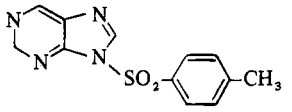
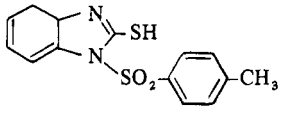
‡The absorptions (λ) obtained were as follows: 6-mercaptopurine, 325, 217; 8-mercaptopurine, 318, 230.5; the sulfonamide from 6-mercaptopurine, 279.5, 210.

Table I

Compd No.	R	R′	Purification[a]	Formula[b]	Analysis	Mp, °C
			RSO_2SR'			
1	CH_3	$CH_2CH_2NH_2 \cdot HCl$	T ($CHCl_3$–abs EtOH)	$C_3H_9NO_2S_2 \cdot HCl$	H, N, S; C[c]	170
2	CH_3	$CH_2CHCOOH$ $NH_2 \cdot HCl$	T($(Et)_2O$)	$C_4H_9NO_4S_2 \cdot HCl$	C, H, N, S	100
3	CH_3	$-C(CH_3)_2$ $HOOCHNH_2$	T($(Me)_2CO$–$CHCl_3$)	$C_6H_{13}NO_4S_2$[d]	C, N, S; H[d]	148–150
4	CH_3		R(petr ether)	$C_8H_8O_4S_2$	C, H, N; S[e]	145–147
5	p-$CH_3C_6H_4$	$CH_2CH_2NH_2 \cdot HCl$	R(abs EtOH)	$C_9H_{13}NO_2S_2 \cdot HCl$	C, H, N, S	159.5–160.5
6	p-$CH_3C_6H_4$		Tlc	$C_{13}H_{18}O_2S_2$	C, H, S	
7	p-$CH_3C_6H_4$	$CH_2CHCOOH$ $NH_2 \cdot HCl$	T($(Et_2)_2O$)	$C_{10}H_{13}NO_2S_2 \cdot HCl$	N, S; C,[f] H[f]	140–141
8	p-$CH_3C_6H_4$		R(petr ether)	$C_{13}H_{13}NO_2S_2$	C, H, N; S[g]	102–104

[a] "R" refers to recrystn (solvent in parentheses); "T" refers to trituration (solvent in parentheses). [b] Analyses were performed by Galbraith Laboratories and A. Bernhardt, Microanalytisches Laboratorium, West Germany, and were within 0.4% of the calcd values, unless noted otherwise. [c] C: calcd, 18.79; found, 19.86. [d] H: calcd, 5.34; found, 4.74. [e] S: calcd, 27.61; found, 26.60. [f] C: calcd, 38.51; found, 39.08. H: calcd, 4.52; found, 5.09. [g] S: calcd, 22.96; found, 21.30.

Table II. Sulfonamides

No.	Compound	Purification[a]	Formula	Analysis	Mp, °C
9		R(MeOH)	$C_6H_6N_4O_2S$	C, H, N, S	201–202 dec
10		T(petr ether–C_6H_6–abs EtOH)	$C_6H_6N_4O_2S$	H, N, S; C[b]	145–148
11		R(*i*-PrOH)	$C_{12}H_{10}N_4O_2S$	H, S; C,[c] N[c]	168–169
12		R($(CH_3)_2CO$–abs EtOH)	$C_{14}H_{12}N_2O_2S_2$	C, H, N, S	161–163

[a] *Cf.* Table I for abbreviations. [b] C: calcd, 36.36; found, 33.89. [c] C: calcd, 52.54; found, 50.57. N: calcd, 20.42; found, 19.47.

Table III. X-Ray Survival of Bacteria and Mice

Compound No.	Bacterial test				Mouse test	
	Toxicity ED_{50}, *M*	Concn, *M*	Solvent[c]	DRF[a]	Dose, mg/kg	% survivors
MEA[b]	$>10^{-1}$	1×10^{-1}	H_2O	3.7		
AET[b]					160	100
1	3×10^{-2}	1×10^{-2}	H_2O	1.7	20	0
2	1×10^{-3}	5×10^{-4}	H_2O	0.71	200	0
3	$>10^{-1}$	3×10^{-2}	1.8×10^{-2} *M* NaOH	4.5	40	92
4	3×10^{-4}	1.5×10^{-4}	A	0.98	200	0
5	2×10^{-2}	5×10^{-3}	B	1.4		
6	1×10^{-2}	3×10^{-3}	A	1.0		
7	5×10^{-3}	3×10^{-3}	90% 0.05 *M* Na_2HPO_4 A	2.1		
8	1.5×10^{-3}	1×10^{-3}	A	0.91	120	0
9	$>6 \times 10^{-2}$	3×10^{-2}	B	1.3		
10	3×10^{-2}	5×10^{-3}	B	1.3	160	10
11	3×10^{-2}	2×10^{-2}	B	1.5		
12	3×10^{-4}	1×10^{-4}	A	2.9	160	0

[a] Dose required to inactivate a given fraction of cells (chose $N/No = 0.37$) in the presence of a protective agent to that required in the presence of solvent alone. N/No is the surviving fraction, or number of cells surviving any radiation dose divided by the original number of cells. Thus DRF of solvent alone is 1.0. [b] MEA (mercaptoethylamine) and AET (aminoethylisothiuronium bromide) were employed as standards in the bacterials and mouse tests, respectively. [c] A = 10% Me_2CO; B = 5×10^{-2} *M* Na_2HPO_4.

oxidation of the mercapto compounds. These various products were identified by elemental analyses, ir spectra, and melting points where literature comparisons were possible.

The reaction of trifluoromethyl *p*-toluenethiolsulfonate and 2-mercaptobenzimidazole produced an excellent yield of the sulfonamide without loss of SH. This again demonstrates the anomalous behavior of the SH group attached to C bonded to two N atoms, for no such structure gave a thiolsulfonate.

Preparation of asymmetrical disulfides using the thiolsulfonates and mercaptans followed previously described methods.[7,8] Recent work[9] has shown how polyfunctional disulfides, as for example the disulfide of cysteine and glutathione, can be synthesized from thiolsulfonates. It can be seen that the reaction with mercaptans is usually reliable.[7] In this work two disulfides were prepared by the thiolsulfonate reaction viz, the disulfide made from cysteine and penicillamine and the disulfide made from cysteine and *p*-thiocresol. However, only the latter gave good elemental analysis.

Biological Results. Organic compounds containing S and N such as cysteine, mercaptoethylamine, and aminoethyl isothiuronium bromide have demonstrated biological protection against ionizing radiation, and because of the structural similarity of some of the compounds it was of interest to determine whether the compounds described in this paper might have similar properties. The thiolsulfonate derived from penicillamine and trifluoromethyl methanethiolsulfonate was effective in protecting both bacteria and mice, even though the concentration at which it had to be used, limited to toxicity, was necessarily quite low.

Experimental Section

The general prepn of thiolsulfonates of possible biologic interest can be illustrated by the synthesis of the compd derived from cysteine and trifluoromethyl *p*-toluenethiolsulfonate. To cysteine·HCl (3.59 g, 0.0205 mole) and 40 ml of 95% EtOH, with stirring, was added 22.0 g (0.086 mole) of trifluoromethyl *p*-toluenethiolsulfonate.[3] The mixt was stirred for 1 hr, 50 ml of cold H_2O was added, and a small amt of solid was removed by filtration. This was identified as cystine, mp 260°. The soln was put into a separatory funnel to remove the trifluoromethyl *p*-toluenethiolsulfonate and 13.4 g was recovered. The remaining soln was evapd at room temp. An oil formed which eventually crystd and 6.35 g of thiolsulfonate product was recovered (90% yield crude). The crude material was triturated 3 times with Et_2O and dried in a desiccator over H_2SO_4, mp 140–141°; it was not further purified. The attempted prepns of purine and pyrimidine thiolsulfonates followed the same procedures. In these cases, however, the solid products were identified as sulfonamides.

Prepns of thiolsulfonates in an acid medium can be illustrated by the syntheses of *o*-carboxyphenyl methanethiolsulfonate. An EtOH soln (0.0185 g of H_3PO_4/ml of 95% EtOH) was stirred. To this was added 0.77 g (0.005 mole) of thiosalicylic acid. After the solid had dissolved, 1.80 g (0.01 mole) of methyl trifluoromethanethiolsulfonate was added. The soln was stirred for 1 hr, chilled H_2O was added, and this yielded a small amount of solid, identified as the disulfide, mp 195–197°, which was removed by filtration. The soln was extd with Et_2O, the ext was sepd, dried (Na_2SO_4), and then placed in a crystallizing dish. After Et_2O had evapd, a semisolid was collected which was recryst from 60–110° petr ether–abs EtOH (8:2), mp 145–147°.

Synthesis of the disulfide of *p*-thiocresol and cysteine was effected as follows. α-Amino-β-carboxyethyl *p*-toluenethiolsulfonate (1 g, 0.32 mole) was dissolved in 15 ml of abs EtOH and to this was added in one addition 0.4 g (0.32 mole) of *p*-thiocresol in 5 ml of abs EtOH. The reaction mixt was stirred for 20 min, H_2O was added and the solid was filtered, washed (H_2O), and dried. The crude material had mp 170° and when recrystd once from AcOH had mp 172° dec. Although the starting thiolsulfonate was cysteine·HCl the disulfide apparently, according to elemental analysis, was the zwitterion. *Anal.* (15), ($C_{10}H_{13}NO_2S_2$) C, H, N, S. The disulfide of cysteine and penicillamine (16) was prepared similarly. *Anal.* ($C_8H_{16}N_2O_4S_2$) S.

Microbiological Assays of Toxicity and Radiation Protection. The bacterium *Escherichia coli* B/r ORNL was obtained from Dr. H. I Adler who characterized it.[10] The organisms were grown by a technique similar to that of Elias[11] except that diln of the inoculum in H_2O was employed rather than centrifugation and washing, and the exp was performed at room temp rather than at 0°. Other differences were as follows: weekly stock cultures of approximately 3×10^9 cells/ml in nutrient broth were prepd from refrigerated, nutrient broth–agar slants. The cultures were grown for 17 hr at 34.5° in a Warburg shaker (aeration through cotton plug) before being cooled at 15°. Daily cultures for actual use were made from the stock culture and grown for 17 hr in the same way.

A weighable quantity of chemical was dissolved in an aqueous (H_2O or 0.05 *M* Na_2HPO_4) or organic (Me_2CO or EtOH) solvent. Direct dilns in H_2O were made for the H_2O-sol compds to obtain a desired series of concs for detg compd toxicity, and following that, to determine the X-ray protection offered by the chemical at a nontoxic molarity. The organic solvent-soluble compds were dild in their respective solvents. From these solns, 0.1-ml aliquots were respectively added to 0.9 ml of sterile distd H_2O (10% by vol of Me_2CO or EtOH were found to be nontoxic). Finally, to these solns 0.1 ml of inoculum which had been dild by 10^3 in sterile distd H_2O, was added and these inoculated solns were used.

For toxicity detns, the dild chemical was incubated with the organisms at room temp for the time period of the X-ray test. Then each diln of chemical with inoculum and each solvent control was diluted by 10^3. Of this final diln 0.1 ml was plated on Difco nutrient broth–agar and the plates were incubated overnight at 35° before colonies were counted.

The X-irradiation technique was that of Stapleton, *et al.*,[12] with the following modifications. A solvent control and 2 chemical solns (at 0.5 their highest nontoxic concns) were irradiated in glass shell vials which were spaced in a ring in a plastic support. A loose-fitting petri dish cover kept contaminants out of the 3 shell vial solns, and a turntable provided uniform irradiation from the G. E. Maxitron 300. Operating settings were 250 kVP, 20 mA, 3-mm Al filter, 17 cm target distance, and a dose rate of 1595 R/min as measured at midline in air by a Victoreen thimble chamber. The inoculated 1.0 ml of chemical soln or the solvent control was incubated at room temp for 0.5 hr. A 0.7-ml portion was irradiated at 10 kR, aliquots totaling 0.2 ml were withdrawn from it, and the approximately 0.5 ml that remained was further irradiated for a total dose of 30 kR. The 0.3-ml portion of the original 1.0 ml was retained as unirradiated control.

The irradiated suspensions were withdrawn at 10 and 30 kR and, with the unirradiated controls, were appropriately dild in sterile distd H_2O. Aliquots were cultured and counted as in the toxicity detns.

Tests with Mice. Female mice, strain BC3 F_1/C, 12–15 weeks old (approx 25 g), were employed. One-half the max dose of chemical that did not kill any mice in toxicity tests was employed in the radiation tests. The compds were dissolved and given ip. Batches of 10 or 20 mice were employed for each irradiation test. The mice, rotated on a turntable, received 900 R of X-irradiation at 100 R/min from a G. E. Maxitron X-ray tube at 300 kVP and 20 mA with 3 mm Al filter at a distance of 70 cm from the tube to the animals. The dose of irradiation was sufficient to kill all of the untreated mice always in less than 30 days following radiation, but mice receiving 160 mg/kg of aminoethyl isothiuronium bromide were all alive in 30 days.

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Synthesis and Biologic Evaluation of 7-Hydroxymethotrexate, 7-Methylaminopterin, and 7-Methylmethotrexate†

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Chemistry. Although apparently not metabolized in man, the important antitumor folate antagonist methotrexate (MTX, 4-amino-4-deoxy-N^{10}-methylpteroylglutamic acid) undergoes biotransformation in several species, notably the rabbit.[1,2] The biotransformation proves to be an oxidative process mediated by aldehyde oxidase (EC 1.2.3.1).[3] On the strength of degradative, chromatographic, and spectral evidence, the product of both the *in vivo* and *in vitro* oxidation of MTX has been assigned the structure, 7-OH-MTX (**1**).[4]

In this report we describe the synthesis of 7-OH-MTX, which constitutes a confirmation of the structure of MTX metabolite. In addition, we report the preparation of 4-amino-4-deoxy-7-methylpteroylglutamic acid (7-methylaminopterin or 7-Me-AM) (**2**) and 7-Me-MTX (**3**). Some preliminary observations regarding the biochemical behavior of these new compounds are included.

7-OH-MTX (**1**) has been synthesized by the straightforward condensation of 2,4-diamino-7-hydroxy-6-pteridine(α-bromo)acetic acid (**6**)[5] with *p*-methylaminobenzoylglutamic acid (**7**).[6]

However, the 7-methyl compounds **2** and **3** were prepared by 2 routes. In the first, 2,4,5,6-tetraaminopyrimidine was allowed to react simultaneously with 3,4-dibromo-2-butanone and *p*-aminobenzoylglutamic acid (**9**) or **7** in the presence of KI and I_2. Since the yields were low, the following approach was adopted. In the second method, the desired intermediate, 2,4-diamino-6-bromomethyl-7-methylpteridine was prepared by the selective reduction of 2,4-diamino-6,7-bis(bromomethyl)pteridine (**8**) with KI. To prepare **8**, 2,4,5,6-tetraaminopyrimidine was condensed with dibromodiacetyl.[7] Finally, reaction of the monobromo compound (not isolated) with either **9** or **7** afforded 7-Me-AM (**2**) and 7-Me-MTX (**3**), respectively. Degradative oxidation of both **2** and **3** gave 2,4-diamino-7-methylpteridine-6-carboxylic acid which has been identified with an authentic specimen.[8]

The nmr spectra of **2** and **3** are entirely in accord with the assigned structures. Thus, the only significant difference between the spectra of **2** and **3** and those of their parent compounds AM and MTX is that the low field (τ1.20 and 1.35, respectively) singlet absorptions attributable to the 7-H in AM and MTX have been replaced by singlet absorptions at τ7.38 and 7.44 as a result of the substitution of the 7-hydrogen atoms by 7-methyl groups in **2** and **3**.

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Table I. Inhibition of Dihydrofolate Reductase

Compound	I_{50}, n*M*
MTX	23
7-OH-MTX	4000
7-Me-AM	39
7-Me-MTX	26

Biological Evaluation. As inhibitors of dihydrofolate reductase (EC 1.5.1.3) of rat liver, the concentrations (I_{50}) for 50% inhibition of enzyme activity by these compounds are presented in Table I.

Because of their favorable I_{50}, 7-Me-AM and 7-Me-MTX were also screened against mouse L1210 leukemia in comparison with MTX (Table II). 7-Me-AM shows only marginal activity while 7-Me-MTX is inactive when tested under the experimental conditions summarized in the table. The cause of this unexpected inactivity is presently under investigation. It may be the result of failure of the 7-methyl compounds to penetrate into tumor cells or the rapid elimination of these drugs from the body, to mention but two of the many possibilities.

Experimental Section

Nmr spectra were determined in DMSO-d_6 (TMS) with a Varian Model A-60 spectrometer at 60 MHz. Uv spectra were recorded on a Cary Model 14 spectrophotometer. Column chromatography was performed on DEAE-cellulose (Calbiochem, 1.02 mequiv per g) and tlc on cellulose-coated plates (Mann Research Laboratories). Paper chromatography was conducted in a descending manner on Schleicher and Scheull paper (470-A-C) and the chromatograms were viewed under long wavelength uv. Solvent systems used were A: *n*-BuOH–AcOH–H_2O ((5:2:3), B: 5% aqueous Na_2HPO_4, C: 5% NH_4OH–3% aqueous NH_4Cl. Elemental analyses were performed by Dr. William Alford, National Institutes of Health, Bethesda, Md., and, where indicated only by the symbols of elements, were within ±0.4% of the theoretical values.

Ethyl 2,4-Diamino-7-hydroxypteridine-6-acetate (4). A modification of the method of Renfrew, *et al.*,[9] was employed. A suspension of 2,4,5,6-tetraaminopyrimidine sulfate (10.0 g, 42 mmoles) in glacial AcOH (800 ml) was heated to 90° and diethyl sodiooxaloacetate (10.0 g, 48 mmoles) was added with rapid stirring. The mixt was refluxed for 90 min. After cooling to room temp the pale yellow solid was collected by filtn, washed thoroughly with H_2O followed by EtOH, and dried *in vacuo*: 9.5 g (85%).

2,4-Diamino-7-hydroxypteridine-6-acetic acid (5) was prepared by hydrolysis of **4** as described by Renfrew, *et al.*[9]

2,4-Diamino-7-hydroxypteridine-6-(α-bromo)acetic acid (6) was prepared from **5** according to the method of Tschesche, *et al.*[5]

***N*-(*p*-{[(2,4-Diamino-7-hydroxy-6-pteridinyl)methyl]methylamino}benzoyl)-L-glutamic Acid (7-OH-MTX) (1).** Condensation of **6** (2.15 g, 6.8 mmoles) with *p*-methylaminobenzoylglutamic acid hydrobromide **7** (3.0 g, 8.3 mmoles) in dry ethylene glycol (130 ml) in the presence of anhyd NaOAc (3.6 g) according to the procedure of Tschesche, *et al.*,[5] afforded 2.05 g (61%) of crude **1**.

A sample of the crude product (1.0 g) was suspended in H_2O (20 ml) and NH_4OH soln (28%) was added dropwise, with stirring, until a clear soln resulted. The soln (pH 7.8) was applied to a column (5 × 35 cm) of DEAE-cellulose which had been equilibrated previously with 0.01 *M* NH_4HCO_3. The column was eluted in a stepwise fashion by applying a series of NH_4HCO_3 buffers of progressively increasing molar strength (1000 × 0.10 *M*, 1000 × 0.15 *M*, 2000 × 0.20 *M*, 2000 × 0.25 *M*, 2000 × 0.30 *M*). Fractions of 10 ml were collected and the elution of successive components was monitored by uv absorption. Fractions containing 7-OH-MTX (0.25–0.30 *M*) were combined, filtd, and evapd at below 50° *in vacuo* while protected from light. The amorphous