[CONTRIBUTION FROM THE DEPARTMENT OF PHARMACOLOGY, SCHOOL OF MEDICINE, YALE UNIVERSITY]

Potential Metabolic Antagonists of Orotic Acid: 6-Uracilsulfonamide and 6-Uracil Methyl Sulfone[1]

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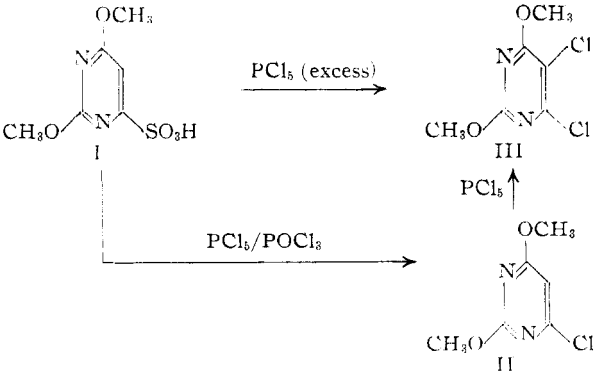
6-Uracilsulfonamide and 6-uracil methyl sulfone were prepared for testing as metabolic antagonists of orotic acid. The sulfonamide was not available through 2,4-dimethoxy-6-pyrimidinesulfonic acid, since attempts to prepare the corresponding sulfonyl chloride were not successful. Phosphorus pentachloride gave either 2,4-dimethoxy-6-chloropyrimidine or 2,4-dimethoxy-5,6-dichloropyrimidine depending on the conditions employed. The sulfonamide was prepared by converting 2,4-dimethoxy-6-pyrimidinethiol to the sulfenamide with ammoniacal hypochlorite and then oxidizing and demethylating the product. The sulfone was prepared from 2,4-dimethoxy-6-methylmercaptopyrimidine by an oxidation and demethylation.

The interest in compounds which might serve as metabolic antagonists of orotic acid (6-uracilcarboxylic acid) has been mentioned in a previous report dealing with the synthesis of 6-uracilsulfonic acid.[2] Although this simple analog has not shown the type of biological activity desired, several related compounds of much greater promise have now been prepared. Preliminary biologic studies of two of these compounds, 6-uracilsulfonamide (VII) and 6-uracil methyl sulfone (X), have indicated that they are potent inhibitors of the growth of orotic acid-requiring strains of *Lactobacillus bulgaricus*, and that they are also inhibitors of the enzymatic decarboxylation of orotic acid (or its appropriate metabolic derivative) by an avian liver system.[3] The preparation of the sulfonamide and of the sulfone is described in this report.

Although the most obvious approach to the 6-uracilsulfonamides appeared to be through a sulfonyl halide derived from 2,4-dimethoxy-6-pyrimidinesulfonic acid (I) (an intermediate used in the preparation of the above-mentioned 6-uracilsulfonic acid), various attempts to convert I to its corresponding sulfonyl halide have not met with success. The compound did not react with thionyl chloride under reflux and fusion with a large excess of phosphorus pentachloride afforded only a sulfur-free substance which melted at 103–104°. The elementary analysis data and the typical pyrimidine ultraviolet absorption spectrum (λ_{max}^{EtOH} 277 mμ, ε 6,780) identified this reaction product as the previously unreported 2,4-dimethoxy-5,6-dichloropyrimidine (III).



In an additional experiment, only a bare excess of phosphorus pentachloride was employed and phosphorus oxychloride was used as a solvent. This modification eliminated the chlorination at the 5-position, but did not eliminate replacement of the sulfonic group; the product obtained under these conditions was the known 2,4-dimethoxy-6-chloropyrimidine (II).[4] There was no reaction with phosphorus oxychloride alone or with cold phosphorus pentachloride.[5]

The ability of phosphorus pentachloride or thionyl chloride to cause replacement of a sulfonic group by chloride has been encountered elsewhere.[6] If it is assumed that the formation of a sulfonyl halide is the first step in such a reaction,[6] then it would appear that the sequence of events in the case at hand is I $\xrightarrow{(1)}$ (sulfonyl chloride) $\xrightarrow{(2)}$ II $\xrightarrow{(3)}$ III, and that reaction 2 proceeds at such a rate as to destroy rapidly the sulfonyl halide even in the absence of excess reagent. Reaction 3 was confirmed by the isolation of III as the product of a reaction of II with excess phosphorus pentachloride. In connection with these negative results, it might be noted that Pyman and co-workers were unable to convert various 4-imidazolesulfonic acids to their sulfonyl chlorides.[7]

The oxidation of sulfenamides to sulfonamides has been described in the patent literature[8] and this alternate approach to the 6-uracilsulfonamides became feasible when it was found that the intermediate 2,4-dimethoxy-6-pyrimidinethiol (IV) could be converted to a stable sulfenamide V in 67% yield upon treatment of its sodium salt with an ice-cold ammoniacal hypochlorite (*i.e.*, chloramine) solution. These conditions are similar to those described by Carr, Smith and Alliger for the preparation of 2-benzethiazolesulfenamide.[9]

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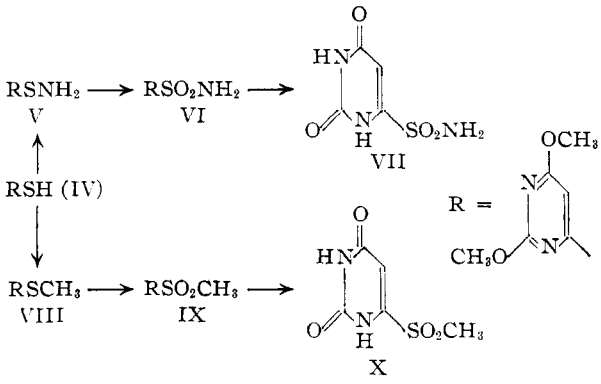
Oxidation of V with potassium permanganate readily produced the sulfonamido derivative VI in similar yield, and a demethylation with dilute acid gave the desired 6-uracilsulfonamide.



2,4 - Dimethoxy - 6 - methylmercaptopyrimidine (VIII) was prepared simply by the action of dimethyl sulfate on the thiol IV. An oxidation with performic acid produced the corresponding methyl sulfone IX in 82% yield; demethylation with dilute hydrochloric acid gave the desired 6-uracil methyl sulfone (X).

The ultraviolet absorption characteristics of the 6-uracilsulfonyl derivatives thus far prepared have been collected in Table I. It may be noted that although the spectra of the derivatives are displaced toward the visible with respect to the spectra of uracil itself, the magnitude of the bathochromic shift caused by a change from acid to basic solvent is roughly the same in each case (27 ± 2 mμ).

TABLE I

ULTRAVIOLET ABSORPTION

	0.1 *N* HCl		0.1 *N* NaOH	
	λ_{max}, mμ	ϵ_{max}	λ_{max}, mμ	ϵ_{max}
Uracil[10]	259	8200	284	6150
6-Uracilsulfonic acid	264	8470	293	9190
6-Uracilsulfonamide	267	7700	292	9180
6-Uracil methyl sulfone	270	7080	296	6730

Solutions of the 6-uracil sulfone and sulfonamide are markedly acidic, and the solubility of these substances in water is greatly increased by the addition of alkali.

In view of the biological results obtained with these new compounds, the synthesis of a number of substituted 6-uracil sulfones and sulfonamides has been undertaken in this Laboratory and will be presented in a future publication.

Acknowledgment.—The author wishes to express his appreciation to Professor Arnold D. Welch for his continued interest and encouragement in these investigations.

Experimental

The absorption spectra were determined with a Beckman model DU quartz spectrophotometer using matched silica cells. The melting points were taken with a calibrated thermometer but are otherwise uncorrected. Microanalyses were performed by the Huffman Microanalytical Laboratories, Wheatridge, Colorado.

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Action of PCl_5 on 2,4-Dimethoxy-6-pyrimidinesulfonic Acid and 2,4-Dimethoxy-6-chloropyrimidine.—(a) A mixture of 0.5 g. of 2,4-dimethoxy-6-pyrimidinesulfonic acid[2] and 2.25 g. of phosphorus pentachloride was heated slowly to 130°. When this temperature was reached the mixture fused and refluxing began. After 30 minutes, the mixture was cooled and extracted with benzene. The extract was shaken with concentrated ammonia, washed with water and dried. Following the removal of the solvent at room temperature, the residue was dissolved in ethanol (95%) and crystallization was induced by the addition of an equal volume of water. This afforded 0.26 g. of a sulfur-free compound, m.p. 103–104°. The substance was identified by its analysis and ultraviolet spectrum as **2,4-dimethoxy-5,6-dichloropyrimidine**; absorption λ_{max}^{EtOH} 277 mμ, ε 6,780.

Anal. Calcd. for $C_6H_6Cl_2N_2O_2$: C, 34.47; H, 2.89; Cl, 33.92; N, 13.10. Found: C, 34.64; H, 3.04; Cl, 33.63; N, 13.10.

(b) A mixture of 0.5 g. of the sulfonic acid, 0.5 g. (5% excess) of phosphorus pentachloride and 5 ml. of phosphorus oxychloride was heated to 120° in an oil-bath. At this temperature refluxing began and solution occurred within 30 minutes. The cooled solution was poured onto ice and the product was isolated and recrystallized in the manner described under (a). This procedure afforded 0.2 g. of 2,4-dimethoxy-6-chloropyrimidine, m.p. 73–74°. The melting point was not depressed by admixture with an authentic sample prepared from trichloropyrimidine and sodium methylate, m.p. 74–75° (lit.[4] 76°).

(c) A mixture of 0.5 g. of 2,4-dimethoxy-6-chloropyrimidine and 2.25 g. of phosphorus pentachloride was heated for one hour at 140°. The reaction mixture was worked up as in (a) and afforded 0.3 g. of crude benzene extract. Three recrystallizations from aqueous ethanol (as above) gave 0.1 g. of 2,4-dimethoxy-5,6-dichloropyrimidine, m.p. 103–104°. The melting point was not changed by admixture with a sample of the compound obtained in (a).

2,4-Dimethoxy-6-pyrimidinethiol (IV).—The thiol was prepared from 2,4-dimethoxy-6-chloropyrimidine and absolute alcoholic sodium hydrosulfide by a modification of the procedure previously described.[2] It was found that a good yield was more consistently obtained if the well stirred reaction mixture was first heated overnight at 50–55° and then heated under reflux for an additional four hours.

2,4-Dimethoxy-6-pyrimidinesulfenamide (V).—A chloramine solution was prepared from a well-stirred 180-ml. portion of cold concentrated ammonia (d. 0.9) by the dropwise addition of 90 ml. of cold 5.25% sodium hypochlorite (Chlorox). This was followed by the dropwise addition (during a period of 15 minutes) of a cold clear solution of 10.3 g. of 2,4-dimethoxy-6-pyrimidinethiol in 63 ml. of 1 *N* sodium hydroxide. The temperature was kept below 15° during both reactions. After an additional 5 minutes of stirring, the precipitate of sulfenamide was collected by filtration, washed free of alkali with ice-water and dried *in vacuo* at room temperature. Recrystallization was effected by dissolving the compound in benzene at room temperature and adding two volumes of petroleum ether (30–60°). The yield of finely powdered sulfenamide was 7.3 g. (67%), m.p. 123–124°. The analytical sample was recrystallized an additional time and dried in the same manner.

Anal. Calcd. for $C_6H_9N_3O_2S$: C, 38.49; H, 4.84; N, 22.45; S, 17.13. Found: C, 38.62; H, 4.86; N, 22.24; S, 17.00.

2,4-Dimethoxy-6-pyrimidinesulfonamide (VI).—A well-stirred suspension of 7.5 g. (0.04 mole) of the above sulfenamide in 160 ml. of water was oxidized by the dropwise addition of 161 ml. of 0.4 *M* potassium permanganate over a period of 15 minutes. (The temperature was not allowed to rise above 30° during the reaction.) After an additional 15 minutes of stirring, the manganese dioxide was destroyed with an excess of saturated sodium bisulfite solution. The tan precipitate was collected, washed first with 150 ml. of cold 1 *N* hydrochloric acid and then with cold water. The dried sulfonamide was dissolved in absolute ethanol, treated with Norit and precipitated by the addition of two volumes of petroleum ether (30–60°); yield 5.51 g. (64%), needles, m.p. 188–189°. The analytical sample was recrystallized twice more in the same manner; m.p. 189–190°.

Anal. Calcd. for $C_6H_9N_3O_4S$: C, 32.88; H, 4.14; N, 19.17; S, 14.63. Found: C, 33.02; H, 4.14; N, 19.29; S, 14.49.

6-Uracilsulfonamide (VII).—The dimethoxysulfonamide (above) was demethylated by autoclaving a suspension of 5.5 g. in 750 ml. of 0.1 *N* hydrochloric acid for two hours at 250°F. (15 p.s.i.). The solution was brought to dryness under reduced pressure and the residue was recrystallized from water. The fine crystals of 6-uracilsulfonamide gradually decomposed above 235°; the yield (including work-up from mother liquor) was 2.8 g. (58%). The analytical sample was recrystallized twice more from the same solvent. The absorption is given in Table I.

Anal. Calcd. for $C_4H_6N_3O_4S$: C, 25.13; H, 2.64; N, 21.98; S, 16.77. Found: C, 25.20; H, 2.67; N, 21.86; S, 16.57.

2,4-Dimethoxy-6-methylmercaptopyrimidine (VIII).—A solution of 6.88 g. (0.04 mole) of 2,4-dimethoxy-6-pyrimidinethiol[2] in 80 ml. of 2.5% sodium hydroxide was treated dropwise with 7.2 g. (0.056 mole) of dimethyl sulfate. The mixture was stirred for 1.5 hours with the occasional addition of enough 10% sodium hydroxide to keep the mixture alkaline. The insoluble methylmercapto derivative crystallized when the reaction mixture was cooled in ice, and was apparently reasonably pure after it was collected and washed with water; yield 5.9 g. (80%), m.p. 44–46°. Recrystallization was effected by dissolving the compound in hot ethanol (95%) and then adding an equal volume of water to the cooled solution. This returned 5 g., m.p. 45–46°. The analytical sample was recrystallized an additional time.

Anal. Calcd. for $C_7H_{10}N_2O_2S$: S, 17.22. Found: S, 17.31.

2,4-Dimethoxy-6-pyrimidine Methyl Sulfone (IX).—A solution of 5 g. of the above methylmercapto derivative in 135 ml. of 88% formic acid was treated with 13.5 ml. of Superoxol and allowed to stand for 3 hours. It was then diluted with an equal volume of water and allowed to stand overnight. The residue obtained by evaporating the solution to dryness under reduced pressure was collected by means of a small amount of cold absolute ethanol and sucked dry. A recrystallization from absolute ethanol afforded the sulfone in the form of long needles; yield 4.81 g. (82%), m.p. 122–123°. The analytical sample was recrystallized a second time.

Anal. Calcd. for $C_7H_{10}N_2O_4S$: C, 38.52; H, 4.62; N, 12.84; S, 14.69. Found: C, 38.48; H, 4.80; N, 12.87; S, 14.60.

6-Uracil Methyl Sulfone (X).—The dimethoxy sulfone (above) was demethylated by autoclaving a suspension of 4.8 g. in 700 ml. of 0.1 *N* hydrochloric acid for two hours at 250°F. (15 p.s.i.). The solution was brought to a small volume under reduced pressure and the crystalline material which separated out was collected (3 g.) and recrystallized from water. By reworking the mother liquor a total of 1.9 g. (45%) of the recrystallized product was obtained, m.p. 299–300° dec. The analytical sample was recrystallized from glacial acetic acid and washed with petroleum ether, m.p. 307–308°. The absorption data are found in Table I.

Anal. Calcd. for $C_5H_6N_2O_4S$: C, 31.58; H, 3.18; N, 14.73; S, 16.86. Found: C, 31.73; H, 3.37; N, 14.44; S, 16.70.

In later work it was found more advantageous to demethylate the demethoxy sulfone by heating it with a 90% acetic acid solution which was 0.2 *N* with respect to hydrochloric acid. This procedure afforded a somewhat higher yield and gave a product which was chromatographically homogeneous after only a single recrystallization. Thus a mixture of 21.8 g. of the demethoxy sulfone (IX), 1575 ml. of glacial acetic acid and 175 ml. of 2 *N* hydrochloric acid was heated under reflux for three hours. The solution was evaporated to dryness under reduced pressure and the residue was recrystallized from water. The uracil methyl sulfone was obtained in the form of fine rods; yield 10.1 g. (54%), m.p. 307–308° dec.

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[Contribution from the Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health]

Influence of Optically Active Acyl Groups on the Enzymatic Hydrolysis of N-Acylated-L-amino Acids

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L- and D-alanine were converted to the corresponding optically active α-chloropropionic acids without loss of configuration, and the halogenated acids combined through their acid chlorides with glycine, L-alanine, L-butyrine and L-norvaline for studies with renal acylase I, and with L-phenylalanine and L-tyrosine for studies with pancreatic carboxypeptidase. The acylated amino acids with L-acyl substituents were hydrolyzed by renal acylase at greater rates than those with D-acyl substituents, but the difference between the rates for the L- and D-acyl substituents was very much greater when the acyl groups were alanyl than when they were α-chloropropionyl. With increasing length of the side-chain of the terminal L-amino acid this difference increased in the case of the α-chloropropionyl derivatives and was practically constant in the case of the alanyl derivatives. In the case of pancreatic carboxypeptidase, the difference in rates between L- and D-acyl derivatives was relatively small except for the carbobenzoxyalanyl derivatives. Unlike renal acylase I, pancreatic carboxypeptidase attacks the L-chloropropionyl residue much more readily than it does the L-alanyl or propionyl.

Acylase I is a soluble, intracellular carboxypeptidase which catalyzes the hydrolysis of a wide variety of N-acetylated amino acids at rates which are dependent upon the nature of the N-acyl group and of the terminal amino acid residue.[1-8] N-Chloroacetyl-L-amino acids are hydrolyzed by acylase I at faster rates than are corresponding N-glycyl-L-amino acids.[3] On the other hand, N-DL-chloropropionyl-L-alanine is hydrolyzed at a much slower rate than is either L-alanyl-L-alanine,[3] or N-propionyl-L-alanine,[3] and it would appear that electronic effects alone are insufficient to account for the influence of the nature of substituents in the N-acyl group on the susceptibility of the substrate to acylase I. Moreover, although L-alanyl amino acids are hydrolyzed much more rapidly than are D-alanyl amino acids, inspection of the hydrolytic curve of DL-chloropropionyl-L-alanine[2] suggests that the susceptibility of the two optical forms to acylase I is not greatly different. Yet one of these two forms corresponds in optical configuration to that of L-alanyl-L-alanine, and the other to that of D-alanyl-L-alanine.

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