[CONTRIBUTION FROM THE RESEARCH DEPARTMENT, THE ARMOUR LABORATORIES]

Synthesis of Compounds Related to Thymine. III. Chlorosulfonation of Uracil

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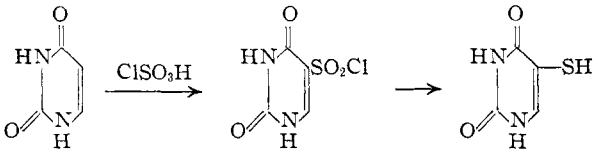
Uracil has been chlorosulfonated, in good yield, to 5-uracil-sulfonyl chloride. Reduction of this product with zinc and aqueous acid leads to 5-uracilyl disulfide or to the zinc salt of 5-mercaptouracil. The latter can be converted to the free thiol.

It has been reported recently[1] that 5-mercaptouracil and some of its derivatives are competitive antagonists of thymine. These compounds originally were synthesized by us from 5-aminouracil by way of the diazonium salt.[2]

In the course of an exploration of alternative routes of synthesis which might be more applicable to the preparation of larger quantities, the synthesis of 5-mercaptouracil by way of 5-uracilsulfonyl chloride was investigated. Since both nitration and bromination of uracil give the corresponding 5-substituted derivatives, *i.e.*, 5-nitrouracil and 5-bromouracil, respectively,[3] it seemed reasonable to expect that if uracil could be chlorosulfonated, the product would be 5-uracilsulfonyl chloride (II). From reduction of this sulfonyl chloride, 5-mercaptouracil (III) could be obtained



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By using a large excess of chlorosulfonic acid, with no other solvent, and heating the reaction mixture at reflux for several hours, a product was obtained which appeared to be the sulfonyl chloride. This material is high-melting (>300°) and difficult to purify because of its insolubility in non-reactive solvents. Elemental analysis of the crude material gave percentages approximating those calculated for a uracilsulfonyl chloride. Reaction of this sulfonyl chloride with aniline gave the sulfonanilide which could be recrystallized from water. Analysis confirmed the identity of the sulfonanilide. Reaction of the sulfonyl chloride with dimethylamine and with sodium phenoxide gave the N,N-dimethylsulfonamide and phenyl sulfonate ester, respectively.

Reduction of 5-uracilsulfonyl chloride was effected with zinc and dilute sulfuric acid. The product of this reaction is either 5-uracilyl disulfide or the zinc salt of 5-mercaptouracil, depending upon the conditions of the reaction. If a small excess of zinc is used, allowed to dissolve, and the reaction mixture is heated for several hours thereafter, the major product is the disulfide. If, however, a large excess of zinc is used and the reaction is stopped before the metal is completely dissolved, the product is isolated predominantly as the zinc salt of 5-mercaptouracil.[4] This material is bright yellow in color and insoluble in water and organic solvents. It gives a positive test with sodium nitroprusside[5] but its infrared absorption spectrum does not show the S–H band characteristic of the thiol.[2]

The same substance may be obtained from the thiol by treating a solution of the mercaptan with a solution of a soluble zinc salt. An immediate bright yellow precipitate of the zinc salt of 5-mercaptouracil is formed.

The zinc salt is soluble in base, and may be converted to the mercaptan by dissolving in sodium hydroxide and adding sodium sulfide to form zinc sulfide, which may be removed by centrifugation. Acidification of the cold supernatant liquid precipitates 5-mercaptouracil. This process is best carried out in the cold and under an atmosphere of nitrogen, since the mercaptan is easily oxidized to the disulfide in basic solution.

An easier procedure for obtaining the free mercaptan from its zinc salt consists of heating the zinc salt with 6 *N* hydrochloric acid (or sulfuric) until it dissolves. A solid separates on cooling which may be recrystallized from water or dilute acid to give 5-mercaptouracil.

When these reactions are used for the preparation of 5-mercaptouracil or the disulfide, the sulfonyl chloride need not be isolated since, when the cooled chlorosulfonation mixture is added to the suspension of zinc dust in water, the hydrolysis products of the excess chlorosulfonic acid serve as the acid in the reduction. Over-all yields up to 40% of the theoretical, based on uracil as the starting material, have been obtained using this procedure.

The 5-mercaptouracil and the disulfide prepared by the chlorosulfonation procedure are identical to those prepared from 5-aminouracil through the diazonium salt.[2] This fact confirms the assumption that chlorosulfonation results in substitution in the 5-position of the uracil nucleus.

The procedure described here has been used by us as a convenient and practical method for the synthesis of 5-mercaptouracil or the disulfide. We were, therefore, interested to read in a recent paper by Barker, *et al.*,[6] that they obtained by the chlorosulfonation of uracil and zinc reduction in "very low yields," the disulfide, and in one experiment the zinc salt of 5-mercaptouracil. The unsatisfactory results reported by these authors may have been

(1) T. J. Bardos, G. M. Levin, R. R. Herr and H. L. Gordon, THIS JOURNAL, **77**, 4279 (1955).

(2) T. J. Bardos, R. R. Herr and T. Enkoji, *ibid.*, **77**, 960 (1955).

(3) H. L. Wheeler and H. F. Merriam, *Am. Chem. J.*, **29**, 486 (1903).

(4) In the reduction of the disulfide with zinc and acetic acid previously described,[2] the product is predominantly in the form of the zinc salt.

(5) H. Meyer, "Analyse und Konstitutionermittlung Organische-Verbindungen," V. Auflage, J. Springer Verlag, Berlin, 1931, p. 631.

(6) G. R. Barker, N. G. Luthy and M. M. Dhar, *J. Chem. Soc.*, 4206 (1954).

due to the much milder reaction conditions which they used.

Acknowledgment.—We wish to thank Dr. A. S. Hussey of Northwestern University and Mr. C. W. Hoerr and P. Michalczyk of Armour and Company, Research Division, for the infrared spectra used in this work. We also wish to thank Dr. J. P. Dailey for his helpful interest during the course of this program.

Experimental[7]

5-Uracilsulfonyl Chloride.—To 11.2 g. (0.1 mole) of uracil was added 105 g. (60 cc., 0.9 mole) of practical grade chlorosulfonic acid. The temperature immediately rose to 50°. After the initial reaction subsided, the mixture was refluxed for eight hours, cooled to room temperature and carefully poured onto 500 g. of crushed ice. When the ice had melted, the precipitate was collected on a buchner funnel and washed with a small amount of water. The dried crude 5-uracilsulfonyl chloride weighed 10.4 g. This material was purified by recrystallization from glacial acetic acid, but much of it was lost in this purification process.

Anal. Calcd. for $C_4H_3ClN_2O_4S$: C, 22.8; H, 1.44; Cl, 16.8; N, 13.3; S, 15.2. Found: C, 23.3; H, 1.70; Cl, 15.3; N, 11.9; S, 15.1.

Alternatively, the sulfonyl chloride was purified by precipitation from acetone solution by the addition of benzene or *n*-hexane, but again the recovery was low.

Anal. Calcd. for $C_4H_3ClN_2O_4S$: C, 22.8; H, 1.44; Cl, 16.8; N, 13.3; S, 15.2. Found: C, 23.4; H, 1.89; Cl, 15.3; N, 11.7; S, 15.4.

5-Uracilsulfonanilide.—To 0.3 ml. of aniline in 5 ml. of 10% sodium hydroxide solution was added 0.4 g. of crude 5-uracilsulfonyl chloride. The mixture was shaken vigorously for 15 minutes. Heat was evolved. After cooling to room temperature, the mixture was filtered and the filtrate acidified with concentrated hydrochloric acid. On chilling, a white precipitate formed which was collected by filtration and dried. The 5-uracilsulfonanilide weighed 0.14 g. and melted at 263–266°. Recrystallization from water gave material which melted at 267–268°.

Anal. Calcd. for $C_{10}H_9N_3O_4S$: C, 44.9; H, 3.39; N, 15.7; S, 12.0. Found: C, 44.8; H, 3.43; N, 15.5; S, 11.8.

5-(N,N-Dimethylsulfamyl)-uracil.—One gram of crude 5-uracilsulfonyl chloride was added portionwise to 5 cc. of 40% aqueous dimethylamine. The reaction was vigorous. After standing for 10 min. the mixture was acidified with concentrated hydrochloric acid. Crystals formed on cooling. The 5-(N,N-dimethylsulfamyl)-uracil was collected by filtration and dried; weight 0.25 g. A sample recrystallized from water melted with decomposition at 340–342°.

Anal. Calcd. for $C_6H_9N_3O_4S$: C, 32.9; H, 4.14; N, 19.2; S, 14.6. Found: C, 33.0; H, 4.05; N, 19.0; S, 14.5.

Phenyl 5-Uracilsulfonate.—To 0.45 g. of phenol dissolved in 10 cc. of 5% sodium hydroxide solution was added 1.1 g. of crude 5-uracilsulfonyl chloride. The mixture was shaken, allowed to stand for 10 min., and acidified with concentrated hydrochloric acid. Crystals formed in cooling. The phenyl 5-uracilsulfonate was collected by filtration and dried; weight, 0.3 g. Recrystallization of this material from water gave a sample which melted with decomposition at 250–253°.

Anal. Calcd. for $C_{10}H_8N_2O_5S$: C, 44.8; H, 3.01; N, 10.4; S, 11.9. Found: C, 44.8; H, 2.89; N, 10.3; S, 11.8.

Reduction of 5-Uracilsulfonyl Chloride. a. Zinc Salt of 5-Mercaptouracil.—A suspension of 10.5 g. (0.05 mole) of 5-uracilsulfonyl chloride in 100 cc. of 20% aqueous sulfuric acid was cooled to 0° and 20 g. (0.3 mole) of zinc dust was added portionwise while stirring. After the addition was completed, the mixture was stirred for an hour in the ice-bath, then allowed to warm to room temperature, and finally heated gently on the steam-bath for one hour. The mixture was a bright yellow-green at this point, and unreacted zinc was present. After cooling to room temperature, the solids were collected by filtration and washed with water. The solid was dissolved in 5% sodium hydroxide solution and filtered to remove unreacted zinc. The filtrate was acidified with glacial acetic acid and the bright yellow precipitate of the zinc salt of 5-mercaptouracil was collected by filtration. The product was washed with water, then ethanol and finally dried to give 5.1 g. of the zinc salt. A sample was reprecipitated twice from sodium hydroxide solution with glacial acetic acid and dried for analysis.

Anal. Calcd. for $C_8H_6N_4S_2O_4Zn \cdot H_2O$: C, 26.0; H, 2.18; N, 15.2; S, 17.4; ash (as ZnO), 22.0. Found: C, 26.8; H, 2.28; N, 15.1; S, 18.1; ash, 24.3.

Two grams of the zinc salt was dissolved in 30 cc. of 5% sodium hydroxide under an atmosphere of nitrogen and cooled in an ice-bath and 20 cc. of a 20% solution of $Na_2S \cdot 9H_2O$ was added slowly. A precipitate of zinc sulfide formed and was separated by centrifugation. Again under an atmosphere of nitrogen, the cold supernatant liquid was acidified with concentrated hydrochloric acid. The 5-mercaptouracil crystallized and was collected by filtration. This product was recrystallized twice from hot water; weight 0.8 g. A sample was dried for analysis.

Anal. Calcd. for $C_4H_4N_2O_2S$: C, 33.3; H, 2.80; N, 19.4; S, 22.2. Found: C, 33.4; H, 2.75; N, 19.4; S, 22.4.

Two grams of the zinc salt was treated with 30 cc. of 6 *N* HCl and heated to boiling. The resultant colorless solution was cooled, and the solid which separated was collected by filtration and recrystallized twice from water to give 1.2 g. of 5-mercaptouracil. A sample was dried for analysis.

Anal. Calcd. for $C_4H_4N_2O_2S$: C, 33.3; H, 2.80; N, 19.4; S, 22.2. Found: C, 33.4; H, 2.67; N, 19.5; S, 22.3.

To a solution of 0.3 g. of 5-mercaptouracil in 15 cc. of hot water was added a solution of 0.6 g. of zinc chloride in 5 cc. of water. An immediate yellow precipitate (0.34 g.) of the zinc salt of 5-mercaptouracil formed. The infrared spectrum of this material was identical with that of the zinc salt obtained in the reduction described above.

b. 5-Uracilyl Disulfide.—A suspension of 38 g. (0.18 mole) of 5-uracilsulfonyl chloride in 400 cc. of 20% sulfuric acid was reduced with 60 g. (0.9 mole) of zinc dust as described above. In this case, however, the reduction mixture was refluxed for four hours. During this time the zinc dissolved and the bright yellow color changed to a light grayish-tan. After chilling the mixture, the solid was collected by filtration, dissolved in 5% sodium carbonate solution and filtered. The filtrate was acidified with concentrated hydrochloric acid and cooled in the refrigerator. The resultant flocculent precipitate was collected by filtration, washed with water and dried in a vacuum desiccator over sodium hydroxide to give 19.0 g. (82% of theoretical) of 5-uracilyl disulfide. This material gave a negative test with sodium nitroprusside.[5] A sample was recrystallized from water and dried for analysis.

Anal. Calcd. for $C_8H_6N_4O_4S_2$: C, 33.6; H, 2.12; N, 19.6; S, 22.5. Found: C, 33.5; H, 2.01; N, 19.7; S, 22.4.

Preparation of 5-Mercaptouracil and 5-Uracilyl Disulfide. a. 5-Mercaptouracil.—To 300 cc. (525 g., 4.5 moles) of practical grade chlorosulfonic acid was added 56 g. (0.5 mole) of uracil, and the mixture was refluxed for four hours. After cooling to room temperature, this mixture was added dropwise to a stirred suspension of 125 g. of zinc dust in 1800 cc. of water and 15 cc. of concentrated sulfuric acid. The reaction vessel was cooled in an ice-bath to maintain the temperature below 20°. After the addition was completed, 75 g. of zinc dust was carefully added (a small amount of ether was added to control the foaming) the mixture was stirred at room temperature for four hours and allowed to stand overnight. The following morning, another 50 g. of zinc dust was added and the mixture was stirred for an additional three hours. The solid was collected by filtration, dissolved in 400 cc. of 5% sodium hydroxide under an atmosphere of nitrogen, and 300 cc. of a 40% solution of $Na_2S \cdot 9H_2O$ was added slowly. The precipitated zinc sulfide was separated by centrifugation. The supernatant liquid, again under nitrogen and cooled in an ice-bath, was acidified by the dropwise addition of 12 *N* sulfuric acid. The 5-mercaptouracil which crystallized was collected by filtration, washed with a small amount of cold water, and dissolved in 400 cc. of boiling water. The resultant solu-

(7) Elemental analyses by Galbraith Laboratories, Knoxville, Tenn.

tion was filtered while hot to remove a small amount of solid, probably disulfide, which did not dissolve. The solid which separated from the filtrate on cooling was collected by filtration and dried in a vacuum desiccator to give 18.0 g. (25% of theoretical) of 5-mercaptouracil.

Anal. Calcd. for $C_4H_4N_2O_2S$: C, 33.3; H, 2.80; N, 19.4; S, 22.2. Found: C, 33.2; H, 2.56; N, 19.1; S, 22.5.

b. 5-Uracilyl Disulfide.—Fifty-six grams (0.5 mole) of uracil was chlorosulfonated and reduced as described above with the exception that only 125 g. of zinc was used and the reduction mixture was refluxed for 5 hours. The crude product was collected by filtration, precipitated from 5% sodium carbonate solution with acetic acid and recrystallized from water using a Soxhlet extractor to give 29.6 g. (41% of theoretical) of 5-uracilyl disulfide.

Anal. Calcd. for $C_8H_6N_4O_4S_2$: C, 33.6; H, 2.12; N, 19.6; S, 22.5. Found: C, 33.2; H, 2.22; N, 19.3; S, 22.7.

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[CONTRIBUTION FROM THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH]

Jervine. IX. Miscellaneous New Derivatives

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The steric course of the reduction of the 11-keto group in tetrahydrojervine (I) parallels that in normal 11-keto-steroids in that reduction with sodium and butanol leads to the 3,11α-diol II (the "β-tetrahydrojervinol" of Jacobs and Huebner),[2] in which the 11-hydroxyl group is unhindered, while with lithium aluminum hydride the hindered 11β-epimer IV is formed. Jervine itself is reduced by the latter reagent to a mixture of dihydrojervine and the unsaturated diol V in which the 11-hydroxyl group appears to be likewise β-oriented. Two, probably stereoisomeric, forms of diacetyljervine 5,6-dibromide and the 3-monoacetates of jervine, dihydrojervine and tetrahydrojervine are described.

The purpose of this paper is to place on record several new derivatives of jervine which were prepared in an early phase of our investigations (1950) but played no direct role in the elucidation of its structure. They all can be formulated readily in terms of the now accepted structure for jervine.

One of our objects at that time was to clarify the relationship between two supposedly 11-epimeric, singly unsaturated diols which Jacobs and Huebner[2] had obtained by reduction with sodium and butanol of jervine and (13,17a)-dihydrojervine, respectively. The diol obtained in this manner from jervine itself ("α-dihydrojervinol") was believed to have suffered reduction at the 11-keto group as well as at the double bond conjugated with it, that from dihydrojervine ("β-dihydrojervinol") at the keto group only. "α-Dihydrojervinol," save for the preparation of a N-acetate, was not further investigated. "β-Dihydrojervinol" formed a triacetate, showing that the new hydroxyl derived from the inert keto group was not hindered. On catalytic reduction it gave a saturated diol ("β-tetrahydrojervinol"), which could also be obtained by sodium–butanol reduction of tetrahydrojervine, and on Oppenauer oxidation an α,β-unsaturated monoketone ("Δ^4-β-dihydrojervinol").[3]

When "α-dihydrojervinol" was prepared in our laboratory, it soon became apparent that the properties and reactions of this compound were incompatible with its formulation as a singly (Δ^5)-unsaturated 3,11-diol, and that it was in fact a dienic triol in which the oxidic ring of jervine has been opened by hydrogenolysis.[4] A reinvestigation also of the "β"-series seemed then in order, the more so as it had meanwhile been shown[5] that jervine could not be a normal 11-keto-steroid. To avoid complications in the projected reoxidation to ketonic derivatives, the saturated ketone, tetrahydrojervine (I), rather than dihydrojervine, was used as the starting material. On reduction with sodium and butanol it readily afforded "β-tetrahydrojervinol" (II) as described by Jacobs and Huebner. This diol formed on acetylation with acetic anhydride and pyridine a triacetate (IIa, m.p. 175–178°, $[\alpha]^{23}D$ + 69°), behaving in this respect like "β-dihydrojervinol." The N-acetate (IIb, m.p. 257–259°, $[\alpha]^{25}D$ +84°), prepared by selective N-acetylation, was oxidized with chromic acid to the 3,11-diketone, N-acetyltetrahydrojervone (III, m.p. 267–271°, $[\alpha]^{25}D$ +27.5°), identical with the product obtained in the same manner from N-acetyltetrahydrojervine. The two specimens yielded the same mono-2,4-dinitrophenylhydrazone, m.p. 255–256°. It was clear, then, that the oxidic linkage in tetrahydrojervine, unlike that in jervine, had not been affected by the reduction with sodium, and that "β-tetrahydrojervinol" was the normal saturated diol with an unhindered 11α-hydroxyl group.

On reduction with lithium aluminum hydride tetrahydrojervine as expected yielded the 11-epimer of II, the 3β,11β-diol IV (m.p. 246.5–248.5°, $[\alpha]^{21}D$ +25°). Careful chromatographic fractionation showed that the epimer II was not present in appreciable amounts.[5a] The acetylation product of IV was amorphous, but its acetyl content and the fact that on chromic acid oxidation it gave diacetyltetrahydrojervine (Ia) left no doubt that it was the diacetate IVa in which the 11-hydroxyl group had remained unacetylated.

It is clear, therefore, that the reduction of the 11-keto group in tetrahydrojervine takes the same steric course as with normal 11-keto-steroids, in that reduction with sodium (or lithium) establishes the thermodynamically more stable unhindered α-

(1) Ciba Research Laboratories, Basel, Switzerland.

(2) W. A. Jacobs and C. F. Huebner, *J. Biol. Chem.*, **170**, 635 (1947).

(3) W. A. Jacobs and Y. Sato, *ibid.*, **175**, 57 (1948).

(4) J. Fried and A. Klingsberg, to be published.

(5) O. Wintersteiner, M. Moore, J. Fried and B. M. Iselin, *Proc. Nat. Acad. Sciences*, **37**, 333 (1951).

(5a) This is in contrast to the experience in the lithium aluminum hydride reduction of cortisone; *cf.* R. Antonucci, S. Bernstein, M. Lenhard, R. Littell and J. H. Williams, *J. Org. Chem.*, **18**, 70 (1953).