[CONTRIBUTION FROM THE CHEMOTHERAPY DIVISION, STAMFORD RESEARCH LABORATORIES, AMERICAN CYANAMID COMPANY]

Studies in Chemotherapy. XI. Oxidation of 2-Thiouracil and Related Compounds by Iodine

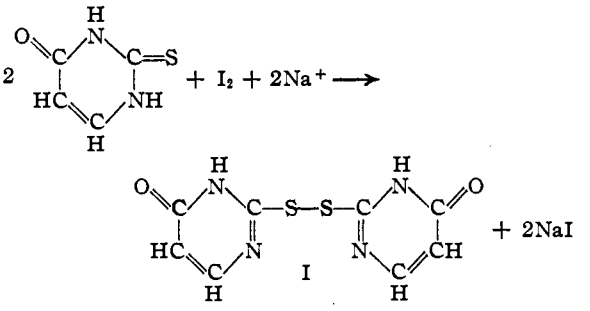
BY WILBUR H. MILLER, RICHARD O. ROBLIN, JR., AND E. B. ASTWOOD[1]

A number of thiourea type compounds and ring-substituted anilines inhibit thyroid activity in animals.[2,3] Thiourea and 2-thiouracil in particular have been used in the treatment of hyperthyroidism in man.[4] Although it has been shown that these compounds promptly block thyroid hormone formation,[2,3,5] little is known about the actual mechanism of this inhibitory effect.

A possible mode of action for the thiourea type compounds is suggested by their rapid reaction with iodine. Thyroxine synthesis can be accomplished *in vitro* by iodination of tyrosine followed by oxidation of the diiodotyrosine.[6] Iodination of proteins has also been shown to lead to the formation of thyroxine.[7] If similar reactions take place in the thyroid gland, 2-thiouracil and related compounds might effectively reduce the iodine liberated there before it could iodinate the specific thyroid protein and thus prevent the formation of the hormone. Although some type of reaction with iodine has been suggested as an explanation for the antithyroid activity of thiourea[8,9] and the thiouracils,[10,11] no experimental evidence has been provided for this hypothesis with respect to 2-thiouracil. Furthermore, a proper distinction has not been made between possible reactions of these compounds with the small amounts of iodine liberated within the thyroid gland and postulated reactions with iodide ion. Rapid interaction between iodine and 2-thiouracil has been considered to be the mechanism by which the exchange between radioiodide and diiodotyrosine was inhibited.[12]

The interaction of thiourea and iodine was studied extensively by Werner[13] who found that an equilibrium existed between thiourea and the reaction product, formamidine disulfide hydroiodide, in acid solution. The free disulfide was too unstable to isolate, neutralization of the acid solution causing an immediate precipitation of sulfur. Other decomposition products were thiourea and cyanamide.

The nature of the reaction of thiouracils with iodine has not been described previously. We have found that equivalent quantities of 2-thiouracil and iodine react rapidly in 0.5 *M* bicarbonate buffer (*p*H 7.4) or alkaline solutions to yield the disulfide of 2-thiouracil.



Unlike the products from thiourea[13] and ethylene thiourea[14] which are basic, I is an acid which can be isolated from the reaction mixture as the disodium salt by acetone precipitation. This salt yielded over 90% of 2-thiouracil when reduced by sodium bisulfite in alkaline solution. When a 0.03 *M* solution of the disodium salt of I is acidified, a solid separates from which 2-thiouracil can be isolated in an amount equivalent to about 45% of the free disulfide.

The instability of the free acid, I, was confirmed in titration experiments (see Experimental) and in ultraviolet absorption spectra studies as indicated in Fig. 1. At *p*H 3.4, a change of absorption spectra with time indicated the rapid decomposition of the disulfide, I. Using the final absorption (after fifteen minutes) and the time rate of change of absorption at a single wave length (270 mμ), the decomposition was found to follow a first order reaction for at least the first eight minutes, the calculated half life of I being 220 seconds. Comparing curves 1 and 2 with 4 and 5, and assuming that the shift in the latter is due chiefly to 2-thiouracil, the regenerated 2-thiouracil is equivalent to 40–60% of the original disulfide. Slight decomposition of the disodium salt of I occurred on standing at room temperature for three hours in a solution of *p*H 11.6.

Reaction of the isomeric 4-thiouracil with iodine at *p*H 6.8 resulted in the nearly quantitative separation of a free disulfide, which was soluble in

(1) Guest Investigator, Summer 1943.

(2) Mackenzie and Mackenzie, *Endocrinology*, **32**, 185 (1943).

(3) Astwood, Sullivan, Bissell and Tyslowitz, *ibid.*, **32**, 210 (1943); Astwood, *J. Pharmacol.*, **78**, 79 (1943).

(4) Astwood, *J. Am. Med. Assoc.*, **122**, 78 (1943); Williams and Bissell, *Science*, **98**, 156 (1943); Himsworth, *Lancet*, **II**, 465 (1943).

(5) Astwood and Bissell, *Endocrinology*, **34**, 282 (1944).

(6) Harington, *J. Chem. Soc.*, 193 (1944).

(7) (a) Ludwig and von Mutzenbecker, *Z. physiol. Chem.*, **258**, 195 (1939); (b) Reineke and Turner, *J. Biol. Chem.*, **149**, 555, 563 (1943).

(8) Baumann, Metzger and Marine, *Endocrinology*, **34**, 44 (1944).

(9) Campbell, Landgrebe and Morgan, *Lancet*, **I**, 630 (1944).

(10) Williams, Weinglass and Kay, *Am. J. Med. Sci.*, **201**, 701 (1944).

(11) Chapman, *Quart. J. Pharm. Pharmacol.*, **17**, 314 (1944).

(12) Miller, Anderson, Madison and Salley, *Science*, **100**, 340 (1944).

(13) Werner, *J. Chem. Soc.*, **101**, 2166 (1912).

(14) Johnson and Edens, THIS JOURNAL, **64**, 2706 (1942).

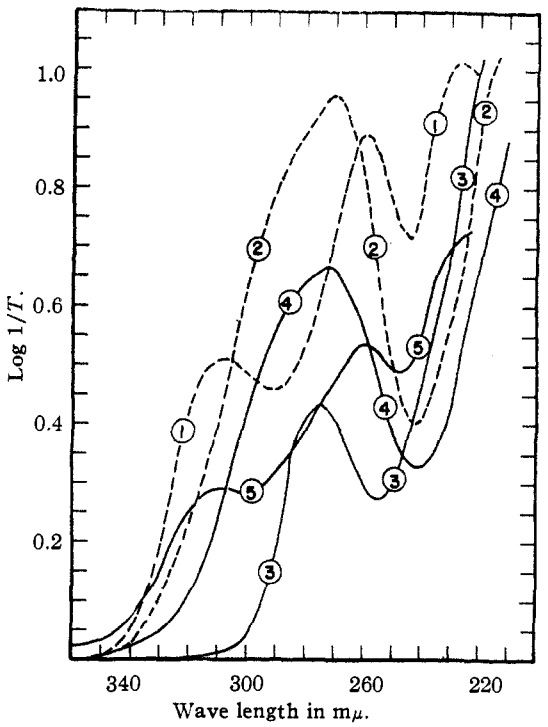


Fig. 1.—Ultraviolet absorption spectra of 2-thiouracil and its disulfide under varying *p*H conditions. thiouracil (10.2 mg./liter. 0.05 *M* phosphate buffer): Curve 1. *p*H 11.6: Curve 2. *p*H 7.5 (curve nearly the same at *p*H 3.4). Disodium salt of thiouracil disulfide monohydrate (11.2 mg./liter. 0.05 *M* phosphate buffer): Curve 3. *p*H 11.6; Curve 4. *p*H 3.4. after standing fifteen minutes; Curve 5. *p*H 11.6. after standing fifteen minutes at *p*H 3.4: Beckmann Model DU ultraviolet spectrophotometer.

alkaline solution. The free disulfide reacted very slowly with iodine in excess of one equivalent at *p*H 6.8, probably because of low solubility.

In contrast to the behavior of 4-thiouracil, 2-thiouracil and other thiourea type compounds reacted rapidly with several equivalents of iodine (Table I). Although a total of eight to ten hours was allowed for the reaction, in most cases the first 4 to 5 equivalents of iodine reacted within fifteen minutes. The extent of the reaction of these compounds with iodine may vary with the concentration as illustrated by 2-mercaptothiazoline (Table I). This compound takes up a greater amount of iodine, due probably to the reactivity of the breakdown products.[15]

Since Werner has shown that thiourea reacts readily with iodine in excess of one equivalent,[13] these observations were not unexpected. However, such results were apparently not considered in relation to the antithyroid action of this class of compounds.[8,9] The reaction with iodine is not the only factor to be considered, since inactive compounds such as 6-amino-2-thiouracil and 5-carbethoxy-2-thiouracil are equally reactive toward iodine. It is conceivable that some inactive compounds are oxidized by enzyme systems in the body and thus may not reach the thyroid gland in effective concentrations. Other factors such as solubility and distribution in body fluids and tissues might greatly modify *in vivo* effectiveness.

(15) Gabriel, *Ber.*, **22**, 1152 (1889).

TABLE I

THE REACTIVITY OF THIO COMPOUNDS TOWARD IODINE[a]

Compound	Antithyroid activity	Concn., mg./100 cc.	Equivalents of iodine
Thiourea	0.10	32	5.3
N.N'-Dimethyl thiourea	0.10	33	6.5
Tetramethyl thiourea	0.30	32	7.6
Ethylene thiourea	0.40	34	6.6
2-Thiouracil	1.00	29	7.7
6-Amino-2-thiouracil	0.001	30	6.0
5-Carbethoxy-2-thiouracil	<0.001	40	7.7
2-Mercaptothiazoline	1.3	34.5	8.6
		16.2	9.7
2-Mercaptoöxazoline	0.2	16.7	4.1

[a] 0.25 *M* Sörensen phosphate buffer. *p*H 6.8–7.0.

Under comparable conditions, the ring-substituted anilines were much less reactive toward iodine than the thiourea type compounds. Sulfanilamide, sulfaguanidine, sulfadiazine and *p*-aminobenzoic acid reacted with only a fraction of one equivalent in eight hours. Thus, although iodine reduction is a possible mechanism of action of thiourea type compounds, the aminobenzene derivatives presumably interfere with thyroid hormone synthesis in some other way.

If thiourea type compounds block the synthesis of thyroid hormone by reacting with liberated iodine, they must compete successfully for this iodine with hormone precursors. When equimolecular quantities of tyrosine and 2-thiouracil in alkaline solution were treated with one equivalent of iodine, the disodium salt of I was obtained. Only iodine-free tyrosine could be isolated from the supernatant liquid. Similarly, 2-thiouracil inhibited the iodination of casein (Table II). When the concentration of the antithyroid compound was varied with respect to iodine, the inhibition exceeded that anticipated from a reaction of 2-thiouracil with only one equivalent of iodine. Further, it is seen that once the casein is iodinated, thiouracil does not influence the iodine content of the protein.

TABLE II

EFFECT OF 2-THIOURACIL ON THE IODINATION OF CASEIN[a]

Expt.	Equiv. of thiouracil[b]	% Iodine content of the protein
1	0	3.4
2	0	2.4
3	0.18	1.1
4	.36	0.3
5	.72	.02
	.72	.08
6	.72[c]	3.9

[a] In 0.7% sodium bicarbonate solution. [b] Based on the iodine used. [c] Added four hours after the iodine.

These results indicate that reaction with iodine is a plausible explanation for the action of thiourea type antithyroid compounds. These compounds may also decrease iodine liberation by action on the appropriate oxidative enzymes. Thiourea[16] and sulfanilamide,[17] representatives of the two types of antithyroid compounds, do inhibit peroxidase action and the activity of the second type might be explained by such an effect.

Experimental[18]

Preparation of the Disulfide Disodium Salt of 2-Thiouracil (Alkaline Solution).—Twelve and eight-tenths grams (0.1 mole) of 2-thiouracil was dissolved in 200 cc. of *N* sodium hydroxide solution and then chilled in ice. One hundred cc. of aqueous *N* iodine solution (containing potassium iodide in the proportion of 2 parts potassium iodide to 1 part of iodine by weight) was added rapidly with constant stirring. The solution quickly became colorless and was diluted to the point of cloudiness with several volumes of acetone. Large flaky white crystals were deposited slowly. These were filtered, washed with acetone, and dried at 70°. Water of crystallization was evolved during the drying and 13–15 g. of product resulted. The product was recrystallized three times from water–acetone mixtures, dried in the oven at 70° and finally for five hours at 100°. The sodium salt was hygroscopic, dissolving in water with the evolution of heat. Analysis indicated that even after prolonged drying at 100°, the compound was the monohydrate of disodium 2-thiouracil disulfide. However, drying at 110° removed the water of hydration as indicated by the sulfur analysis.

Anal. Calcd. for $C_8H_4N_4O_2S_2Na_2H_2O$: C, 30.4; H, 1.9; N, 17.7; Na, 14.5. Found: C, 30.0, 29.8; H, 2.0, 2.1; N, 17.6, 17.6; Na, 14.6, 14.4. Calcd. for $C_8H_4N_4O_2S_2Na_2$: S, 21.5. Found: S, 21.2, 21.1.

Ultraviolet spectroscopic examination of the buffered solutions of the sodium salt of 2-thiouracil and of the disodium 2-thiouracil disulfide showed that the disulfide sample contained at the most 2% of 2-thiouracil (based on the extinction at 3100 Å. and *p*H 11.6).

Reaction of 2-Thiouracil with Iodine in Neutral Solution.—To determine whether a similar reaction would occur at the *p*H of body tissues, similar experiments were carried out in an excess of bicarbonate buffer. Twelve and eight-tenths grams (0.1 mole) of 2-thiouracil was suspended as a fine powder in 200 cc. of 5% sodium bicarbonate and the *p*H adjusted to 7.4 with carbon dioxide. One hundred cc. of *N* iodine solution was added slowly with constant stirring over a period of five to ten minutes. As the reaction proceeded the thiouracil dissolved, the iodine was decolorized, and carbon dioxide was liberated. The final *p*H was 6.8. The reaction product isolated as above by dilution of the solution with acetone weighed 13 g. and was largely disodium 2-thiouracil disulfide monohydrate.

Reduction of Disodium 2-Thiouracil Disulfide by Sodium Bisulfite.—Three and sixteen-hundredths grams (0.01 mole) of disodium 2-thiouracil disulfide monohydrate was dissolved in 15 cc. of water to give a solution of *p*H 9.3. The addition of 1.25 g. (0.012 mole) of sodium bisulfite in 10 cc. of water to the stirred solution caused some 2-thiouracil to precipitate immediately. The precipitate increased when carbon dioxide was bubbled through the mixture for one and one-half hours. After filtration and washing of the solid on the filter, 1.45 g. of white powder was obtained. The combined filtrate and washings were evaporated *in vacuo* at 40–45° and the residue dissolved in 15 cc. of water. The solution was acidified with *N* sulfuric acid to *p*H 2, cooled, filtered and the precipitate was washed to yield 1.0 g. of white 2-thiouracil. Melting points taken simultaneously with a recrystallized sample of 2-thiouracil (m. p. 315–316° dec.) were: 1.45 g. dec. 315–316°; 1.0 g. dec. 312–313°. Mixed melting points with 2-thiouracil were not depressed in either case; 2.45 g. corresponds to 96% recovery of 2-thiouracil.

Preparation of the Disulfide of 4-Thiouracil.—4-Thiouracil (0.319 g., 0.0025 mole) was dissolved by warming with 50 cc. of water and 75 cc. of 0.25 *M* Sörensen phosphate buffer, *p*H 6.8. Iodine solution equivalent to 1.02 mole of 4-thiouracil (2.56 cc. of 0.98 *N* iodine) was added forming an intermediate dark blue complex which changed quickly to a white precipitate. The last 10% of the iodine was taken up slowly. The solution was filtered after being cooled for one hour in an ice-bath. About 0.3 g., softening at 222° cor. and decomposing at 251° cor., was obtained. To recrystallize, 0.1 g. was suspended in 25 cc. of water and ammonium hydroxide—about 10 drops— added for solution. This was treated with Norit A at room temperature for a few minutes and filtered. The yellow filtrate was acidified with glacial acetic acid and yielded a white, flocculent precipitate which, after drying at 60°, decomposed at 216–223° cor. After one more recrystallization, the sample decomposed slightly at 218° and more completely at 221–223° (cor.).

Anal. Calcd. for $C_8H_6N_4O_2S_2$: C, 37.8; H, 2.4; S, 25.2. Found: C, 37.8, 37.7; H, 2.6, 2.4; S, 25.2, 25.5; satisfactory nitrogen analyses were not obtained due to difficulty in burning the sample.

Determination of Equivalent Weights.—Samples of disodium 2-thiouracil disulfide monohydrate of about 0.1 g. were dissolved in 100–125 cc. of distilled water previously boiled and cooled to room temperature with nitrogen bubbling into the water. The solutions were stirred and titrated with 0.1 *N* hydrochloric acid or sodium hydroxide. Measurements were made with the Beckmann *p*H meter. The results varied with the time taken for titration since an equivalent weight of as little as 159 was observed during a fifteen-minute period while a maximum value of 182 was found for fifty minutes and beyond. In the shorter time interval it was possible to back-titrate partially with alkali before any breakdown products settled out of solution. The curve so obtained differed from the acid titration curve and indicated a shift from a stronger to a weaker acid. This could be explained if 2-thiouracil were a product of the disulfide breakdown.

Ultraviolet Absorption.—Solutions of known concentration (about 10 mg. per liter) were prepared by dissolving samples in 0.05 *M* phosphate buffer adjusted to *p*H 11.6. The amount of acid necessary to bring this solution to a given *p*H was pre-determined so that *p*H changes could be made quickly and the reaction followed immediately using the Beckmann spectrophotometer. At the end of the reaction, the *p*H was checked with the Beckmann *p*H meter.

Reaction of Thio Compounds with Several Equivalents of Iodine.—Samples of thio compounds (16 to 100 mg.) weighed to the nearest 0.1 mg. were dissolved in about 100 cc. of 0.25 *M* Sörensen phosphate buffer, *p*H 6.8. These compounds were titrated with *N* iodine, the triiodide solution being added in portions over a period of eight hours so that no large excess of iodine remained when the reaction was completed. Although usually the first equivalents of iodine were added one every fifteen minutes until color remained in solution, those compounds reactive toward several equivalents of iodine could react with the first four to five in fifteen minutes. In some cases, it was necessary to add sodium hydroxide solution to bring the *p*H back to 6.8. The total number of equivalents added was finally estimated by titration of any excess iodine with sodium thiosulfate.

Attempted Iodination of Tyrosine in the Presence of 2-Thiouracil.—Ten millimoles each of tyrosine and 2-thiouracil were dissolved in 20 cc. of *N* sodium hydroxide. Ten cc. of *N* iodine solution was added quickly with stirring. The disodium 2-thiouracil disulfide monohydrate was isolated in the usual manner from the solution by acetone precipitation. The tyrosine, isolated from the

(16) Sumner and Somers, "Chemistry and Methods of Enzymes," Academic Press, New York, N. Y., 1943.

(17) Lipmann, *J. Biol. Chem.*, **139**, 977 (1941).

(18) Microanalyses were carried out in these Laboratories under the direction of Dr. J. A. Kuck.

supernatant fluids and purified by recrystallization, was found to contain no iodine.

Iodination of Casein.—The method was that of Reineke and Turner[7b] except that a solution of iodine was used instead of powdered iodine. To 1-g. portions of casein in 40 cc. of 0.7% sodium bicarbonate solutions containing 0, 0.00025, 0.0005 and 0.001 equivalent of 2-thiouracil was added 0.0014 equivalent of iodine in the form of a 0.28 *N* solution (in aqueous potassium iodide). One fifth of the iodine solution was added slowly every fifteen minutes with stirring which was continued for a total of four hours. To prevent oxidation of iodide by the 2-thiouracil disulfide present, acidification was avoided and the casein was precipitated with 2 *M* ammonium sulfate. After washing the protein free of iodide it was further purified by isoelectric precipitation followed by drying with acetone. Iodine determinations[5] on the purified protein gave the values shown in Table II.

Acknowledgment.—The measurement and interpretation of the ultraviolet spectral data were made by Paul H. Bell and J. Foster Bone of the Chemotherapy Division.

Summary

A study of the reaction between iodine and 2-thiouracil and related compounds has been carried out. The disulfides of the isomeric 2-thiouracil and 4-thiouracil were obtained by iodine oxidation. The former compound was very unstable and was isolated only as the disodium salt while the latter was obtained as the stable free acid.

The mercapto compounds have been found to react with several equivalents of iodine at *p*H 6.8. In the case of 2-thiouracil, this reaction rate was such that tyrosine and casein were protected against iodination by this compound. These facts support the hypothesis that the thio compounds may prevent thyroid hormone synthesis in the gland by blocking the iodination of hormone precursors.

STAMFORD, CONN. RECEIVED AUGUST 29, 1945

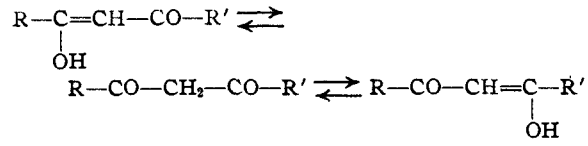
[CONTRIBUTION FROM THE THOMPSON LABORATORY OF THE PHILLIPS EXETER ACADEMY]

The Alkaline Cleavage of Unsymmetrical β-Diketones

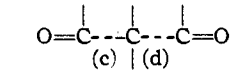
BY CHARLES L. BICKEL

β-Diketones are cleaved both by bases[1,2] and by acids.[2,3] Unsymmetrical β-diketones, R—CO—CH_2—CO—R′, can be cleaved in either of two ways and an analysis of the products of fission can be used to find the direction of the cleavage.

β-Diketones with terminal aryl groups are highly enolic and the solution of an unsymmetrical diaryl β-diketone may consist of an equilibrium mixture of the two enolic forms and the diketonic form



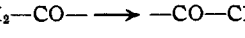
It is therefore possible that the direction of cleavage is determined by the proportion of the two enolic forms present in the mixture. Bradley and Robinson,[1] using aqueous sodium hydroxide, concluded (1) that the direction of cleavage is determined by the strength of the two possible aryl acids, the stronger acid being formed in the larger proportion, and (2) that the direction of enolization is not a factor in the fission. However, Adkins and co-workers,[2,3] using alcoholic hydrogen chloride, concluded that the acid cleavage is probably directed by the enolic forms and that the strength of the two acids has little to do with the course of the reaction. Most significant, however, is the final conclusion of Adkins,[4] based on a great deal of evidence, that "the relative lability of the two bonds (c) and (d)



in an unsymmetrical diketone seems to be approximately the same irrespective of whether the reaction is alcoholysis, hydrolysis or hydrogenolysis."

Bradley and Robinson's conclusions were based on the analysis of the mixture of the two aryl acids formed by alkaline cleavage. As will be shown in the experimental section of this paper, a rather large quantity of sodium chloride was present as an impurity in the mixtures which they analyzed. In consequence, their analytical results were in error and the validity of the conclusions based on these results is open to question. It therefore seemed advisable to check Bradley and Robinson's results[5] and also to attack the problem in a different way.

When one of the central hydrogen atoms of a β-diketone is replaced by an alkyl group, for example



the enolization is almost completely suppressed. Under the ordinary conditions, these alkylated compounds give no coloration with ferric chloride and do not yield copper salts.[6,7]

This paper is concerned with the alkaline cleavage of certain diaryl β-diketones and their methyl

(1) Bradley and Robinson, *J. Chem. Soc.*, **129**, 2356 (1926).
(2) Kutz and Adkins, THIS JOURNAL, **52**, 4036, 4391 (1930).
(3) Adkins, Kutz and Coffman, *ibid.*, **52**, 3212 (1930).
(4) Sprague and Adkins, *ibid.*, **56**, 2675 (1934).
(5) In the experimental part of this paper.
(6) Kohler, Tishler and Potter, THIS JOURNAL, **57**, 2518 (1935).
(7) Sprague and Adkins, *ibid.*, **56**, 2672 (1934).