[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF COLORADO]

The Synthesis of 5-Halogeno-2-thiouracil and 6-Methyl-5-halogeno-2-thiouracil Derivatives[1]

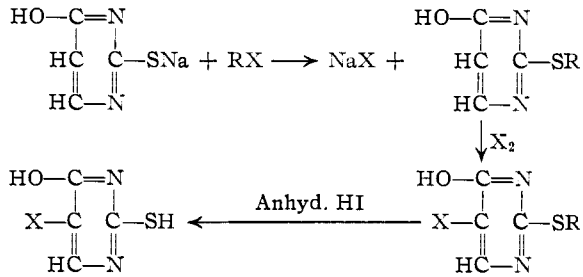
BY HAROLD W. BARRETT,[2] IRVING GOODMAN AND KARL DITTMER

Following Astwood's discovery in 1943 of the relatively high antithyroid activity and low toxicity of 2-thiouracil,[3] numerous derivatives of this compound were prepared and tested for physiological activity.[4-6] In general, it has been found that substitution on either the sulfur[4] or the nitrogen[5] of the molecule decreased or destroyed the antithyroid potency of the parent compound, while substitution of a small alkyl group in either the 5- or the 6-position enhanced such activity, as did substitution of a benzyl, phenethyl or thenyl group in the 6 position.[4,6] A large variety of other substituents in either of these positions, including saturation of the 5,6-double bond, diminished the activity so that the resulting compound was no longer useful as an antithyroid agent.

Clinically, the more highly active derivatives have not corrected the basic defects of 2-thiouracil itself; they frequently provoke toxic reactions which limit or preclude their use, and also induce thyroid hyperemia and friability consequent to blocking hormone synthesis. Attempts to overcome these defects by simultaneous administration of iodine,[7,8] thyroxine,[8] folic acid,[9,10] or various other vitamins[11] have not been uniformly successful; hence there has been a continuing search for new types of derivatives for both research and clinical purposes.

In view of the effect of 5-substitution on the activity of thiouracil, it seemed of interest to prepare the 5-halogeno-2-thiouracils, and to test their physiological action. It seemed especially desirable to obtain the 5-iodo derivative which would permit the simultaneous administration of an organic iodide and a possible antithyroid compound. In this paper, we wish to report the synthesis of 5-chloro, 5-bromo, and 5-iodo-2-thiouracil, and the corresponding halogeno derivatives of 6-methyl-2-thiouracil. The antithyroid potency of three of these halogenated derivatives was compared with that of 2-thiouracil using the rat as the assay animal. Assigning an arbitrary value of 100% to 2-thiouracil, the relative potencies of the 5-chloro-, 5-bromo-, and 5-iodo compounds in producing increased thyroid weights were +125, −2 and +35%, respectively. The relative potencies in producing decreased thyroid iodine levels were 86, 68 and 66%, respectively. The detailed results of these physiological tests will be reported later.

The synthesis of these derivatives is summarized by the following steps



5-Halogeno-2-thiouracil

Although Johnson and Johns[12] prepared the 5-bromo derivative of 6-amino-4-oxy-2-mercaptopyrimidine by direct bromination of the pyrimidine in glacial acetic acid, it appears that 2-thiouracil can be halogenated only when the sulfur is blocked by an alkyl or aryl group. Attempts to obtain the halogenated derivatives by condensation methods or by halogenation of the unsubstituted molecule failed; and, although 2-thiouracil will react with 7.7 equivalents of iodine in neutral or alkaline solution,[13] no iodine can be found in the product following purification. The 2-thiocyanate (m. p. 148–149° with decomposition) was formed by adding an alcoholic solution of cyanogen bromide to an aqueous solution of sodium thiouracil, but this derivative could not be brominated in the ring. Attempts were made to form the S-benzoyl and the S-sulfonyl derivatives by the Schotten–Baumann method, but the products were too unstable to isolate.

In preliminary work with the S-alkyl derivatives, a sample of 5-bromo-2-ethylthiouracil was prepared by the method of Wheeler and Johnson,[14] and treated with dry hydrogen chloride to remove the ethyl group.[15] However, this compound slowly decomposed at the melting point, and when the hydrogen chloride was passed through for a few minutes, only a red tar remained. The same result was obtained with the analogous methyl

(1) This work was supported in part by a research contract with the Office of Naval Research.

(2) Present address: Department of Chemistry, Colorado A. & M. College, Fort Collins, Colorado.

(3) Astwood, *J. Pharmacol. Exptl. Therap.*, **78**, 79 (1943).

(4) Astwood, Bissell and Hughes, *Endocrinology*, **37**, 456 (1945).

(5) Bywater, McGinty and Jenessel, *J. Pharmacol. Exptl. Therap.*, **85**, 14 (1945).

(6) Anderson, Halverstadt, Miller and Roblin, THIS JOURNAL, **67**, 2197 (1945).

(7) Christian, *Med. Clinics N. Am.*, **30**, 283 (1946); from "Yearbook of General Therapeutics," 1946, p. 350.

(8) Danowski, Mann and Winkler, *Am. J. Med. Sci.*, **210**, 777 (1945).

(9) Goldsmith, Gordon, Finkelstein and Charipper, *J. Am. Med. Assoc.*, **125**, 847 (1944).

(10) Newman and Jones, *ibid.*, **132**, 77 (1946).

(11) Fishberg and Vorzimer, *ibid.*, **128**, 915 (1945).

(12) Johnson and Johns, *Am. Chem. J.*, **34**, 186 (1905).

(13) Miller, Roblin and Astwood, THIS JOURNAL, **67**, 2201 (1945).

(14) Wheeler and Johnson, *Am. Chem. J.*, **31**, 591 (1904).

(15) Wheeler and Liddle, *ibid.*, **40**, 537 (1908).

derivative. A quantity of 2-benzylthiouracil was prepared[15] and brominated in the same manner as the ethyl derivative to yield 5-bromo-2-benzylthiouracil (m. p. 184–185° with decomposition). This compound could be partially debenzylated, but with considerable decomposition, by passing dry hydrogen chloride through a tube of the crystals at 120° or by dissolving the compound in glacial acetic acid and passing dry hydrogen bromide through the solution which was kept near the boiling point. An attempt was made to split this derivative with cyanogen bromide[16] in glacial acetic acid, but little reaction took place even when the mixture was heated to 90–100° in a sealed tube.

Finally it was determined that 5-bromo-2-benzylthiouracil, as well as the corresponding S-alkyl derivatives, could be split to give fair yields of the desired 5-halogeno-2-thiouracils by dissolving the intermediate in glacial acetic acid and treating the solution with dry hydrogen iodide at the appropriate temperature. Since a controlled flow of hydrogen iodide gas was difficult to obtain, the splitting was originally carried out by adding 50% hydriodic acid to a large excess of acetic acid–acetic anhydride mixture, and adding the resulting solution dropwise to the hot glacial acetic acid solution of the pyrimidine. Upon subsequent cooling, a part of the final product precipitated out, and the remainder was obtained by evaporating the excess solvent under reduced pressure. During the preparation of a larger quantity of 5-iodo-2-thiouracil the splitting reaction with anhydrous hydrogen iodide was greatly improved by carrying out this reaction in the apparatus illustrated in Fig. 1. This apparatus provides a convenient method for the preparation of anhydrous hydrogen iodide from 50% hydriodic acid without the possibility of introducing water into the reaction mixture, and also permits working with much less solvent. This technique was used in the preparation of 5-iodo-2-thiouracil and 6-methyl-5-iodo-2-thiouracil in better yields than was obtained by the method employed for the other halogeno compounds.

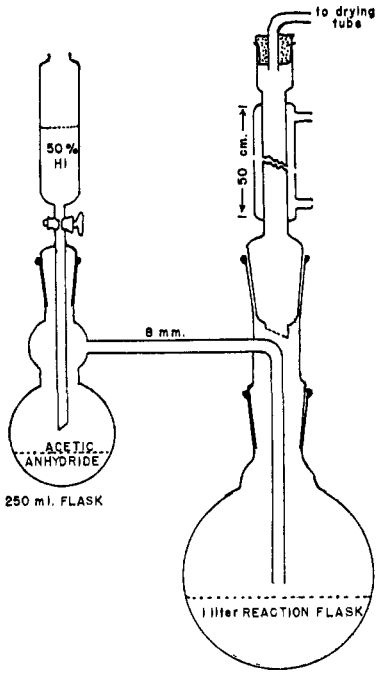


Fig. 1.—The apparatus used for the preparation of anhydrous hydrogen iodide.

Although bromine was very readily introduced in the 5 position of the S-alkyl derivatives of 2-thiouracil, the same was not true of chlorine. When an acetic acid solution of the pyrimidine was treated with chlorine, it appeared that either a hydrochloride salt[14] or a sulfonium chloride was formed,[17] and when the product was taken up in water it decomposed to form uracil and a mercaptan. It was found necessary to use a ferric chloride catalyst with heating to introduce the chlorine into the 5-position. The product was then taken up in a water–pyridine mixture to avoid decomposition.

Experimental[18]

2-Methylthiouracil.—Wheeler and McFarland[19] prepared 2-methylthiouracil by the action of methyl iodide on 2-thiouracil in a solution of absolute alcohol and sodium alcoholate. It was found more convenient to prepare the intermediate in the manner described here.

A mixture of 12.8 g. of 2-thiouracil and 4.3 g. of sodium hydroxide was placed in a 500-ml. Erlenmeyer flask, and dissolved on the steam-bath with a minimum amount of water. Twice the volume of 95% alcohol was then added, the solution cooled to about 30°, and 6.3 ml. of methyl iodide added. The solution was reheated to 50–60° for twenty minutes, then cooled to room temperature. The precipitate was filtered off, and, after acidifying the filtrate with acetic acid, the excess solvent was removed *in vacuo*. The combined precipitates were thoroughly washed with water and recrystallized from alcohol to give a final yield of 9.0 g. (63% of the theoretical yield) of 2-methylthiouracil, capillary m. p. 198°.

5-Chloro-2-methylthiouracil.—Nine grams of 2-methylthiouracil was dissolved in an excess of glacial acetic acid containing 5% acetic anhydride to remove any moisture. A trace of ferric chloride was added as catalyst. The solution was then treated with a 20% excess of chlorine in carbon tetrachloride. The solution became warm and the temperature was maintained at 50–60° until most of the hydrogen chloride fumes were evolved. After cooling, a small amount of precipitate formed; more was obtained when the filtrate was evaporated *in vacuo* to a small volume. The combined precipitates were taken up in excess aqueous pyridine, and the solvent allowed to evaporate at room temperature. The residue was taken up in water, acidified with glacial acetic acid, filtered, and washed several times with water. After several recrystallizations from alcohol the final yield of 5-chloro-2-methylthiouracil was 2.2 g. (20%), m. p. 258–260°.

Anal. Calcd. for $C_5H_5N_2OSCl$: Cl, 20.08. Found: Cl, 20.02.

5-Chloro-2-methylthiouracil was obtained in the same yield when the chlorination was carried out by adding a

(16) Braun and Englebertz, *Ber.*, **56**, 1573 (1923).

(17) Fromm and Raiziss, *Ann.*, **374**, 90 (1910).

(18) All melting points reported in this paper, unless otherwise indicated, were determined on a Dennis melting point bar.

(19) Wheeler and McFarland, *Am. Chem. J.*, **42**, 101 (1909).

20% excess of sulfuryl chloride to the acetic acid–acetic anhydride solution of 2-methylthiouracil, with ferric chloride as the catalyst.

5-Chloro-2-thiouracil.—Two and two-tenths grams of 5-chloro-2-methylthiouracil was dissolved in 100 ml. of glacial acetic acid containing 20% acetic anhydride. This solution was placed in a round bottom flask provided with a ground glass joint and reflux condenser with a funnel at the top. While the solution was kept at its boiling temperature, a mixture consisting of 3.5 ml. of 50% hydriodic acid (specific gravity 1.5), 60 ml. of glacial acetic acid, and 20 ml. of acetic anhydride was added dropwise through a reflux condenser. Heating was continued for an hour after all the hydriodic acid was added. Crude 5-chloro-2-thiouracil precipitated on cooling, and more was obtained when the remaining solution was concentrated to a small volume. The combined precipitates were taken up in dilute ammonium hydroxide and heated until solution was complete. The hot solution was acidified with acetic acid, cooled and the 5-chloro-2-thiouracil collected on the filter. It was recrystallized first from alcohol and then from water to yield 1.5 g. (72%) of pure 5-chloro-2-thiouracil, m. p. 264–270° with decomposition.

Anal. Calcd. for $C_4H_3N_2OSCl$: Cl, 21.80; N, 17.23; S, 19.71. Found: Cl, 21.90; N, 17.54; S, 19.52.

6-Methyl-5-chloro-2-ethylthiouracil.—A sample of 6-methyl-2-ethylthiouracil was prepared by the method of Johns,[20] and chlorinated in glacial acetic acid and acetic anhydride as described for the preparation of 5-chloro-2-methylthiouracil. A 10-g. sample of 6-methyl-2-ethylthiouracil was chlorinated and the product recrystallized from alcohol. A 24% yield of 6-methyl-5-chloro-2-ethylthiouracil, m. p. 188–190°, was obtained.

Anal. Calcd. for $C_7H_9N_2OSCl$: Cl, 17.32. Found: Cl, 17.20.

6-Methyl-5-chloro-2-thiouracil.—The 6-methyl-5-chloro-2-ethylthiouracil was split and the product isolated in the same manner as has been described for the preparation of 5-chloro-2-thiouracil, except that after all the hydriodic acid was added the reaction mixture was vigorously boiled for two hours. The yield from 10 g. of 6-methyl-5-chloro-2-thiouracil was 50–60%. The 6-methyl-5-chloro-2-thiouracil had a m. p. of 265–270° with decomposition.

Anal. Calcd. for $C_5H_5N_2OSCl$: Cl, 20.08; N, 15.86. Found: Cl, 20.18; N, 15.52.

6-Methyl-5-chloro-2-isopropylthiouracil.—6-Methyl-2-isopropylthiouracil, m. p. 155°, was prepared in the same manner as the S-ethyl analog, and upon chlorination gave a 36% yield of 6-methyl-5-chloro-2-isopropylthiouracil, m. p. 162–163°.

Anal. Calcd. for $C_8H_{11}N_2OSCl$: Cl, 16.21. Found: Cl, 16.39.

Unfortunately, the isopropyl group was so difficult to remove that this derivative was not used further.

5-Bromo-2-methylthiouracil.—The intermediate 5-bromo-2-methylthiouracil was prepared by the method described by Wheeler and Johnson[14] for the preparation of the S-ethyl analog. It is best to repeat the bromination, since some unreacted material precipitates out during the first bromination. Fourteen grams of 2-methylthiouracil was dissolved in glacial acetic acid containing 5% acetic anhydride. To this solution was added 7.3 ml. of bromine in 15 ml. of glacial acetic acid. The precipitated product was filtered off, washed with glacial acetic acid and suspended in hot glacial acetic acid. To this suspension was added 1 ml. of bromine in 5 ml. of glacial acetic acid. The product was collected on the filter, washed with glacial acetic acid and recrystallized from ethyl alcohol. The yield of 5-bromo-2-methylthiouracil was 17.4 g. (80%), m. p. 255°. When heated in a capillary it turned yellow at 205°, red at 217°, and decomposed completely to a red liquid at 219°.

Anal. Calcd. for $C_5H_5N_2OSBr$: Br, 36.15. Found: Br, 36.07.

5-Bromo-2-thiouracil from the S-Methyl Intermediate.—The methyl group was removed by hydriodic acid, and the 5-bromo-2-thiouracil isolated, in the same way as described for the chlorinated derivative. Yields obtained, when 10 g. of 5-bromo-2-methylthiouracil was split, varied from 30–49%. The 5-bromo-2-thiouracil crystallized out of either water or alcohol as long, colorless prisms, m. p. 270° with decomposition; when heated slowly in a capillary it turned brown around 170° and decomposed to a red liquid near 200°. As with the chloro derivative, it is necessary to identify this compound and verify its purity by analysis.

Anal. Calcd. for $C_4H_3N_2OSBr$: Br, 38.59; N, 13.53; S, 15.48. Found: Br, 38.70; N, 13.44; S, 15.30.

5-Bromo-2-thiouracil from the S-Benzyl Intermediate.—5-Bromo-2-thiouracil was prepared from 5-bromo-2-benzylthiouracil in the same manner as from the S-methyl derivative but at a temperature of 100°. This preparation, however, was not employed because 2-benzylthiouracil decomposed appreciably during bromination. The 5-bromo-2-benzylthiouracil, m. p. 184° with decomposition, was usually obtained in yields of 40 to 50%.

Anal. Calcd. for $C_{11}H_9N_2OSBr$: Br, 26.9. Found: Br, 26.8.

6-Methyl-5-bromo-2-methylthiouracil.—Ten grams of 6-methyl-2-methylthiouracil was brominated and isolated in the same manner as 5-bromo-2-methylthiouracil to yield 13 g. (91%) of 6-methyl-5-bromo-2-methylthiouracil, m. p. 255–256° with decomposition.

Anal. Calcd. for $C_6H_7N_2OSBr$: Br, 34.00. Found: Br, 34.08.

6-Methyl-5-bromo-2-thiouracil.—Eleven grams of 6-methyl-5-bromo-2-methylthiouracil was treated with hydriodic acid in acetic acid–acetic anhydride solution according to the above described directions to yield 1.8 g. of 6-methyl-5-bromo-2-thiouracil, m. p. 268–272° with decomposition; capillary melting point was 230° with decomposition.

Anal. Calcd. for $C_5H_5N_2OSBr$: Br, 36.15. Found: Br, 36.28.

5-Iodo-2-benzylthiouracil.—This compound was made by iodinating 2-benzylthiouracil according to the method of Johnson and Johns[21] for the preparation of 5-iodo-2-ethylthiouracil. When a quantity of 10.5 g. of 2-benzylthiouracil was iodinated, a yield was obtained of 9.3 g. (56%) of 5-iodo-2-benzylthiouracil, m. p. 178–180°.

Anal. Calcd. for $C_{11}H_9N_2OSI$: I, 36.87. Found: I, 36.83.

5-Iodo-2-thiouracil.—A sample of 5-iodo-2-ethylthiouracil was prepared according to the method of Johnson and Johns,[21] but in attempting to de-ethylate the compound with hydriodic acid as previously described, it was found that practically all the iodine was lost from the ring, while little splitting occurred. Essentially the same result was obtained with the S-methyl derivative. For this reason the 5-iodo-2-benzylthiouracil was used, since it could be satisfactorily split by keeping the temperature between 90 and 100° while adding anhydrous hydrogen iodide by the use of the apparatus illustrated in Fig. 1.

Sixty-five and four-tenths grams of 5-iodo-2-benzylthiouracil was dissolved in 400 ml. of glacial acetic acid containing 10 ml. of acetic anhydride and placed in the reaction vessel. In the side flask was placed 95 ml. of acetic anhydride, and in the dropping funnel 75 ml. of 50% hydriodic acid. While maintaining the temperature of the reaction flask at approximately 100° with a boiling water-bath, the hydriodic acid was added dropwise to the acetic anhydride in the side flask. This mixture became hot, and the hydrogen iodide as it was liberated was conducted into the reaction flask. As the hydrogen iodide came in contact with the solution of the S-benzyl deriva-

(20) Johns, *Am. Chem. J.*, **40**, 348 (1908).

(21) Johnson and Johns, *J. Biol. Chem.*, **1**, 305 (1905).

tive, a ring of precipitate of the split product formed under the inlet tube. When all the hydriodic acid was added to the acetic anhydride, the remaining hydrogen iodide was forced over by heating the hydrogen iodide generator with a small flame. The reaction was considered complete when no more precipitate formed in the reaction flask. After the reaction mixture was cool and the precipitation complete, the supernatant liquid was poured off and the 5-iodo-2-thiouracil was washed on the Buchner funnel with peroxide-free ether to remove the residual iodine. It was then twice extracted with hot glacial acetic acid to remove unreacted material, then washed alternately with water and alcohol to remove the acetic acid. The almost pure product was purified by dissolving in dilute sodium hydroxide with gentle warming; the addition of acetic acid precipitated 27 g. of 5-iodo-2-thiouracil (57%).

The supernatant liquid from the reaction mixture was concentrated *in vacuo* and 7.4 g. of unreacted 5-iodo-2-benzylthiouracil was recovered.

When the 5-iodo-2-thiouracil was heated in a capillary the product became discernibly yellow at 190°; slowly darkened to brown at 210°; and decomposed to a black tar at 214–215°. On the Dennis melting point bar it decomposed slowly with melting at 231–236° and melted instantaneously with decomposition at 278–280°.

Anal. Calcd. for $C_4H_3N_2OSI$: I, 49.95; N, 11.03; S, 12.62. Found: I, 50.08; N, 10.9; S, 12.77.

5-Iodo-2-thiouracil and 5-iodo-2-benzylthiouracil are light sensitive and are best dried over phosphorus pentoxide *in vacuo*.

6-Methyl-5-iodo-2-benzylthiouracil.—Twelve grams of 6-methyl-2-benzylthiouracil was iodinated as described above but, since some of the material escaped iodination, the product was washed thoroughly with water, and re-iodinated. Eleven grams (58%) of 6-methyl-5-iodo-2-benzylthiouracil was obtained, m. p. 180–181° with decomposition.

Anal. Calcd. for $C_{12}H_{11}N_2OSI$: I, 35.44. Found: I, 35.65.

6-Methyl-5-iodo-2-thiouracil.—Ten grams of 6-methyl-2-benzylthiouracil was split as described for the preparation of 5-iodo-2-thiouracil. The yield was 3 g. (40%). On the Dennis melting point bar it decomposed slowly above 220°; it melted instantly at 285–289° with decomposition. When heated slowly in the capillary 6-methyl-5-iodo-2-thiouracil began to darken at 175°, progressively decomposed with loss of iodine, and decomposed completely at 195° without melting.

Anal. Calcd. for $C_5H_5N_2OSI$: I, 47.34. Found: I, 47.10.

Acknowledgment.—The authors wish to thank Dr. Noel E. Foss of the Calco Chemical Division of the American Cyanamid Company for generous supplies of thiouracil.

Summary

Methods are described for the preparation of 5-iodo-, 5-bromo- and 5-chloro-2-thiouracil, and the 5-iodo-, 5-bromo-, and 5-chloro-6-methyl-2-thiouracil. These compounds were prepared by the direct halogenation of either the S-methyl or S-benzyl derivatives followed by splitting with anhydrous hydrogen iodide.

An apparatus is illustrated for the convenient preparation of anhydrous hydrogen iodide.

BOULDER, COLORADO RECEIVED DECEMBER 27, 1947

[CONTRIBUTION FROM THE LABORATORY FOR THE STUDY OF HEREDITARY AND METABOLIC DISORDERS, AND THE DEPARTMENTS OF BIOLOGICAL CHEMISTRY AND MEDICINE, UNIVERSITY OF UTAH SCHOOL OF MEDICINE]

The Preparation of D- and L-Homoserine[1]

BY MARVIN D. ARMSTRONG

In the course of the synthesis of some biologically interesting compounds, it became necessary to prepare a considerable amount of pure L-homoserine (α-amino-γ-hydroxybutyric acid). A review of the literature revealed that little work had been accomplished on homoserine since its first preparation by Fischer and Blumenthal[1a] in 1907. Kitagawa's discovery of canavanine[2] and his demonstration that it was α-amino-γ-guanidinoxy-*n*-butyric acid[3–6] was the beginning of an increasing number of references to homoserine in the later literature. The main emphasis in such reports has been in connection with both syntheses and degradations of methionine.[7–12]

The previously reported O-phenylhomoserine[1a] provided a suitable intermediate for the preparation of the optically active homoserines. The N-formyl derivative was easily prepared and was found to give a crystalline strychnine salt; the use of 50% aqueous methanol as a solvent gave a good separation of the two diastereoisomers in one step, the salt of the D-isomer being more insoluble.

That the more soluble strychnine salt was of the L-configuration was shown by an application of the rule of Lutz and Jirgensons[13] to the crude (+)-O-phenylhomoserine obtained by decomposition of the mother liquors from the first crystallization of the strychnine salt. A definite negative maxi-

(1) This research was supported by a grant from the United States Public Health Service. Presented in part before the Division of Biological Chemistry at the 112th meeting of the American Chemical Society, New York, September 16, 1947.

(1a) E. Fischer and H. Blumenthal, *Ber.*, **40**, 106 (1907).

(2) M. Kitagawa and S. Monobe, *J. Biochem. Japan*, **18**, 333 (1933); *C. A.*, **28**, 1021[9] (1934).

(3) M. Kitagawa and S. Monobe, *J. Agr. Chem. Soc. Japan*, **9**, 845 (1933); *C. A.*, **28**, 2678[7] (1934).

(4) M. Kitagawa, *ibid.*, **12**, 871 (1937); *C. A.*, **31**, 1362[2] (1937).

(5) M. Kitagawa and A. Takani, *J. Biochem. Japan*, **23**, 181 (1936); *C. A.*, **30**, 4818[2] (1936).

(6) M. Kitagawa, *ibid.*, **24**, 107 (1936); *C. A.*, **30**, 8162[4] (1936).

(7) L. W. Butz and V. du Vigneaud, *J. Biol. Chem.*, **99**, 135 (1932).

(8) E. M. Hill and W. Robson, *Biochem. J.*, **30**, 248 (1936).

(9) H. R. Snyder, J. H. Andreen, G. W. Cannon and C. F. Peters, THIS JOURNAL, **64**, 2082 (1942).

(10) J. E. Livak, E. C. Britton, J. C. VanderWeele and M. F. Murray, *ibid.*, **67**, 2218 (1945).

(11) G. Toennies and J. J. Kolb, *ibid.*, **67**, 1141 (1945).

(12) W. H. Stein and S. Moore, *J. Org. Chem.*, **11**, 681 (1946).

(13) O. Lutz and B. Jirgensons, *Ber.*, **63**, 448 (1930); **64**, 1221 (1931).