lanosten-3β-yl acetate has been reported to melt at 120–121°.[2]

Anal. Calcd. for $C_{32}H_{54}O_2$: C, 81.63; H, 11.56. Found: C, 82.12; H, 11.76.

Reaction of Δ^8-lanosten-3α-ol with phosphorus oxychloride. (a) To a solution of 0.10 g. of *epi*-dihydrolanosterol in 5.0 ml. of dry pyridine was added 0.20 ml. of phosphorus oxychloride. The homogeneous, colorless reaction mixture was warmed on the steam bath for 2 hr. After about 1 hr. the solution turned milky and deposited a colorless oil. (Compare dihydrolanosterol.) The reaction mixture was poured into water, and extracted twice with ether. The ethereal extracts were washed well with water, and finally 10% hydrochloric acid, and the solvent removed at the water pump. By this procedure approximately 1 mg. of organic material could be recovered. Additional extractions of the aqueous phases with chloroform afforded no organic material.

(b) To a solution of 0.05 g. of *epi*-dihydrolanosterol in 3 ml. of dry pyridine was added 0.10 ml. of phosphorus oxychloride and the mixture heated under reflux for 2 hr. The reaction was worked up as in (a) and a yellow oil obtained which was dissolved in hexane and filtered through an alumina column. On removal of the solvent, a colorless glass was obtained which was crystallized from chloroform methanol to afford 0.010 g. of semicrystalline solid, m.p. 87–94° with previous sintering. Several recrystallizations from the same solvents gave a minute amount of material, m.p. 95–112°.

Reaction of Δ^8-lanosten-3α-ol with phosphorus pentachloride. To a suspension of 0.05 g. of *epi*-dihydrolanosterol in 5.0 ml. of hexane was added 0.05 g. of phosphorus pentachloride. The reaction was stirred 2 hr. at room temperature, and then heated under reflux for an additional hour. The reaction mixture was diluted with ether, washed with successive portions of water, 5% sodium bicarbonate, and again with water, dried, and the solvent removed at reduced pressure. The resulting yellow oil was taken up in hexane and filtered through an alumina column. Removal of the hexane afforded a small amount of colorless oil, which could not be induced to crystallize. Under identical conditions dihydrolanosterol in our hands yields isolanostadiene.

ATLANTA, GA.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, FORDHAM UNIVERSITY]

Studies on Some Oxidation and Reduction Products of Thiamine. II.[1] Thiamine Disulfide-Thioglycolic Acid Reaction.[2–4]

GUIDO E. BONVICINO[5] AND DOUGLAS J. HENNESSY

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Thioglycolic acid in aqueous solution at *p*H 5 reduces thiamine disulfide (I) to thiamine (IIa). When the reaction conditions are more vigorous, thioglycolic acid displaces the thiazole moiety of thiamine and of oxythiamine (IIb) to give (4-amino-2-methyl-5-pyrimidinylmethylthio)acetic acid (IIIa) and (4-hydroxy-2-methyl-5-pyrimidinylmethylthio)acetic acid (IIIb) respectively and 5-(β-hydroxyethyl)-4-methylthiazole (IV). The structures of IIIa and IIIb were established by Raney-nickel desulfurization to give 4-amino-2,5-dimethylpyrimidine (Va) and 2,5-dimethyl-4-hydroxypyrimidine (Vb) respectively and acetic acid. IIIa was converted to IIIb and Va was converted to Vb by 6*N*-hydrochloric acid at reflux temperature. IIIa was synthesized from 4-amino-5-bromomethyl-2-methylpyrimidine hydrobromide (VI) and thioglycolic acid.

The possibility of vitamin B_1 activity in natural products being due, at least in part, to the biologically active oxidation product thiamine disulfide[6,7] and other reversibly oxidized forms of thiamine[8] led us to modify the thiochrome assay[9] by including a reduction step in the procedure. The reduction of thiamine disulfide to thiamine is necessary because thiamine disulfide is not oxidized to thiochrome by alkaline ferricyanide. We used thioglycolic acid for the reduction of thiamine disulfide[10] in the thiochrome procedure. While investigating this reduction, it was observed that the recovery of thiamine disulfide as thiamine decreased when the thioglycolic acid concentration was too high.[10] This low recovery was thought to be caused by a further reaction between thiamine and thioglycolic acid following the reduction. To test this hypothesis, an aqueous solution of thiamine and three molar equivalents of thioglycolic acid was adjusted to *p*H 5, and refluxed for one hour. The crystalline product, which separated in 70–75% yield on cooling the reaction mixture, analyzed for a compound of empirical formula $C_8H_{11}N_3O_2S$ (IIIa or IIIc). The ether extract of the basified aqueous filtrate yielded 5-(β-hydroxyethyl)-4-methylthiazole (IV) in 65–70% yield, identified as the picrate and picrolonate salts.

(1) Paper I: G. E. Bonvicino and D. J. Hennessy, *J. Am. Chem. Soc.*, **79**, 6325 (1957).

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(3) From the dissertation submitted by G. E. Bonvicino in partial fulfillment of the requirements for the degree of Doctor of Philosophy, the Graduate School, Fordham University, 1952.

(4) Presented before the Division of Biological Chemistry, American Chemical Society, (a) 116th Meeting, Atlantic City, N. J., September, 1949; see Abstracts, p. 63C, (b) 117th Meeting, Philadelphia, Pa., April, 1950; see Abstracts, p. 49C.

(5) Present address: Organic Chemical Research Section, Research Division, American Cyanamid Co., Lederle Laboratories, Pearl River, N. Y.

(6) O. Zima and R. R. Williams, *Ber.*, **73**, 941 (1940).

(7) O. Zima, K. Ritsert, and T. Moll, *Z. physiol. Chem.*, **267**, 210 (1941).

(8) M. Fujiwana, H. Watanabe, and K. Matsui, *J. Biochem. (Japan)*, **41**, 29 (1954).

(9) D. J. Hennessy, *Biol. Symposia*, **12**, 111 (1947).

(10) G. E. Bonvicino and D. J. Hennessy, in preparation.

When thiamine disulfide was reacted with thioglycolic acid, under the same conditions as thiamine, the insoluble product which was isolated had the same composition as IIIa or IIIc. From the aqueous solution, IV was again isolated in good yield. Oxythiamine[11] behaved like thiamine and thiamine disulfide in the reaction with thioglycolic acid. The thiazole (IV) was again isolated and identified as its picrate and picrolonate salts. The pyrimidine fraction analyzed for a compound of empirical formula $C_8H_{10}N_2O_3S$ (IIIb or IIId).

The nucleophilic displacement of IV by thioglycolic acid was expected because of the work of Snyder and Speck[12] and of Raison.[13] The 4-amino group of the pyrimidine moiety of thiamine (IIa) or the 4-hydroxy group of the pyrimidine moiety of oxythiamine (IIb), being in the ortho position, exerts an electromeric effect (+E); $H_2\ddot{N}$—C=C—$\overset{\delta+}{C}H_2$—N+, to facilitate the rupture of the methylene bridge carbon-thiazolium nitrogen bond during the attack of the nucleophilic thiol group. This is quite analogous to the sulfite cleavage reaction of thiamine.[14]

Recently Kupstas and Hennessy[15] have postulated a similar mechanism for the biosynthesis of icthiamine.

Setkina and Kursanov[16] have reported reactions of quaternary ammonium salts of the type $ROCH_2$—N+ Y^-, with carboxylic acids and their salts whereby a displacement reaction took place as follows:

$$ROCH_2\text{—}N^+ Y^- + R'COOM \longrightarrow ROCH_2OCOR' + |N + MY$$

The tertiary amine is displaced by the carboxylate radical resulting in the formation of alkoxymethyl esters. If the reaction of thiamine or oxythiamine with thioglycolic acid took place in a similar manner, the structure of the pyrimidine reaction products would be IIIc and IIId respectively. However, when one considers that the reaction product, $C_8H_{11}N_3O_2S$, is amphoteric, insoluble in organic solvents and melts with decomposition at 290°, then the reaction product seems to have the structure IIIa rather than IIIc. That the 4-amino group of the pyrimidine moiety of the vitamin was unaltered in the reaction product $C_8H_{11}N_3O_2S$ (IIIa) was shown by its conversion to $C_8H_{10}N_2O_3S$ by refluxing IIIa with 6*N* hydrochloric acid for six hours. This product was identical with that obtained from the oxythiamine-thioglycolic acid reaction. Both decompose at 230° and have identical infrared spectra. The product of the oxythiamine-thioglycolic acid reaction must be represented as IIIb rather than IIId. Furthermore, the reaction conditions of Setkina and Kursanov were very much different from ours, *i.e.*, anhydrous medium at 150–170° for three or more hours.

The application of the Raney-nickel desulfurization reaction to the product $C_8H_{11}N_3O_2S$ (IIIa or IIIc) was especially satisfactory because it yielded 4-amino-2,5-dimethylpyrimidine (Va)[17] and acetic acid. Under the same conditions, $C_8H_{10}N_2O_3S$ (IIIb or IIId) afforded 2,5-dimethyl-4-hydroxypyrimidine (Vb)[17] and acetic acid. The acetic acid obtained in both cases was characterized as its *p*-bromophenacyl ester.[18] Compounds Va and Vb were identical with authentic samples of 4-amino-2,5-dimethylpyrimidine[17b,19] and 2,5-dimethyl-4-hydroxypyrimidine[16b] respectively. Had the reaction taken the path described by Setkina and Kursanov, the desulfurization reaction should have afforded the acetate esters of 4-amino-5-hydroxymethyl-2-methylpyrimidine (Vc) and of 4-hydroxy-5-hydroxymethyl-2-methylpyrimidine (Vd) respectively. To complete the structural proof of $C_8H_{11}N_3O_2S$ and of $C_8H_{10}N_2O_3S$, fragment Va was converted to Vb with boiling 6*N* hydrochloric acid for six hours.

Compound IIIa was synthesized from 4-amino-5-bromomethyl-2-methylpyrimidine hydrobromide (VI)[20] and thioglycolic acid in the presence of sodium bicarbonate in 50% aqueous ethanol. The elemental analysis and the infrared spectrum of the synthesized product were in agreement with those of IIIa, obtained by reaction of thiamine chloride with thioglycolic acid. The synthesized sample of IIIa gave Va with Raney-nickel, identical with an authentic sample,[17,19] and acetic acid, characterized as before.

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(11) (a) M. Soodak and L. R. Cerecedo, *J. Am. Chem. Soc.*, **66**, 1988 (1944); (b) L. R. Cerecedo and A. J. Eusebi, *J. Am. Chem. Soc.*, **72**, 5749 (1950); (c) H. N. Rydon, *Biochem. J.*, **48**, 383 (1951).

(12) H. R. Snyder and J. C. Speck, *J. Am. Chem. Soc.*, **61**, 688, 2895 (1939).

(13) C. G. Raison, *J. Chem. Soc.*, 2074 (1949).

(14) R. R. Williams, R. E. Waterman, J. C. Keresztesy, and E. R. Buchman, *J. Am. Chem. Soc.*, **57**, 536 (1935).

(15) E. E. Kupstas and D. J. Hennessy, *J. Am. Chem. Soc.*, **79**, 5222 (1957).

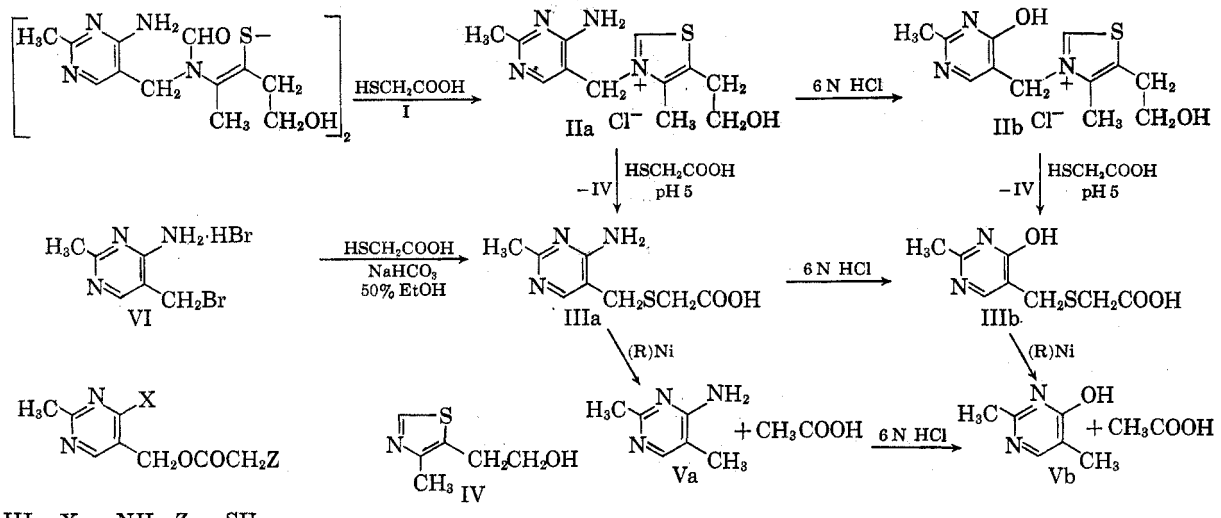
(16) V. N. Setkina and D. E. Kursanov, *Otdel. Khim. Nauk.*, 190 (1949); *Chem. Abstr.*, **43**, 6161 (1949).

(17) (a) J. C. Cline, R. R. Williams, A. E. Ruehle, and R. E. Waterman, *J. Am. Chem. Soc.*, **59**, 530 (1937); (b) R. R. Williams, A. E. Ruehle, and J. Finkelstein, *J. Am. Chem. Soc.*, **59**, 526 (1937).

(18) R. L. Shriner and R. C. Fuson, *The Systematic Identification of Organic Compounds*, 3rd Ed., John Wiley and Sons, Inc., New York, 1948, p. 157.

(19) The authors are indebted to Dr. Ellis V. Brown of the Chemistry Department, Seton Hall University, for an authentic sample of this material.

(20) Kindly furnished by Merck and Co. Inc., Rahway, N. J.



IIIc, X = NH_2, Z = SH
IIId, X = OH, Z = SH
Vc, X = NH_2, Z = H
Vd, X = OH, Z = H

Fermenting yeast did not resynthesize thiamine from IIIa in the presence of added IV.[21]

EXPERIMENTAL[22]

(4-Amino-2-methyl-5-pyrimidinylmethylthio)acetic acid (IIIa). *(a) From thiamine and thioglycolic acid.* A solution of 13.8 g. (0.15 mole) of thioglycolic acid, 16.9 g. (0.05 mole) of thiamine in 50 ml. of water was adjusted to *p*H 5 with 20% sodium hydroxide and refluxed for 1 hr. After the reaction mixture was cooled in an ice bath for several hours, the precipitated product was collected by filtration and dried. The filtrate was saved for the isolation of IV. Recrystallization from hot water afforded 8.0 g. (73%) of product, m.p. 290° dec.

Anal. Calcd. for $C_8H_{11}N_3O_2S$: C, 45.06; H, 5.20; N, 19.71; S, 15.03. Found: C, 45.09; H, 5.20; N, 19.39; S, 14.86.

(b) From thiamine disulfide and thioglycolic acid. Thiamine disulfide, 2.81 g. (0.005 mole) and 2.76 g. (0.03 mole) of thioglycolic acid were dissolved in 10 ml. of water and the solution adjusted to *p*H 5 with 10% sodium hydroxide and heated under reflux for 1 hr. The reaction product was isolated as described in (*a*) above. Recrystallization from hot water afforded 2.0 g. (60%) of product, m.p. 289–291° dec.

Anal. Calcd. for $C_8H_{11}N_3O_2S$: C, 45.06; H, 5.20; N, 19.71; S, 15.03. Found: C, 45.30; H, 5.06; N, 19.91; S, 15.14.

(c) From 4-amino-5-bromomethyl-2-methylpyrimidine hydrobromide[20] *and thioglycolic acid.* To a solution of 5.5 g. (0.06 mole) of thioglycolic acid in 50 ml. of 50% ethanol was added 8.4 g. (0.02 mole) of 4-amino-5-bromomethyl-2-methylpyrimidine hydrobromide. The mixture was refluxed for 2 hr. and then cooled. The precipitated product was collected, washed with hot alcohol, and dissolved in hot water. The hot solution was treated with sodium carbonate to *p*H 8–8.5, Norite and filtered. The colorless filtrate was cooled in an ice bath and was acidified with acetic acid to *p*H 5. The crystalline product which slowly precipitated was collected and recrystallized from hot water. The product, 3.0 g. (70%), melted with decomposition at 291°.

Anal. Calcd. for $C_8H_{11}N_3O_2S$: C, 45.06; H, 5.20; N, 19.71; S, 15.03. Found: C, 44.85; H, 4.96; N, 19.83; S, 15.14.

The products obtained by procedures (*a*), (*b*), and (*c*) above all had identical infrared and ultraviolet spectra.

(4-Hydroxy-2-methyl-5-pyrimidinylmethylthio)acetic acid (IIIb). (*a*) *From* IIIa (*Thiamine-thioglycolic acid reaction product*). A solution of 4.3 g. (0.02 mole) of IIIa in 250 ml. of 6*N* hydrochloric acid was refluxed for 6 hr. The solution was evaporated to dryness on a steam bath at reduced pressure. The residue was dissolved in 25 ml. of water, adjusted to *p*H 4.5, and cooled in an ice bath. A crystalline solid separated, which on recrystallization from 25 ml. of hot water afforded 2.6 g. (60%) of product, m.p. 230° dec.

Anal. Calcd. for $C_8H_{10}N_2O_3S$: C, 44.85; H, 4.71; N, 13.07; S, 14.96. Found: C, 44.78; H, 4.86; N, 12.91; S, 14.76.

(This procedure is essentially that used by Rydon[11c] for the preparation of oxythiamine from thiamine.)

(*b*) *From oxythiamine* (IIb). A solution of 16.9 g. (0.05 mole) of oxythiamine[11c] in 50 ml. of water and 13.8 g. (0.15 mole) of thioglycolic acid was adjusted to *p*H 5 and refluxed for 2 hr. The clear solution was cooled in an ice bath for 1 hr. and then stored overnight in a refrigerator. The crystalline product which had separated was collected by filtration. (The filtrate was saved for the isolation of the thiazole moiety.) Recrystallization of the collected material from 15 ml. of hot water afforded 6.7 g. (63%) of product, m.p. 231° dec.

Anal. Calcd. for $C_8H_{10}N_2O_3S$: C, 44.85; H, 4.71; N, 13.07; S, 14.96. Found: C, 44.77; H, 4.36; N, 13.03; S, 15.07.

5-(β-Hydroxyethyl)-4-methylthiazole (IV). (*a*) *Isolation from the thiamine-thioglycolic acid reaction.* The aqueous filtrate (after separation of IIIa) was adjusted to *p*H 9 with potassium carbonate and extracted with four 50-ml. portions of ether. The combined ether extract was dried over anhydrous magnesium sulfate and evaporated to dryness to give 5.0 g. (70%) of an oil. A sample of this product was converted to the picrate salt in 73% yield, m.p. 163–164° (lit.,[23] 162–163°), and to the picrolonate salt in 80% yield, m.p. 185–186° (lit.,[24] 184°). Mixed melting points with the authentic salts showed no depression.

(*b*) *Isolation from the thiamine disulfide-thioglycolic acid reaction.* The isolation was the same as described in (*a*), above. The yield was 0.7 g. (50%), characterized as the picrate, m.p. 163° and the picrolonate, m.p. 184–185°. The picrate salt was analyzed.

(21) Thiamine regeneration studies were done by Dr. R. J. Moshy, Fordham University.

(22) All melting points were taken on a Fisher-Johns melting point apparatus and are uncorrected.

(23) H. T. Clarke and S. Gurin, *J. Am. Chem. Soc.*, **57**, 1876 (1935).

(24) E. R. Buchman, R. R. Williams, and J. C. Keresztesy, *J. Am. Chem. Soc.*, **57**, 1849 (1935).

Anal. Calcd. for $C_{12}H_{12}N_4O_3S$: C, 38.71; H, 3.25; N, 15.05; S, 8.61. Found: C, 38.88; H, 3.42; N, 15.20; S, 8.31.

(*c*) *Isolation from the oxythiamine-thioglycolic acid reaction.* The filtrate, obtained after the isolation of IIIb (see (*b*)), was basified with excess potassium carbonate and extracted with five 50-ml. portions of ether. The combined etherial extract was dried over anhydrous magnesium sulfate and evaporated to dryness. The residue, 4.3 g. (60%) was characterized as the picrate, m.p. 163–164° (lit.,[22] 162–163°), and the picrolonate salt, m.p. 184–186° (lit.,[23] 184°). Mixed melting points with authentic samples were 163–165° and 184–186° respectively.

4-Amino-2,5-dimethylpyrimidine (Va). (*a*) *Raney-nickel desulfurization of* IIIa (*from the thiamine-thioglycolic acid reaction*). A suspension of 10.7 g. (0.05 mole) of IIIa, 100 g. of alcohol-free Raney-nickel (prepared according to the procedure of Mozingo *et al.*,[25] the weight was estimated as suggested by Adkins)[26] in 350 ml. of water was refluxed for 2.5 hr. The hot mixture was filtered and the filtrate adjusted to *p*H 10–11 with 20% sodium hydroxide and again filtered hot over a bed of filter-aid. The filtrate was evaporated to dryness, redissolved in 100 ml. of hot water, decolorized with charcoal, and filtered. The filtrate on cooling deposited 4.0 g. (65%) of product, m.p. 203–204° (lit.,[17b] 201–202°). The picrate salt from ethanol melted at 224–225° dec. (lit.,[17b] 222°). A sample of the free base was sublimed at 130–135°/0.5 mm. The sublimate melted at 205–206°.

Anal. Calcd. for $C_6H_9N_3$: C, 58.52; H, 7.37; N, 34.13. Found: C, 58.52; H, 7.33; N, 33.94.

The above desulfurization was repeated on a sample of IIIa prepared from 4-amino-5-bromomethyl-2-methylpyrimidine hydrobromide and thioglycolic acid as described above. The product which was obtained was identical with an authentic sample and with the product isolated above. Mixed melting points of the free base and of its picrate salt showed no depression.

(*b*) *Isolation of the acetic acid fragment from the Raney-nickel desulfurization of* IIIa. The desulfurization reaction described above was repeated on a 4.0 g. sample of IIIa with 40 g. of Raney-nickel. The hot reaction filtrate was acidified to *p*H 4 with dilute hydrochloric acid and distilled in order to separate the acetic acid into the distillate. The latter was adjusted to *p*H 9 with 10% sodium hydroxide and evaporated to dryness. The residue was dissolved in 5 ml. of water and treated with one gram of *p*-bromophenacyl bromide according to the procedure described by Shriner and Fuson.[18] The *p*-bromophenacyl acetate was recrystallized from alcohol, m.p. 86–87° (lit.,[18] 85°); the yield was 150 mg.

2,5-Dimethyl-4-hydroxypyrimidine (Vb). (*a*) *Raney-nickel desulfurization of* IIIb (*from oxythiamine and thioglycolic acid*). The desulfurization of 5.2 g. (0.024 mole) of IIIb and 50 g. of Raney-nickel in 175 ml. of water was accomplished as described above for the desulfurization of IIIa. After the second filtration, the filtrate was evaporated to dryness. The residue was extracted with three 50-ml. portions of hot chloroform. The combined chloroform extract was evaporated to dryness and the residue, 1.64 g. (55%), m.p. 175–176°, was sublimed at 135–140°/0.5 mm. The sublimate, m.p. 176–177°, on recrystallization from acetone afforded 1.43 g. (48%) of product, m.p. 176–177°, (lit.,[17b] 174°).

Anal. Calcd. for $C_6H_8N_2O$: C, 58.05; H, 6.49; N, 22.57. Found: C, 57.92; H, 6.47; N, 22.43.

The *p*-bromophenacyl acetate was prepared from the chloroform insoluble residue as described above.

(*b*) *From 4-amino-2,5-dimethylpyrimidine* (Va). A solution of 3.0 g. (0.024 mole) of Va in 100 ml. of 6*N* hydrochloric acid was refluxed for 8 hr. and evaporated to dryness. The residue was dissolved in 50 ml. of water, adjusted to *p*H 5, and evaporated to dryness. This residue was extracted with three 50-ml. portions of chloroform. The chloroform extract yielded 2.7 g. (90%) of product, m.p. 174.5–176°, on evaporation. This material was sublimed at 135–140°/0.5 mm. to give 2.3 g. (77%) of sublimate, m.p. 176–177°. Recrystallization from acetone afforded 2.0 g. (67%) of product, m.p. 176–177°. Mixed melting point with the product obtained from IIIb above showed no depression.

Anal. Calcd. for $C_6H_8N_2O$: C, 58.05; H, 6.49; N, 22.57. Found: C, 58.25; H, 6.58; N, 22.72.

The chloroform insoluble residue when treated with dilute sodium hydroxide and warmed, evolved ammonia.

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NEW YORK 58, N. Y.

(25) R. Mozingo, D. E. Wolf, S. A. Harris, and K. Folkers, *J. Am. Chem. Soc.*, **65**, 1013 (1943).

(26) H. Adkins, "Reactions of Hydrogen," The University of Wisconsin Press, Wis., 1946, p. 22.