carbonated with dry carbon dioxide gas for 20 minutes at 1 atm. The product was hydrolyzed with 50 ml. of water then transferred into a 3-liter flask with ether and 1.5 liters of water. The 2-methyl-C^{14}-naphthalene was steam distilled directly into a 1-liter liquid–liquid extractor. The distillate was extracted for 28 hours using as small a volume of ether as was practicable. The raffinate showed no radioactivity when checked with a counter. The extract (20 ml.) was treated with 15 ml. of redistilled piperidine in a 200 ml. flask equipped with a reflux condenser and a guard tube filled with KOH pellets. The system was heated under gentle refluxing for 16 hours. The solution was then cautiously acidified with 1:4 hydrochloric acid and extracted with ether in a liquid–liquid extractor for 5.5 hours. The ether extract was dried, treated with "Norit," and filtered directly into the oxidation flask. The ether was removed by distillation through a column.

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[CONTRIBUTION FROM THE LOS ALAMOS SCIENTIFIC LABORATORY OF THE UNIVERSITY OF CALIFORNIA (UNDER THE AUSPICES OF THE A.E.C.)]

Micro Syntheses with Tracer Elements. VIII. The Synthesis of Thiamin Labeled with C^{14}

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2-Amino-4-methyl-5-(β-hydroxyethyl)-thiazole-2-C^{14} has been prepared and converted to 4-methyl-5-(β-hydroxyethyl)-thiazole-2-C^{14}, in high yield, by the reduction of the diazonium compound. The deaminated thiazole has been used to prepare C^{14} labeled thiamin with a specific activity of 170 mg./mc. It has been shown that 2-methyl-4-amino-5-bromomethylpyrimidine hydrobromide reacts with butanol to form an ether.

The availability of thiourea[1] labeled with either S^{35} or C^{14} makes possible the labeling of thiamin with these isotopes.

The condensation of thiourea with γ-chloro-γ-acetopropanol in boiling aqueous medium gave 83 to 90% yields of 2-amino-4-methyl-5-(β-hydroxyethyl)-thiazole. In the only published preparation of this compound, from the above intermediates, giving yield data either no solvent[2] or alcohol solvent[3] was used. The reported yields were 72 and 29%, respectively. Hatcher[4] reported 80% yields of this aminothiazole from thiourea and γ-chloro-γ-acetopropyl ether.

A modification of the procedure of Hatcher,[4] who prepared the desired thiazole in 31% yield, for the deamination of this aminothiazole gave reproduceably high yields (70%) of 4-methyl-5-(β-hydroxyethyl)-thiazole.

Published procedures for the condensation of the pyrimidine and thiazole moieties utilize a one to two molar excess of the thiazole. Williams, *et al.*,[5] obtained a 45% yield of thiamin from two moles of thiazole to one of pyrimidine with butanol as solvent. Gravin,[6] using 2.4 moles of thiazole to one of pyrimidine in bromoform solvent, obtained a 54% yield of thiamin, while a 70% yield of a thiamin isomer was obtained by Baumgarten[7] using a 3 to 1 molar ratio of 4-methyl-5-(α-hydroxyethyl)-thiazole to pyrimidine in nitromethane solvent.

Obviously it is not feasible to use an excess of thiazole when this is the isotope labeled moiety. In an effort to determine why an excess of the thiazole is conducive to good yields, an experiment was made to determine if the pyrimidine reacts with itself to form a quaternary base or with butanol to form an ether. A practically quantitative yield of material melting at 135–136° was obtained which was indicated by analysis to be the hydrobromide of 2-methyl-4-amino-5-butoxymethylpyrimidine. The free base, m.p. 84°, was also prepared. These compounds appear not to have been previously described in the literature.

Use of the conditions described by Baumgarten[7] with nitromethane as solvent but with an excess of pyrimidine (3 to 1 molar ratio) gave a lower yield of thiamin than the experiments with butanol as solvent and a 1 to 1 ratio of reactants. All attempts to isolate unreacted thiazole from the mother liquors from the latter experiments were without success. This result was further confirmed when C^{14} labeled thiazole was used in the condensation.

In view of the above results, the major side reactions appear to involve thiazole and possibly some thiamin which has already formed. Both the unreacted thiazole and the desired product, thiamin, have the β-hydroxyethyl group which is capable of reacting with the 5-bromomethyl group of the pyrimidine moiety to form an ether.

Experimental

2-Amino-4-methyl-5-(β-hydroxyethyl)-thiazole-2-C^{14}.—C^{14} Thiourea, 0.1697 g. (0.0022 mole) reacted with γ-chloro-γ-acetopropanol, 0.394 g. (0.0029 mole), in boiling aqueous medium for 2.5 hours. An 86.7% yield (0.3746 g.) of the aminothiazole was isolated as the hydrochloride from dry ether solution. In preliminary experiments with unlabeled thiourea, in which reaction time and ratio of reactants were varied, 83 to 90% yields of the aminothiazole were isolated as the hydrochloride in the same manner, m.p. 153–154°. Inasmuch as Hatcher[4] claimed to have obtained a di-hydrochloride there was some question as to the composition of our compound. Therefore a sample of the unlabeled hydrochloride, m.p. 153°, was analyzed for chlorine.

Anal. Calcd. for $C_6H_{10}N_2OS.HCl$: Cl, 18.2. Found: Cl, 18.3, 18.6.

The free base was prepared and crystallized from benzene in colorless needles, m.p. 93°. This material formed a

(1) C. W. Bills and A. R. Ronzio, THIS JOURNAL, **75**, 5510 (1950).

(2) A. R. Todd, F. Bergel, H. L. Fraenkel-Conrat and A. Jacob, *J. Chem. Soc.*, 1601 (1936).

(3) U. P. Basu and S. J. Das-Gupta, *J. Indian Chem. Soc.*, **15**, 160, 164 (1938) (*C. A.*, **32**, 7040[4] (1938)).

(4) J. B. Hatcher, THIS JOURNAL, **69**, 465 (1947).

(5) Joseph K. Cline, Robert R. Williams and Jacob Finkelstein, *ibid.*, **59** 1052 (1937).

(6) A. I. Gravin, *J. Applied Chem. (USSR)*, **16**, 105 (1943); *C. A.*, **38**, 1239 (1944).

(7) P. Baumgarten, A. Dornow, K. Gutschmidt and H. Krebl, *Ber.*, **75B**, 444 (1942).

mono-picrate which melted at 218° after washing with ether and recrystallization from absolute ethanol. The picrate was analysed for nitrogen.

Anal. Calcd. for $C_{12}H_{13}N_5O_8S$: N, 18.08. Found: N, 18.08, 18.15.

There is considerable discrepancy in the melting points given for all of the above compounds in the literature.[8]

4-Methyl-5-(β-hydroxyethyl)-thiazole-2-C^{14}.—The aminothiazole hydrochloride, 0.3746 g. (0.00193 mole) was dissolved in 9.6 ml. of concentrated hydrochloric acid while the flask was immersed in a dry ice–acetone-bath maintained at −5°. During 15 minutes, 2.9 ml. of 1 *N* sodium nitrite solution, precooled to 0°, was added to the well-stirred solution of amine hydrochloride. A white crystalline compound, probably the diazonium chloride, began to precipitate when one half of the sodium nitrite solution was added. The mixture was allowed to stand at −5° for 30 minutes, then was diluted slowly with 9.6 ml. of water cooled to 0°. The colorless precipitate slowly dissolved. With the cooling bath still maintained at −5°, the diazonium salt was reduced by the addition of 4.2 ml. (0.0023 mole) of 32% hypophosphorous acid (during 13 minutes) to the rapidly stirred solution. Rapid evolution of nitrogen took place. The solution was placed in a refrigerator maintained at about 2°. The evolution of nitrogen was complete after 16 hours after which 6 *N* sodium hydroxide was added slowly to the cooled (−5°) acidic solution. When the solution was only weakly acidic, a quantitative transfer was made, with water, into an ether extractor. The solution was then adjusted to *p*H 10–12 with 6 *N* sodium hydroxide and was extracted with ether for 40 hours. The ether extract was dried over anhydrous magnesium sulfate and filtered into a cone-shaped flask. The drying agent was washed five times with 20-ml. portions of dry ether. The washings were reduced in volume, combined with the ether extract, and the volume of the entire solution was reduced to 1–2 ml.

In the preliminary experiments it was found that the maximum yield of 70% was reproducibly obtained when the diazotization was done in concentrated hydrochloric acid solution followed by the reduction with 32% hypophosphorous acid in about 6 *N* hydrochloric acid. The optimum molar ratio of hypophosphorous acid to the amine was 12 to 1. In these experiments, the products were isolated and weighed as the picrate. Ether was removed from the ethereal extracts on the water-bath. The oily residues were then treated with a slight excess of a saturated solution of picric acid in absolute ethanol. The picrates formed immediately and the alcohol was then evaporated under vacuum. Since the thiazole picrate was found to be very slightly soluble in dry ether, the picrates were washed twice with 5-ml. portions of dry ether to remove picric acid and then dried *in vacuo*. The picrates of the products from ten experiments melted from 162 to 163°; the literature gives the melting point of the picrate of the thiazole from natural thiamin as 162–163° from ether[9] and 163° for synthetic material recrystallized from ethanol.[10] The picrate from a 70.1% yield of thiazole, m.p. 163°, was analyzed for nitrogen:

Anal. Calcd. for $C_{12}H_{12}N_4O_8S$: N, 15.04. Found: N, 14.68, 15.20.

(8) Basu and Das-Gupta give 70° for the free base and 138° for the hydrochloride. Todd, *et al.*, give 85–90° for the crude base and 213° for the picrate. E. M. Gibbs and F. A. Robinson, *J. Chem. Soc.*, 925 (1945) give 154° for the hydrochloride and 210° for the picrate. K. A. Jensen and Th. Thorsteinsson, *C. A.*, **35**, 5109[4] (1941), give 150° for the hydrochloride. Adolf Wentz, *C. A.*, **33**, P177[15] (1939), gives 153–154° for the free base and 162–163° for the picrate.

(9) E. R. Buchman, R. R. Williams and J. C. Keresztesy, THIS JOURNAL, **57**, 1849 (1935).

(10) E. R. Buchman, *ibid.*, **58**, 1803 (1936).

C^{14} Thiamin Hydrobromide.—One ml. of butanol was added to the C^{14} thiazole and a short water-cooled condenser was attached to the flask which was then immersed in an oil-bath heated to 125°. During 20 minutes, 0.382 g. (0.00134 mole) of 2-methyl-4-amino-5-bromomethylpyrimidine hydrobromide was added in small portions. When the addition was complete, the condenser was washed with 0.5 ml. of absolute alcohol. Heating was continued for 15 minutes. The reaction mixture was let stand at room temperature for 15 hours and was then filtered. The colorless crystals of crude thiamin which had separated were washed first with 0.5 ml. of absolute alcohol then twice with 2-ml. portions of dry ether. After drying in the vacuum desiccator, the crude product weighed 0.3341 g. (50% yield based on thiazole). The crude thiamin was dissolved in 2 ml. of hot absolute methanol and the solution, while still being warmed, was diluted with 8 ml. of butanol then was allowed to cool slowly and was kept at about 2° for 24 hours. The yield of recrystallized product was 26.6% of theory based on thiazole. The product was dissolved in absolute methanol and transferred to a smaller container. The solvent was removed under vacuum leaving a residue which melted at 223–224°. In all the preceding experiments the products recrystallized from methanol-butanol melted at 238–239°. An authentic sample (furnished by Merck and Company) melting at 226–227°, melted at 239–240° when recrystallized from methanol–butanol. This behavior is not without parallel in the literature and the melting point appears to be a function of the solvent used. Gravin[6] reported 215–220° for the compound recrystallized from acetone-water, Williams, *et al.*,[5] reported 229–231° when methanol–ethanol was used as solvent and Andersag and Westphal[11] obtained a product melting at 220° from dilute ethanol. Williams and Cline[12] noted that synthetic vitamin B_1 hydrochloride melting at 232–243° was identical with the natural compound, m.p. 246°, in physiological dosage and composition. The two compounds also gave identical absorption spectra. These investigators also noted that the natural vitamin displayed two crystalline habits, the synthesized product corresponding to one of the forms observed. The C^{14} thiamin hydrobromide possessed an activity of one millicurie per 170 milligrams.

2-Methyl-4-amino-5-butoxymethylpyrimidine and Hydrobromide.—The pyrimidine hydrobromide (0.322 g.) was heated with 2 ml. of *n*-butanol in an oil-bath at 121–122°. The pyrimidine dissolved completely within 25 minutes. Upon cooling the solution, colorless crystals formed, 0.233 g., m.p. 135–136°. Dilution of the mother liquor with ether precipitated additional material, m.p. 135°. Ultimate analysis indicated the compound is 2-methyl-4-amino-5-butoxymethylpyrimidine hydrobromide.

Anal. Calcd. for $C_{10}H_{18}N_3OBr$: C, 43.48; H, 6.56; N, 15.21. Found: C, 43.69, 44.09; H, 6.57, 6.40; N, 15.09, 15.13.

About 0.2 g. of the hydrobromide salt was dissolved in a minimum of water and 6 *N* sodium hydroxide was added dropwise until the solution remained cloudy. After a few minutes, the free base separated in fine needles which melted at 84° after recrystallization from *n*-hexane.

Anal. Calcd. for $C_{10}H_{17}N_3O$: C, 61.50; H, 8.77; N, 21.52. Found: C, 61.35; H, 8.75, 8.94; N, 21.65, 21.61.

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(11) H. Andersag and K. Westphal, *Ber.*, **70B**, 2035 (1937).

(12) R. R. Williams and J. K. Cline, THIS JOURNAL, **58**, 1504 (1936).