[CONTRIBUTION FROM THE RADIOCARBON LABORATORY, UNIVERSITY OF ILLINOIS]

Synthesis of DL-2-Mercaptohistidine-α-C^{14} and DL-Ergothioneine-α-C^{14} [1]

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A new synthesis of DL-2-mercaptohistidine (VI), the key intermediate for the synthesis of ergothioneine, is described. N-3-Bromoacetonylphthalimide (I) was treated with diethylamine to give 1-diethylamino-3-phthalimidopropanone (II) in 80% yield. Hydrolysis of II with hydrochloric acid and subsequent cyclization of the crude 1-amino-3-diethylaminopropanone hydrochloride (III) with potassium thiocyanate afforded 2-mercapto-4(5)-diethylaminomethylimidazole (IV). This compound was condensed with ethyl acetamidocyanoacetate-2-C^{14} in the presence of sodium ethoxide and methyl sulfate. 2-Methylthiolhistidine-α-C^{14} (V) thus obtained in 83% yield was cleaved with sodium in liquid ammonia yielding DL-2-mercaptohistidine-α-C^{14} (VI). The conversion of VI into ergothioneine-α-C^{14} (VII) was performed in two steps with an over-all yield of 11.2% calculated on ethyl acetamidocyanoacetate-2-C^{14}.

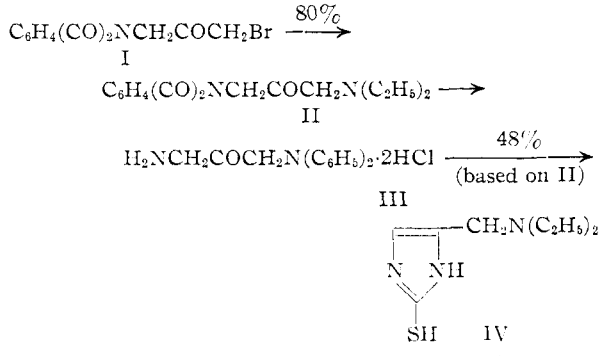
Though ergothioneine was found to be present in red blood cells more than 30 years ago,[2] and its wide-spread occurrence in animals is well established,[3] very little is known about its role and fate in the organism. Investigations along these lines received a new impetus after synthetic ergothioneine,[4] and S^{35}-labeled 2-mercaptohistidine and ergothioneine,[5] became available. However, as pointed out by Heath,[4] studies with the S^{35}-labeled compound could not furnish an explanation of its function and metabolism.

The objective of the work described in this paper was to develop a suitable method for the synthesis of radioactive ergothioneine labeled with C^{14} in such a way as to facilitate the biochemical studies of the metabolic pathways of this compound. For this purpose labeling in the α-position seemed to be more promising than the preparatively much simpler labeling in the 2-position of the imidazole ring or in the quaternary methyl groups.

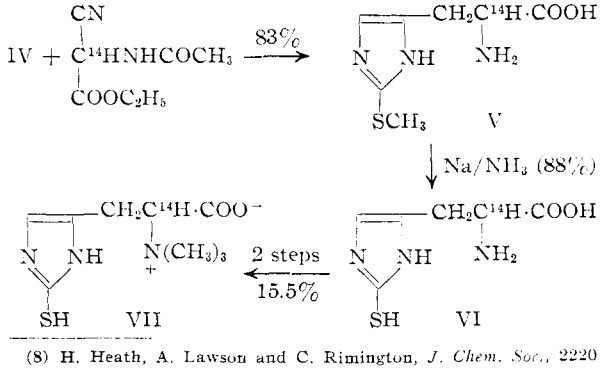
This aim could be achieved either by making use of the synthetic approaches already described in the literature, or by developing an entirely new synthesis. In the first case, only two methods are at present available, *i. e.*, (1) the one by Heath, *et al.*,[4] starting from histidine, which could be labeled in the α-position according to Wolf,[6] and (2) the synthesis by Hegedus[7] starting from bromoacetone and diethyl acetamidomalonate. Unfortunately, neither one of these methods seems to be suitable for the preparation of the radioactive compound, because the labeled component has to be introduced at an early stage of the synthesis, consequently resulting in a low yield of the final product. For this reason, the development of a new synthesis was highly desirable.

It was obvious that 2-mercaptohistidine (VI) has to be considered as the key intermediate for the synthesis of ergothioneine (VII). In any synthesis of mercaptohistidine where the labeled side chain should be introduced as late as possible, the imidazole ring has to be formed first. Thus, our efforts were concentrated upon the synthesis of 2-mercaptoimidazole substituted in the 4(or 5)-position in such a way that the subsequent introduction of the side-chain moiety could be effected with a good yield. Heath's synthesis of 2-mercapto-4(5)-methylhistidine[8] is a good example of how such a scheme might work.

Bearing in mind the fact that 2-mercapto-4-(5)-chloromethylimidazole could not be prepared from the corresponding 4(5)-hydroxy compound,[9] 2-mercapto-4(5)-diethylaminomethylamidazole (IV) was considered as the most promising intermediate. The synthesis of this compound was accomplished in the following way



The condensation of 2-mercapto-4(5)diethylaminomethylimidazole (IV) with ethyl acetamidocyanoacetate-2-C^{14} was accomplished under con-



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(2) S. R. Benedict, E. B. Newton and J. A. Behre, *J. Biol. Chem.*, **67**, 267 (1926); E. B. Newton, S. R. Benedict and H. D. Dakin, *ibid.*, **72**, 367 (1927); G. Hunter and B. A. Eagles, *ibid.*, **65**, 623 (1925); **72**, 123 (1927).

(3) For a general review see: H. Heath, C. Rimington, C. E. Searle and A. Lawson, *Biochem. J.*, **50**, 530 (1952).

(4) H. Heath, A. Lawson and C. Rimington, *J. Chem. Soc.*, 2215 (1951).

(5) H. Heath, *Biochem. J.*, **54**, 689 (1953).

(6) G. Wolf, *J. Biol. Chem.*, **200**, 637 (1953).

(7) B. Hegedus, *Helv. Chim. Acta*, **38**, 22 (1955).

(8) H. Heath, A. Lawson and C. Rimington, *J. Chem. Soc.*, 2220 (1951).

(9) A. O. Jackson and C. S. Marvel, *J. Biol. Chem.*, **103**, 191 (1933); see also H. Heath, A. Lawson and C. Rimington, *J. Chem. Soc.*, 2223 (1951).

ditions similar to those applied by Heath, *et al.*,[8] for the synthesis of 2-methylthiol-4(5)-methylhistidine, but the yields were considerably improved by using ion-exchange resins instead of the rather tedious isolation procedure *via* the mercury complex. 2-Mercaptohistidine was converted into ergothioneine-α-C^{14} (VII) in two steps by Heath's method.[4] Many attempts were made to increase the yields in the methylation step by changing the reaction conditions and the isolation procedure, but no improvements over their original method could be achieved. In the actual radioactive run a 15.5% yield of ergothioneine based on mercaptohistidine was accomplished as compared with a 21% yield reported by Heath.[4] Although it was beyond the scope of this work to investigate the quaternization of the α-amino group more thoroughly, it might be of some interest to point out that paper chromatograms of the crude product revealed that at least three different compounds were formed in unequal amounts in the last step of the synthesis.

The over-all yield of labeled ergothioneine prepared as here reported is 11.2% based on ethyl acetamidocyanoacetate which represents a considerable improvement over the yield of 2 to 5% which can be expected by the already known synthetic procedures.[4,7]

Experimental

All melting points were determined on a micro hot-stage and are uncorrected. The microanalyses were performed by Clark Microanalytical Laboratory, Urbana, Ill. Radioactivity was determined by dry combustion by the Pregl method, the volume of carbon dioxide measured manometrically and radioactivity by the vibrating reed electrometer.[10]

1-Diethylamino-3-phthalimidopropanone (II).—A mixture of N-3-bromoacetonylphthalimide,[11] m.p. 147° (6.0 g.), anhydrous ethanol (60 ml.) and diethylamine (6.7 ml.) was stirred while the temperature was allowed to rise to about 40°. After one hour, the pale yellow solution was evaporated to dryness *in vacuo*. The residue was dissolved in chloroform and shaken with water in order to remove the diethylamine hydrobromide. The chloroform solution was evaporated to dryness and the oily residue treated with anhydrous ethanol whereupon the product became crystalline. The crystals were separated and the mother liquor concentrated giving an additional amount of the product. The total yield was 4.71 g. (80%), m.p. 86–88°. For analysis it was recrystallized from ethanol; colorless crystals, m.p. 91°.

Anal. Calcd. for $C_{15}H_{18}N_2O_3$: C, 65.68; H, 6.62; N, 10.21. Found: C, 65.93; H, 6.36; N, 9.96.

2-Mercapto-4(5)-diethylaminomethylimidazole (IV) Hydrochloride.—Thirty grams of the phthalimidoketone II was dissolved in 6 *N* hydrochloric acid (330 ml.) and heated with stirring to 90–95° for 9 hours. Higher temperature, or even refluxing, decreased the yield considerably due to tar formation. After standing overnight in the cold the separated phthalic acid was filtered off. The filtrate was evaporated to dryness *in vacuo* and the crude 1-amino-3-diethylaminopropane (III) thus obtained in the form of a sirupy hygroscopic hydrochloride was not isolated in a pure state or further characterized. It was dissolved in water (30 ml.) and to this solution a solution of potassium thiocyanate (11.4 g.) in water (10 ml.) was added, and the mixture heated on the steam-bath for one hour. The resulting solution was then evaporated to dryness *in vacuo* and the residue extracted with three 50-ml. portions of boiling methanol. The inorganic salts remained undissolved. The solvent was removed *in vacuo*, the residue dissolved in 50 ml. of water, treated with charcoal, and the filtrate concentrated to a small volume. Upon cooling, 2-mercapto-4(5)-diethylaminomethylimidazole hydrochloride crystallized out. By concentrating the mother liquor a second crop of crystals was obtained; colorless prisms, m.p. 185–187°, yield 13.6 g. (48%).

Anal. Calcd. for $C_8H_{16}ClN_3S$: C, 43.33; H, 7.27; N, 18.95. Found: C, 43.26; H, 7.12; N, 18.59.

The free base was obtained by dissolving the hydrochloride (10 g.) in water (30 ml.) and adding a solution of sodium carbonate (4.1 g.) in water (25 ml.). The base crystallized as colorless needles, m.p. 169°, 5.6 g. (78%). It decomposes when recrystallized from hot water.

DL-2-Methylthiolhistidine-α-C^{14} (V).—The condensation was performed on the vacuum line in an atmosphere of helium under strictly anhydrous conditions. 2-Mercapto-4(5)-diethylaminomethylimidazole (725 mg., 3.92 mmoles) and 666 mg. of ethyl acetamidocyanoacetate-2-C^{14} (0.75 mc./mmole)[12] were suspended in anhydrous ethanol (6 ml.). To the stirred suspension a solution of sodium (200 mg.) in ethanol (8 ml.) was added. After dissolution was effected, methyl sulfate (1.2 ml.) was added and the temperature allowed to rise as the reaction proceeded. After stirring for 16 hours at room temperature 8 ml. of water was added and the solution concentrated to a small volume at 20° (0.1 mm.). The residue was hydrolyzed by refluxing for 10 hours with 6 *N* hydrochloric acid (20 ml.). The solution was concentrated *in vacuo*, and the hydrochloric acid removed by subsequent additions of water and evaporation. The crude product was dissolved in water (20 ml.) and the solution brought to *p*H 6.5–7 with sodium hydroxide solution. It was then passed through an Amberlite IR-120 (H^+ form, 20–40 mesh) ion exchange resin column (16 × 150 mm.). The column was washed with water until acid free. The product was then eluted with 1% aqueous pyridine solution. The ninhydrin positive fractions were combined and evaporated to dryness *in vacuo*. Thus, 654 mg. (83%) of almost pure 2-methylthiolhistidine-α-C^{14} was obtained. Only traces of glycine could be detected by paper chromatography and the compound was sufficiently pure for the next step; m.p. 255–260° dec., R_f 0.28–0.29 (1-propanol–*N* acetic acid, 3:1). For analysis a non-radioactive sample prepared in the same way was recrystallized from water; m.p. 259–261° dec.

Anal. Calcd. for $C_7H_{11}N_3O_2S$: C, 41.77; H, 5.51; N, 20.88. Found: C, 41.70; H, 5.09; N, 20.62.

DL-2-Mercaptohistidine-α-C^{14} (VI).—2-Methylthiolhistidine-α-C^{14} (654 mg., 3.25 mmoles) was dissolved in about 40 ml. of liquid ammonia, and sodium was added in small pieces until a permanent blue color developed. Ammonium chloride (about 0.1 g.) then was added and the ammonia allowed to evaporate. The residue was dissolved in 50 ml. of water and the solution passed through an Amberlite IR-120 (H^+ form, 20–40 mesh) ion exchange resin column. The product was isolated by elution with aqueous pyridine in the same manner as described for the S-methyl compound. The residue obtained after evaporating the pyridine eluates was dissolved in hot water, decolorized with charcoal, and the colorless filtrate concentrated to a small volume at 0° (0.1 mm.). 2-Mercaptohistidine crystallized (539 mg., 88%). The product was identical with an authentic sample. It decomposes above 300° without melting, R_f 0.15 (1-propanol–*N* acetic acid, 3:1); radioactivity, 0.75 mc./mmole.

Anal. Calcd. for $C_6H_9N_3O_2S$: C, 38.49. Found: C, 38.72.

DL-Ergothioneine-α-C^{14} (VII).—The conversion of 2-mercaptohistidine into ergothioneine was performed *via* the 2-carbethoxy derivative following closely the procedure given by Heath, *et al.*[4] When starting with 520 mg. (2.78 mmoles) of 2-mercaptohistidine, 100 mg. of ergothioneine-α-C^{14} (spec. activity 0.74 mc./mmole), *i.e.*, 15.5%, was obtained. The product showed a single spot on the paper chromatogram, R_f 0.26 (1-propanol–*N* acetic acid, 3:1), and gave an ultraviolet spectrum identical with an authentic sample.

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(12) Supplied by Tracerlab, Inc., 1601 Trapelo Rd., Waltham 54, Mass.