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THE SYNTHESIS OF CHOLINE CHLORIDE AND ACETYLCHOLINE CHLORIDE

LABELED IN THE METHYL GROUP WITH C¹⁴

F. Christenson and B. S. Tolbert

July 15, 1949

Berkeley, California

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by

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ABSTRACT

A method is described for the preparation of choline-methyl-C¹⁴
chloride and acetylcholine-methyl-C¹⁴ chloride from methyl-C¹⁴ iodide
on a semi-micro scale.

* The work described in this paper was sponsored by the Atomic
Energy Commission.

THE SYNTHESIS OF CHOLINE CHLORIDE AND ACETYLCHOLINE CHLORIDE
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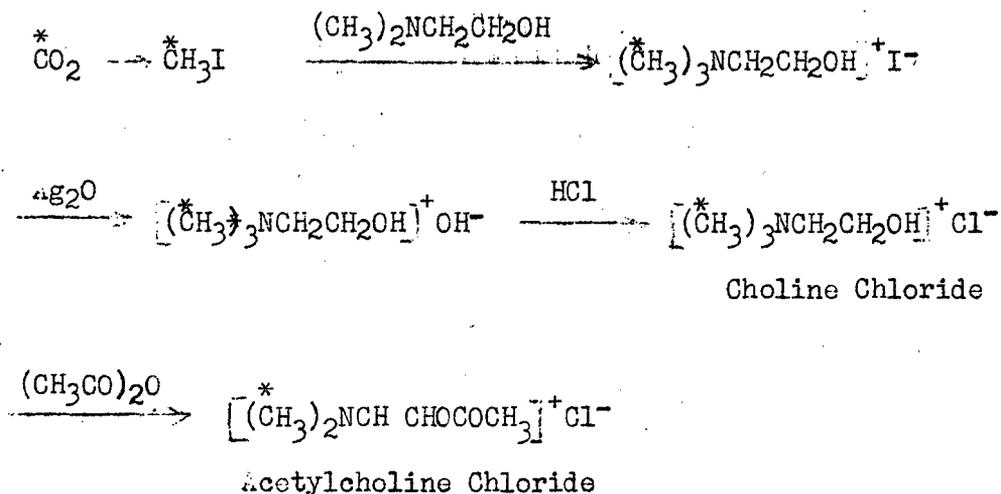
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Choline has been studied not only as an important factor in fatty acid and phospholipid metabolism but also as an intermediate in the formation of acetylcholine, as a component of lecithin and as a source of labile methyl groups. Because of this biological importance the preparation of choline and acetylcholine labeled with C¹⁴ was undertaken. Practically quantitative yields of choline have been obtained by condensing trimethylamine with ethylene chlorohydrin (1), but the method is not readily adaptable to radiosynthesis since neither of the intermediates were available in this laboratory labeled with C¹⁴. However, by the quaternization of β -dimethylaminoethyl alcohol with C¹⁴ labeled methyl iodide, which was available, it appeared possible to prepare choline in good yield and so this method was studied. The chemical steps are readily adaptable to semi-micro work and there is no problem in separation of the final product as a pure compound since there are no important side reactions. The acetylation of the alcohol is also essentially quantitative and also there are no side products. The steps of the reaction may be represented as follows:

(*) The work described in this paper was sponsored by the Atomic Energy Commission.

(1) R. R. Renshaw, J. Am. Chem. Soc., 32, 128 (1909).



EXPERIMENTAL

Methyl iodide: - Carbon dioxide was catalytically hydrogenated to methyl alcohol at high pressures and iodinated with phosphorus and iodine (2). The yield varied from 80-85%.

Choline-methyl-C¹⁴ chloride: - One and nine-tenths grams (13.7 mmoles) of methyl-C¹⁴ iodide was distilled in vacuo into a reaction tube containing a slight excess of β-dimethylamino ethyl alcohol (1.7 g.) dissolved in 10 ml. absolute ethyl alcohol. The reaction tube was constructed of a Carius tube sealed to a 10 mm. stopcock and connected to the vacuum manifold with a 14/35 standard joint (3). After the methyl iodide was added the stopcock was clamped shut and the tube heated at 60° C for one to one and one-half hours.

After heating the tube it was reconnected to the vacuum line and all the volatile compounds, including the excess tertiary amine, distilled off.

(2) B. M. Tolbert, J. Am. Chem. Soc., 69, 1529 (1947).

(3) "Isotopic Carbon" by Calvin, Heidelberger, Reid, Tolbert and Yankwich, p. 142 and 170, John Wiley and Sons, Inc., New York, 1949,

The non-volatile residue was washed with water into a 50 ml. beaker, which solution was chilled in an ice-bath and 5 g. silver oxide added slowly and the mixture thoroughly stirred. The excess silver oxide and the silver iodide was filtered off and the filtrate and wash water combined and titrated to pH 7.4 with N hydrochloric acid. The choline chloride was concentrated to a syrup in vacuo and was then twice crystallized from an absolute alcohol-ether mixed solvent. The recrystallized choline chloride was dried in vacuo.

The calculated specific activity of the choline chloride based on the specific activity of the barium carbonate used to begin the synthesis was 9.75×10^4 dis./min./mg; the observed specific activity of the product was $9.7 \pm 0.3 \times 10^4$ dis./min./mg. The yield based on $C^{14}H_3I$ was 87.0%.
anal. Calcd for $C_5H_{14}ONCl$: C, 43.0; H, 10.0; Cl, 24.5. Found: C, 43.13; H, 10.11; Cl, 24.96.

acetylcholine-methyl- C^{14} chloride: - Approximately one gram anhydrous choline chloride labeled in the methyl group was placed in an ignition tube with 1.5 ml. acetic anhydride. The tube was sealed and heated on the steam bath for two hours. The tube was then cooled, opened and transferred with an excess of absolute ethyl alcohol to a small flask equipped with a condenser and refluxed for one hour. The volatile compounds were evaporated off and the syrup dried in vacuo. The acetylcholine chloride was then crystallized from dry acetone. First crop yield was 42% based on choline chloride.

The calculated specific activity based on the specific activity of

the choline chloride was $7.7 \pm 0.5 \times 10^4$ dis./min./mg; the observed specific activity of the product was $8.7 \pm 0.5 \times 10^4$ dis./min./mg. Anal. Calcd for $C_7H_{16}O_2NCl$: C, 46.2; H, 8.85; Cl, 19.6. Found: C, 45.43; H, 8.94; Cl, 19.2.

Acknowledgment: - The authors would like to thank Prof. M. Calvin for his encouragement in this work.

SUMMARY

A method is described for the preparation of choline-methyl- C^{14} chloride and acetylcholine-methyl- C^{14} chloride from methyl- C^{14} iodide on a semi-micro scale.