

ring substitution products are in the same order as their probable base strengths is readily understandable. Also subject to interpretation on the basis of the above development is the observation recorded by Robinson and Robinson<sup>3</sup> that, of a series of phenylhydrazones derived from structurally similar carbonyl compounds, those phenylhydrazones which are derived from the most readily enolizable aldehydes or ketones give the most facile Fischer reactions. If the tautomeric shift represented in step 2 is closely analogous to the keto-enol change, then the most readily enolizable ketone of a related series should give a phenylhydrazone whose  $K_2$  value should be greatest of the series. Since  $K_1$  and  $k_3$  values would not be expected to vary much throughout a series of phenylhydrazones of related aldehydes or ketones, the changes in  $k_{\text{expt}}$  should be functions mainly of changes in  $K_2$ . In this connection it should be stressed, however, that quantitative data permitting comparisons of degrees of enolization of carbonyl compounds on the one hand with rates of Fischer reactions of their phenylhydrazones on the other are not available. A study of this problem will be carried out in this Laboratory.

Finally, the latter part of the proposed reaction sequence is not fundamentally different from the ring-closure mechanism proposed by Allen and Wilson<sup>4</sup>; it therefore gives a satisfactory accounting for the fact demonstrated by them that the phenylhydrazone nitrogen atom bound to the benzene ring is that which remains in the indole ultimately formed.

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## The Preparation of C<sup>14</sup>-Chain Labeled Choline Chloride

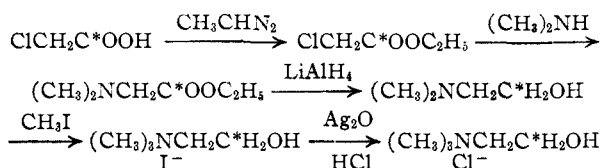
BY WILLIAM G. DAUBEN AND MILDRED GEE

The role of choline in transmethylation reactions and in phospholipid biochemistry has been widely investigated. For the former type of study, choline labeled in the methyl groups with either deuterium or C<sup>14</sup> has been employed.<sup>1,2</sup> Such a labeled compound is of questionable use, however, in phospholipid investigations or in the determination of the metabolic fate of choline itself. In order to pursue problems of the latter type, choline labeled in the chain with C<sup>14</sup> is most desirable. Recently, Fields and his collaborators<sup>3</sup> reported the preparation of such a labeled material starting with barium carbide and progressing through acetylene and ethylene bromohydrin. Choline, so prepared, is labeled in both carbon atoms of the chain. We wish to report an alternate synthetic route which starts with the more readily available and more easily manipulated acetic acid and also allows for specific labeling of the individual carbon atoms.

(1) V. duVigneaud, M. Cohn, J. P. Chandler, J. R. Schenck and S. Simmonds, *J. Biol. Chem.*, **140**, 625 (1941).

(2) J. Dubnoff, *Arch. Biochem.*, **22**, 474 (1949).

(3) D. E. Walz, M. Fields and J. A. Gibbs, *THIS JOURNAL*, **73**, 2968 (1951).



Carboxyl-labeled sodium acetate was converted into chloroacetic acid in the standard fashion<sup>4</sup> and this acid was esterified with diazoethane. The resulting ethyl chloroacetate was then allowed to react with dimethylamine and the product reduced to N,N-dimethylaminoethanol with lithium aluminum hydride. The substituted ethanol was further methylated with methyl iodide and the choline iodide so formed converted into choline chloride.

During this preparation, it was found that when methyl chloroacetate was subjected to aminolysis, not only was the chlorine atom displaced but the ester was also transformed in a large part into a substituted amide. The ethyl ester, however, was found to be sufficiently less reactive<sup>5</sup> so that this latter side-reaction occurred only to a minor amount. The usual mode of preparation of choline from ethylene chlorohydrin could not be used in this synthesis since it is known that when chloroacetic acid is reduced with lithium aluminum hydride, ethanol is the major product.<sup>6</sup>

### Experimental

**Diazoethane.**—N-Nitrosoethylurea was prepared in the manner described for the methyl derivative<sup>7</sup> except 33% aqueous ethylamine was substituted for methylamine; yield 38%. An ethereal solution of diazoethane was prepared by adding 15.0 g. of N-nitrosoethylurea to a chilled mixture of 150 ml. of ether and 45 ml. of 50% aqueous potassium hydroxide. After standing for 20 minutes, the ether was decanted and the solution used directly in the next reaction.

**Carboxyl-labeled Ethyl Chloroacetate.**—Carboxyl-labeled acetic acid, generated from carboxyl-labeled sodium acetate (0.596 g., 7.3 mmoles) containing approximately 4.2 millicuries of C<sup>14</sup>, was diluted with 0.329 g. (4.2 mmoles) of inactive acetyl chloride. The resulting mixture was allowed to react with iodine, phosphorus pentachloride and chlorine as described by Hughes and Tolbert<sup>4</sup> and the reaction diluted first with 1 ml. of water and then with ether. The resulting ethereal solution was added to the chilled solution of diazoethane prepared above. The excess reagent was removed by distillation and the residual ethereal solution of the ester dried over anhydrous potassium carbonate. The radioactive ester was not distilled but in inactive experiments the ester was isolated in yields of 75–90% (based upon acetic acid moieties), b.p. 144° (lit. 144°).

**Ethyl N,N-Dimethylaminoacetate.**—The crude ester prepared above was allowed to react for 12 hours at room temperature with 1.2 g. (26 mmoles) of dimethylamine in 5 ml. of dry benzene in a flask fitted with a Dry Ice condenser.<sup>8</sup> The reaction mixture was filtered and the salt washed with small portions of dry ether. After removal of the solvents, the amino ester was distilled through a micro fractionation column<sup>9</sup> in order to separate the ester from any amide which might have been formed. The product boils at 144–149°. The over-all yield of pure material was 55% based on acetic acid moieties.

(4) D. Hughes and B. Tolbert, UCRL-256 (Univ. of Calif. Radiation Lab.).

(5) L. Claisen, *Ber.*, **38**, 693 (1905).

(6) C. E. Sroog, C. M. Chih, F. A. Short and H. W. Woodburn, *THIS JOURNAL*, **71**, 1710 (1949).

(7) "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 461.

(8) H. Gault, *Compt. rend.*, **145**, 126 (1907); *Bull. soc. chim., France*, [4] **3**, 369 (1908).

(9) C. W. Gould, G. Holzmann and C. Niemann, *Anal. Chem.*, **20**, 361 (1948).

**Choline Iodide.**—The foregoing ester was reduced with a solution of 2.0 g. (5.2 mmoles) of lithium aluminum hydride in 25 ml. of anhydrous diethyl carbitol. The reaction mixture was decomposed with 30 ml. of butyl carbitol and then heated at 160–180° for four hours. The bath temperature was then raised and about 25 ml. of distillate was collected in an ice-cooled receiver. The distillate was redistilled and the fraction boiling up to 180° was collected. In inactive runs it was found difficult to separate small quantities of diethyl carbitol from the dimethylaminoethanol but the presence of the carbitol did not affect the preparation of the methiodide.

The crude dimethylaminoethanol was allowed to reflux for two to three hours on a steam-bath with methyl iodide. The solid was filtered, washed with dry ethanol and dried; yield 0.420 g. (15.8% based upon starting acetic acid derivatives).

*Anal.* Calcd. for  $C_6H_{14}OI$ : C, 25.98; H, 6.10; I, 54.92. Found: C, 26.25; H, 5.93; I, 55.21.

**Choline Chloride.**—A mixture of 0.752 g. (3.3 mmoles) of choline iodide and 1.0 g. (4.3 mmoles) of silver oxide in 5 ml. of water was shaken for a short while and then allowed to stand overnight. The precipitate was centrifuged and the aqueous layer titrated with 1 *N* hydrochloric acid to a pH of 7. The resulting solution of choline chloride was lyophilized and the residual white solid weighed 396 mg. (87%).

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## The Reaction between Uranium and Oxygen

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Uranium tarnishes rapidly at room temperature and its heat of oxidation is large (256 kcal. per mole of  $UO_2$ ). In these respects it is similar to thorium, which has been shown<sup>2</sup> to oxidize by the parabolic law<sup>3</sup> at low temperatures and the linear law at higher temperatures. The present study was made to discover whether the uranium oxidation was similar to that of thorium in changing from parabolic to linear.

### Experimental

The rate of consumption of oxygen by the metal was measured by following the decrease in pressure of oxygen over a sample of metal in a closed container.<sup>2</sup> The one sample used throughout this work was a  $\frac{3}{8}$ " rod of 99.5% pure uranium obtained from the A. D. Mackay Co. under license from the Atomic Energy Commission. The surface of the sample was machined by the shops of the Argonne National Laboratory. Immediately before each oxidation the surface oxide was removed from the sample by abrasion with 400 emery cloth. All oxidations were made at 20 cm. oxygen pressure.

The apparatus used previously<sup>2</sup> was modified because of the large sample size. The sample bulb was a test-tube,  $\frac{5}{8}$ " by 6". It was connected to the measuring manometer with a standard taper ground joint for easy introduction of the sample. The ground joints were outside the furnace. The other end of the manometer was connected to a similar test-tube containing a rod of silicon carbide,  $\frac{3}{8}$ " by 3", used to balance the volume of the uranium rod. The gas volume in the temperature gradient between the furnace and the room was filled as completely as possible with glass rods to minimize fluctuations from changes in the temperature gradient.

(1) North American Aviation, Inc., Downey, California.

(2) P. Levesque and D. Cubicciotti, *THIS JOURNAL*, **73**, 2028 (1951).

(3) For a discussion of the types of oxidation laws see E. A. Gulbransen, *Trans. Electrochem. Soc.*, **91**, 573 (1947). Of interest in the present work are the parabolic law,  $w^2 = kt$ , and the linear law,  $w = k_1t$  in which  $w$  is the weight of oxygen consumed per unit area in time  $t$ ;  $k$  and  $k_1$  are constants. A few of the oxidations of the present work were found to follow an equation  $w^n = k^n t$ , in which  $n < 1$ . This equation is mentioned in the text as a less than first degree equation in the amount of oxygen consumed.

Preliminary experiments showed that a relatively long time was required to heat the sample to temperature. The lower the temperature of a run, the longer heating period necessary. For the lowest temperatures the sample was heated in vacuum for 100 minutes before oxygen was introduced. At the highest temperatures, 30 minute heating periods were used, while at about 160° the period was 60 minutes.

The effect of an insufficient heating period was to introduce a dip in the beginning of the oxidation curve. Campbell and Thomas<sup>4</sup> observed a similar dip with copper from 100 to 300° and showed the cause to be related to outgassing of the sample. To test this idea, one oxidation was stopped by evacuating the oxygen, cooling the sample under vacuum, reheating it for a short period, and then resuming the oxidation by re-admitting oxygen. A dip appeared in that curve. Another oxidation was stopped by evacuating the oxygen and immediately restarting the oxidation without cooling the sample. No dip appeared in that curve. It seemed that the temperature of the sample had more influence on the dip than outgassing.

A possible explanation for the dip in the present work is as follows. If the oxygen is admitted too soon, the temperature of the sample is below that of the furnace and of the reference bulb because the metal absorbs the radiant energy less readily than non-metals. The gas in the reference bulb then expands to its final state before the gas in the sample bulb, causing the initial rise in the oxidation curve. The gas in the sample bulb subsequently expands and lowers the oxidation curve. When the sample finally gets to temperature, it begins to oxidize and gives the curve a parabolic shape.

## Results and Discussion

As with thorium,<sup>2</sup> uranium was found to oxidize according to the parabolic law at low temperatures and according to the linear law at higher temperatures. At still higher temperatures the oxidation curve was lower than first degree in the weight of oxygen consumed. The temperature of the parabolic to linear transition for uranium was lower than that found for thorium.

From 90 to 165° the oxidation was parabolic. The data for several oxidations are given in Fig. 1, plotted as amount of oxygen consumed *versus* the square root of time. Since the curves were straight lines, the oxidations obeyed the parabolic law. From 165 to 215° the oxidations were essen-

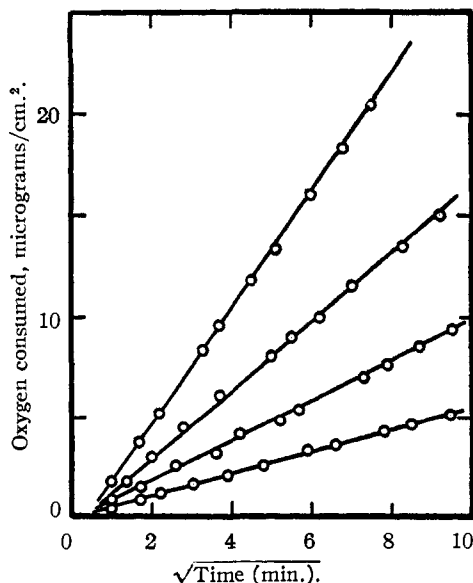


Fig. 1.—The oxidation of uranium at 112, 133, 150 and 167°, parabolic plot.

(4) W. E. Campbell and U. B. Thomas, *ibid.*, **91**, 345 (1947).