619. *Syntheses with Isotopic Tracer Elements. Part II. The Preparation of Ethanol labelled with Carbon Isotopes.*

By J. D. Cox and H. S. Turner.

Methods are described for the conversion of sodium acetate labelled with carbon isotopes into acetyl chloride and ethanol. Excellent yields of good-quality material have been obtained on the 5—15-millimoles scale.

SEVERAL methods suitable for the preparation of carbon-labelled ethanol have been described. Lifson, Lorber, Sakami, and Wood (*J. Biol. Chem.*, 1948, **176**, 1263; 1950, **183**, 518) converted acetic acid (labelled in the carboxyl group) into the *n*-butyl ester, which on hydrogenolysis over a copper–barium–chromium oxide catalyst at 250°/220 atm. gave a mixture of *n*-butanol and ethanol. This was converted into a mixture of iodides, which was separated by distillation, but the yield cannot have been very high since when the ethyl iodide was converted into butyric acid the overall yield of the latter from sodium acetate was 25%. A more satisfactory method in which, however, a 100% dilution with unlabelled material is inherent, is the conversion of the sodium acetate into ethyl acetate followed by hydrogenolysis over copper chromite (Tolbert *et al.*, *J. Org. Chem.*, 1949, **14**, 525; Sakami, unpublished work, quoted by Calvin *et al.*, in " Isotopic Carbon," Chapman and Hall, London, 1949, p. 202). On the 8—12-millimoles scale Tolbert obtained an aqueous ethanol, containing some diethyl ether, which on treatment with phosphorus tribromide gave ethyl bromide of good quality in an overall yield of 67—77% from sodium acetate. The only other recorded preparation of labelled ethanol is that by Lorber, Cook, and Meyer (*J. Biol. Chem.*, 1949, **181**, 475), who state that " ^{14}C-carboxyl-labelled acetate was reduced to ethanol with lithium aluminium hydride," but give no further details.

The reduction of carboxylic acids, esters, and chlorides to alcohols by lithium aluminium hydride has been described by Nystrom and Brown (*J. Amer. Chem. Soc.*, 1947, **69**, 1197, 2548); excellent yields were frequently obtained. We have applied this reagent to the preparation of ethanol and have developed satisfactory methods for the preparation of the necessary intermediates. Anhydrous sodium acetate has been converted, using techniques of vacuum manipulation, into anhydrous acetic acid by action of a 10% excess of anhydrous hydrogen chloride (cf. Tolbert, unpublished work, quoted in " Isotopic Carbon," p. 195). The product was freed from excess of hydrogen chloride by distillation at $-80°$; yields of 97—99% were obtained. No really satisfactory methods for the small-scale preparation of labelled acetyl halides from the acid are to be found in the literature. Several authors have refluxed sodium acetate with benzoyl halides, obtaining yields ranging between 66 and 90% (Anker, *J. Biol. Chem.*, 1948, **176**, 1333; Gould *et al.*, *ibid.*, 1949, **177**, 728; Sakami, unpublished work, quoted in " Isotopic Carbon," pp. 176, 193). Olsen, Hemingway, and Nier (*J. Biol. Chem.*, 1943, **148**, 611) obtained an 80% yield of acetyl chloride by action of phosphorus oxychloride on sodium acetate, while Calvin and Lemmon (*J. Amer. Chem. Soc.*, 1947, **69**, 1232) have used phosphorus tribromide in the preparation of acetyl bromide from acetic acid. We have obtained excellent yields of acetyl chloride by the action of phthaloyl chloride on acetic acid (cf. van Dorp and van Dorp, *Rec. Trav. chim.*, 1906, **25**, 96; Kyrides, *J. Amer. Chem. Soc.*, 1937, **59**, 208). The crude product from this reaction (yield, ~100%) has been shown to consist of acetyl chloride (94—95%), acetic acid (2%), and hydrogen chloride (3—4%), and can be reduced subsequently without further purification. Ethyl acetate has been prepared by heating anhydrous sodium acetate with ethyl sulphate (Sakami, Evans, and Gurin, *ibid.*, 1947, **69**, 1110; Tolbert *et al.*, *J. Org. Chem.*, 1949, **14**, 525). Weight yields of crude ethyl acetate of 102—120% were obtained. Sulphur dioxide was present, and possibly also ethyl ether and ethylene. It was not necessary to remove these before reduction to ethanol.

Acetyl chloride vapour is quantitatively absorbed by a stirred solution of lithium aluminium hydride in diethylene glycol diethyl ether. The reaction is strongly exothermic and it is necessary to admit the acetyl chloride slowly into the reaction vessel and to cool the solution in melting ice. The ethanol was recovered by alcoholysis with ethylene glycol monophenyl ether, followed by distillation under partial reflux in a current of inert gas. In this way the overall weight yield of ethanol (containing 0·5% of water and free from acetaldehyde) from sodium acetate was 96%, the recovery of excess isotope being 91%. A fall of concentration of isotope corresponding to a ~4% dilution with inactive ethanol was recorded. When the temperature of the reaction mixture was permitted to rise the percentage dilution was much greater. Examination of pure diethylene glycol diethyl ether isolated from the residue showed that no isotopic exchange had occurred between the ethoxy-groups of the ether and ethoxides in the reaction mixture.

Pure ethyl acetate vapour is also absorbed by lithium aluminium hydride solutions, and yields of around 100% of ethanol are obtainable. However, the crude ethyl acetate obtained by the action of ethyl sulphate on sodium acetate as previously described was less readily absorbed and there was some production of a permanent gas, probably hydrogen, which hindered diffusion and slowed the reaction. However, by pumping off this permanent gas at intervals through a trap at $-196°$, and condensing the ester on to the reducing solution by cooling the latter to $-80°$, it was possible to effect complete absorption. The ethanol ultimately obtained was contaminated with hydrogen sulphide (derived from sulphur compounds present in the crude ethyl acetate) which was removed by treatment with Raney nickel (cf. Adkins, " Reactions of Hydrogen," Univ. Wisconsin Press, 1937, p. 28). In a single run the overall weight yield of ethanol containing 2·2% of water from sodium acetate was 93·5%. The overall recovery of excess isotope was 84%; the quality of the ethanol was good, but inferior to that obtained through acetyl chloride. The concentration of isotope fell by 55% during the conversion, as compared with the inherent minimum fall of 50%.

Several attempts were made to reduce acetic acid by the same technique but, since the acid reacts with the production of an equimolar quantity of hydrogen, its diffusion became very slow.

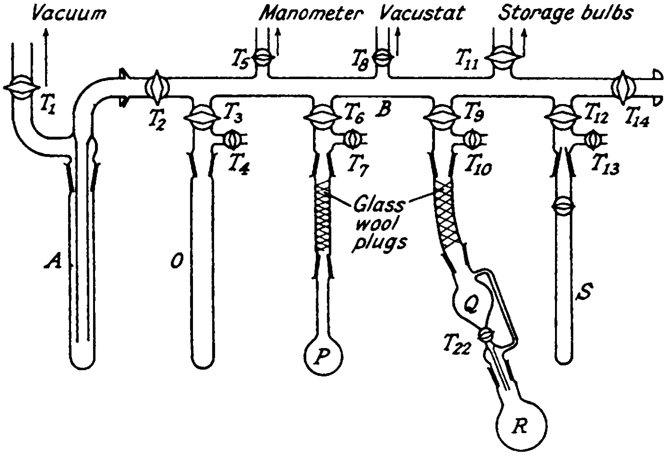
Even when the acetic acid was condensed on to the reducing solution yields of ethanol did not exceed 40%, and this route was not further examined.

EXPERIMENTAL.

Micro-analyses are by Miss M. Corner. In this paper specific activities are expressed in curies per mg.-atom of carbon.

Preparation of Acetic Acid.—Apparatus was attached to the manifold as shown in Fig. 1. In a typical experiment, finely powdered anhydrous $^{14}CH_3{\cdot}CO_2Na$ (1·108 g.; gross activity, 53·6 μc.; specific activity 1·99 × 10^{-6}), was charged into flask *P* (capacity 50 c.c.) and heated for 2 hours *in vacuo* at 200° to expel traces of water. Dry sodium chloride (0·87 g., 10% excess) was placed in flask *R* (capacity 100 c.c.) and concentrated sulphuric acid (15 c.c.) in the funnel *Q*, the bore of the plug of T_{22} being already evacuated; T_9 was opened cautiously and the vessels *Q* and *R* pumped out for a few minutes, after which T_2 was closed. Hydrogen chloride was generated by the cautious addition of the acid to the sodium chloride. When reaction had ceased, the gas was condensed into trap *O* by cooling it in liquid nitrogen, 3 minutes being allowed before closing T_9. A small pressure of a permanent gas remained in the system and was removed by pumping. Trap *O* was warmed to room temperature, T_6 was opened and the hydrogen chloride admitted to *P*. Reaction took place rapidly until the gas pressure had fallen to about 2 cm. Hg. At this stage trap *O* was cooled to −196° while flask *P* was heated to 100°. When the pressure had fallen to 10^{-3} mm. Hg, T_3 was closed and *O* was warmed until the acetic acid melted. Trap *O* was then cooled to −78° and flask *P* to −196°; T_3 was opened and the hydrogen chloride distilled into *P*. T_3 and T_6 were closed. Flask *P* was warmed to room temperature and kept there for ½ hour. Finally trap *O* was cooled to −196° with T_3 and T_6 open. Flask *P* was again heated to 100° until the pressure had fallen to 10^{-3} mm. Hg. The acetic acid in trap *O* was largely freed from hydrogen chloride by repeating the following procedure until the pressure above the acid at −78° was below 0·1 mm. Hg. (*a*) The acid was melted; after 2 minutes it was cooled to −78°. (*b*) With flask *P* at −196° and T_6 open, T_3 was opened and then closed. The acetic acid was distilled into trap *S* and weighed (0·797 g., containing a little hydrogen chloride). The conversion of sodium acetate into sodium chloride was 100%. In other runs, the true yield of acetic acid was 97—99%. The preparation was carried out on scales 5—15 millimoles.

FIG. 1.



Preparation of Acetyl Chloride.—The apparatus illustrated in Fig. 5 of Part I (preceding paper) was used; taps and joints were lubricated with silicone grease in this preparation. $^{14}CH_3{\cdot}CO_2H$ (0·797 g.; see above) was distilled *in vacuo* into flask *M*. Dry air was admitted to the apparatus through T_{13}, then the condenser was raised slightly at J_5 while phthaloyl chloride (9·6 c.c., 400% excess) was quickly run in. The acetic acid was next allowed to melt and the reactants were mixed by gentle shaking. At this stage trap *I* was cooled to −78° while a slow stream of dry nitrogen was passed through T_{21} (the gas-inlet tube *N* was rotated about J_6 until above the liquid surface). When the vessel *M* was heated in an oil-bath to 80°, reaction began and acetyl chloride refluxed freely from the cold finger; the inlet tube *N* was rotated until below the liquid surface and the gas flow was increased slightly. The temperature was raised during ¾ hour to 120° at which it was kept for 1 hour. T_{20} was closed, vessel *M* was detached at J_4, and a stopper quickly inserted in its place. The acetyl chloride in trap *I* was largely freed from hydrogen chloride by the corresponding procedure for purifying acetic acid (see above). Finally the acetyl chloride was distilled into a weighing trap of type *S* (Fig. 1) in which it could be stored for short periods at −78°. For prolonged storage at room temperature, the product was sealed *in vacuo* in a "break-seal" tube. The apparent yield of $^{14}CH_3{\cdot}COCl$ chloride was 1·047 g. (101%). The corresponding yield with unlabelled acetic acid was 100—101%. Hydrolysis of an aliquot followed by titration for chloride and total acid indicated a composition of 94—95% of acetyl chloride, 3—4% of hydrogen chloride, and 2% of acetic acid. The above method was used on scales of 8 to 100 millimoles.

Preparation of Ethanol from Acetyl Chloride.—The apparatus illustrated in Fig. 2 was attached to the manifold; the reaction vessel U contained a magnetic stirrer capsule. $^{14}CH_3\cdot COCl$ from the previous stage (1·047 g., 13·3 millimoles) was contained *in vacuo* in the " break-seal " tube W. 1·86M-Lithium aluminium hydride solution (7·2 c.c., 13·3 millimoles, 100% excess) and pure diethylene glycol diethyl ether (3·6 c.c.) were charged into U through J_9; V was then replaced. The system was next evacuated (through T_{12}) and after a minute's pumping, the solution in U was cooled to $-78°$ (the v. p. of diethylene glycol diethyl ether at 20° is ~0·4 mm. Hg, but pumping at room temperature is necessary to remove dissolved gases). When the pressure had fallen to 10^{-3} mm. Hg, T_{23} was closed and the reducing solution allowed to melt; it was cooled in ice and stirred magnetically. Meanwhile the break-seal tube W was cooled to $-78°$ and the seal broken by the magnetic hammer X. W was warmed slowly to room temperature, the acetyl chloride distilling smoothly during ½ hour into the hydride solution. A trace of liquid (acetic acid ?) then remained in W and distillation had ceased owing to the presence of hydrogen (arising from hydrogen chloride and acetic acid in the acetyl chloride). Trap T was therefore cooled to $-196°$ and U to $-78°$, and T_{23} cautiously opened. When the pressure had fallen to 10^{-3} mm. Hg, T_{23} and T_{24} were closed and trap T was warmed to room temperature while the reducing solution was stirred at 0° for 2 hours; T_{12} was then closed and nitrogen admitted through T_{13} and T_{23}. The bridge V was detached at J_9 and replaced by the apparatus illustrated in Fig. 3. The funnel Y had been previously charged with ethylene glycol monophenyl ether (10 c.c., 50% excess). A stream of dry nitrogen was passed from the inlet tube Z to T_{13}, and trap T was cooled to $-196°$.

FIG. 2.

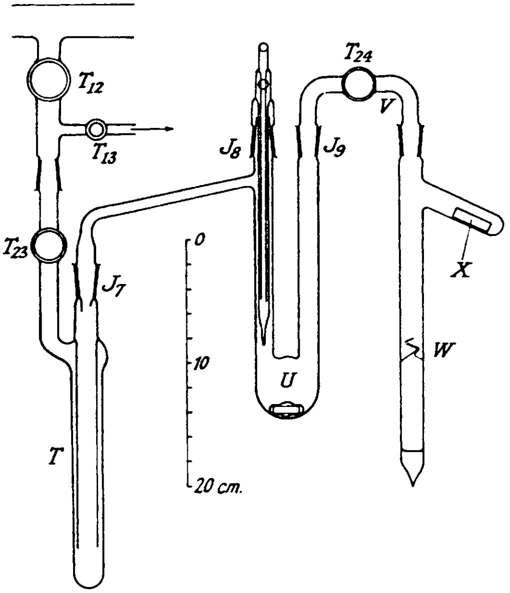
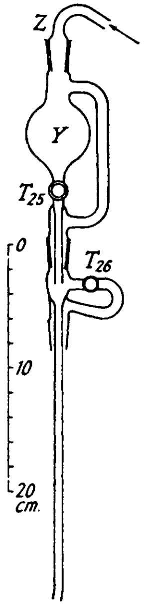


FIG. 3.



With T_{26} closed, the ethylene glycol monophenyl ether was added dropwise to the stirred reaction mixture. The vessel U was then immersed in an oil-bath at 90°. This was heated by a woven resistance wire mat through which magnetic stirring was applied. During ½ hour the bath was heated to 100° and maintained there for 2 hours, T_{26} being opened intermittently. Ethanol released by alcoholysis, together with some solvent, distilled into trap T under conditions of partial reflux.

The vessel U was detached from trap T at J_7 and was quickly replaced by a stopper against a counter-current of dry nitrogen. T_{13} was shut and T, still at $-196°$, was evacuated. The distillate in trap T was then subjected to a single-plate distillation, using the procedure described for the preparation of methanol (see Part I, preceding paper). Successive vapour-pressure readings (with the liquid reservoir at 0° and expanding into a 4-l. system) were : 11·8, 11·6, 11·5, 11·1, 10·1, 6·2, 1·8, 0·2 mm. Hg. The vapours were condensed into a trap of type S (Fig. 1).

0·599 G. of $^{14}CH_3\cdot CH_2\cdot OH$ was obtained, having n_D^{25} 1·3600, v. p. (0°) 12·2 mm. Hg, b. p. 78° (Found : C, 51·3; H, 13·1. Calc. for C_2H_6O : C, 52·2; H, 13·15%). The product contained 0·55% of water (Karl Fischer determination), 1·1% of methanol (determined by Simmonds's method, *Analyst*, 1912, **37**, 16), diethylene glycol diethyl ether (less than 2% on the basis of vapour-pressure calculations), and no detectable amount of acetaldehyde. The weight yield from $^{14}CH_3\cdot CO_2Na$ was 96%. The specific activity was $1·91 \times 10^{-6}$ (a fall of ~4%) and the gross activity 48·8 μc. Thus the recovery of ^{14}C from sodium acetate was 91%.

The residue from the alcoholysis (gross activity 1·36 μc.) was fractionally distilled to give pure diethylene glycol diethyl ether. A sample of the latter was oxidised to carbon dioxide which had no measurable radioactivity.

Preparation of Ethyl Acetate.—The apparatus shown in Fig. 5 of Part I (preceding paper) was used. J_5 was lubricated with silicone grease and J_6 with ethyl sulphate. Finely powdered anhydrous $^{14}CH_3\cdot CO_2Na$ (0·510 g.; gross activity 0·233 μc., specific activity $1·87 \times 10^{-8}$) was charged into flask *M*, followed by freshly distilled ethyl sulphate (10 c.c.). With T_{12} closed, and T_{13}, T_{20}, and T_{21} open, a slow stream of dry nitrogen was passed through T_{21}; the bubbler tube *N* was below the liquid surface. Next, trap *I* was cooled to $-196°$ while *M* was immersed in an oil-bath initially at 160°. The temperature was raised during 10 minutes to 190° and there maintained; reaction began at 170° and after 15 minutes refluxing had almost ceased. After a further 10 minutes distillation was complete. The reaction vessel *M* was detached at J_4 and replaced by a stopper. Traps *O* and *S* (see Fig. 1) were attached to the manifold below taps T_3 and T_6; the system was evacuated and T_2 was closed. Trap *I* was warmed to $-78°$ and the contents were submitted to distillation (see preparation of ethanol) until the vapour pressure of the product had fallen to 0·1 mm. Hg, vapour being condensed into trap *O* by cooling it to $-196°$. Trap *I* was next warmed to 0° and the distillation was again carried out (in a 4-l. system); vapour was condensed into the detachable trap *S*. When the vapour pressure of the liquid in *I* had fallen to 0·5 mm. Hg, *S* was removed for weighing.

0·598 G. of product was obtained (theor., 0·547 g.), having v. p. (0°) 39·2 mm. Hg in a 500-c.c. system and 32·2 mm. Hg in a 1250-c.c. system. Yields with unlabelled materials were variable but always high (102—120% by weight). Sulphur dioxide was present. The product was finally sealed *in vacuo* in a break-seal tube.

Preparation of Ethanol from Ethyl Acetate.—The procedure was similar to that described for the reduction of acetyl chloride; the crude $^{14}CH_3\cdot CO_2Et$ (0·598 g.) was contained *in vacuo* in the break-seal tube *W*. Quantities were as follows: 1·86M-lithium aluminium hydride solution, 3·65 c.c.; diethylene glycol diethyl ether, 2·5 c.c.; ethylene glycol monophenyl ether, 10 c.c. Reaction was slow, so it was necessary at intervals (*a*) to condense ethyl acetate into *U* by cooling it to $-78°$ and then to melt and stir the reaction mixture, and (*b*) to cool trap *T* to $-196°$ and pump out permanent gas by opening T_{23}, after which T_{23} was closed and *T* warmed to room temperature. After 1½ hours the ethyl acetate had been absorbed. T_{23} was re-opened and the system pumped out for a few minutes, after which the normal procedure was followed. Vapour-pressure readings (at 0°) during single-plate distillation of the crude product were: 11·6, 11·1, 11·0, 10·9, 6·6, 2·6, 0·5 mm. Hg (~4-l. system). The yield of crude $^{14}CH_3\cdot CH_2\cdot OH$ containing hydrogen sulphide [n_D^{25} 1·3608; v. p. (0°) 14·5 mm. Hg and (17°) 40·6 mm. Hg] was 0·556 g. (Found: C, 51·0; H, 13·1. Calc. for C_2H_6O: C, 52·2; H, 13·15%). Part of the product (0·435 g.) was treated with dry Raney nickel for 10 minutes at room temperature. Recovery, by the usual vacuum techniques, gave 0·427 g. of ethanol [n_D^{25} 1·3604; v. p. (0°) 12·6 mm. Hg] free from hydrogen sulphide [Found: C, 51·0; H, 13·0. H_2O (Karl Fischer), 2·2%; specific activity $8·45 \times 10^{-9}$]. At the same percentage recovery, desulphurization of the whole of the ethanol would have given 0·546 g., with a gross activity of 0·196 μc., a recovery of ^{14}C of 84% (from $^{14}CH_3\cdot CO_2Na$). The overall weight yield was 93·5% (of *anhydrous* ethanol); the dilution with unlabelled material was ~122% (theor., 100%), corresponding to a fall in excess isotope concentration of 55%.

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CHEMICAL RESEARCH LABORATORY, D.S.I.R.,
TEDDINGTON, MIDDLESEX.

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