S 70. *The Microsynthesis of Some ^{14}C-Labelled Organic Compounds.*

By W. J. ARROL* and R. GLASCOCK.

Carbon dioxide can be converted in high overall yield into ethyl iodide on the 10—20-micromole scale, and on the same scale into ethylmagnesium iodide in moderate yield. The methods described are suitable for producing ethylmagnesium iodide, both carbon atoms of which are labelled with ^{14}C at the highest possible specific activity.

SYNTHESES now described were carried out between October 1946 and August 1948 with the object of developing techniques of microsynthesis with ^{14}C and also as steps in the labelling of a compound of biological interest. The compound chosen was stilboestrol in which it would be necessary to produce a molecule of the highest possible specific activity in order to study in animals the metabolism and mode of action of the substance at very low dose levels at which it is effectively œstrogenic. The final stages of the synthesis have not yet been carried out, but methods are described for the preparation of ethylmagnesium iodide fully labelled in the ethyl group, this being the intermediate used for introducing the second ethyl group in the commercial synthesis of stilboestrol.

The isotope ^{14}C is usually made available in the form of barium carbonate and is in an isotopic abundance of about 4%. The scale of 10—20 micromoles was originally chosen as being about the amount of one millicurie of isotopically pure $^{14}CO_2$. When it became known

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that the ^{14}C was unlikely to be available at more than 4% isotopic abundance, the scale was retained, as 50 microcuries of ^{14}C would still give about 50 rat doses of the final material at a specific activity probably high enough for its metabolism to be investigated.

The overall scheme for preparing ethylmagnesium iodide from labelled carbon dioxide is

$$C^*O_2 \xrightarrow[600^\circ]{\text{Ba at}} BaC^*_2 \xrightarrow{H_2O} C^*_2H_2 \xrightarrow{\text{reduction}} C^*_2H_4 \xrightarrow{HI} C^*_2H_5I \longrightarrow C^*_2H_5{\cdot}MgI$$

The best yield of Grignard reagent yet obtained in terms of the labelled carbon dioxide is about 40%.

EXPERIMENTAL.

Conversion of Carbon Dioxide into Acetylene.—We have already described this conversion by way of barium carbide (*J.*, 1948, 1534), the yield in this stage being generally above 90%.

Conversion of Acetylene into Ethylene.—Acetylene can be reduced almost quantitatively to ethylene by Traube's method (Traube and Passarge, *Ber.*, 1916, **49**, 1692; Patterson and du Vigneaud, *J. Biol. Chem.*, 1938, **123**, 327), in which it is shaken for an hour with a large excess of freshly prepared chromous chloride solution at room temperature. The chromous chloride is prepared by the rapid reduction with zinc wool of a solution of one part of green chromic chloride hexahydrate in two parts of 25% hydrochloric acid until the solution becomes almost a copper sulphate blue. About 0·4 ml. of this solution is pipetted into a glass reaction vessel (Fig. 1) frozen in liquid nitrogen and evacuated. The acetylene is condensed into the tube, which is then sealed off at *A* and allowed to warm to room temperature. Shaking of the solution is carried out for one hour by holding the vessel horizontally in a chuck attached to an electric motor and rotating it intermittently. After the reaction has been completed, the vessel is sealed on to the high-vacuum apparatus at *C*, and the break-seal *B* opened. All the dissolved gases are boiled out from the chromous chloride solution, and ethylene condensed at liquid nitrogen temperature (—196°) into the trap connected with a McLeod Gauge, water and any residual acetylene being held back in a U-tube trap at liquid oxygen temperature (—183°). The yield in terms of standard gas volume is 96—98% in this stage, and the remaining few units % of acetylene can be recovered from the oxygen-cooled trap. Ethylene can be identified by absorption into 22% mercuric sulphate in 22% sulphuric acid (Francis and Lukasiewicz, *Ind. Eng. Chem. Anal.*, 1945, **17**, 703).

FIG. 1.

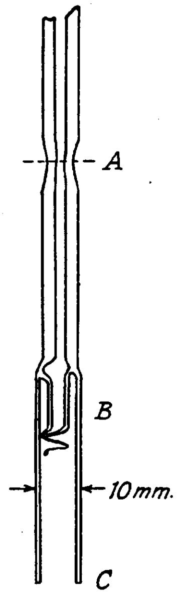


FIG. 2.

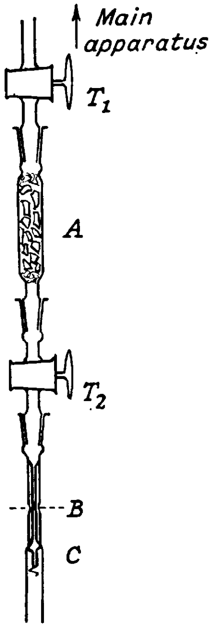
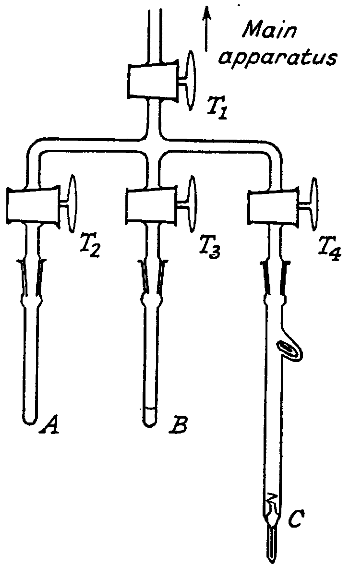


FIG. 3.



Catalytic reduction of acetylene to ethylene has been found to be possible on this scale by using palladium-black at room temperature, and yields are about 75%. The palladised palladium catalyst is easily poisoned, however, and chromous chloride reduction is to be preferred.

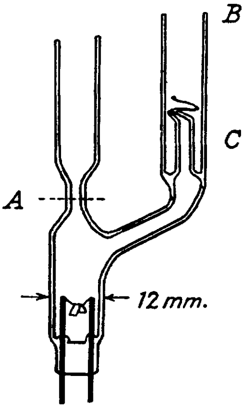
Conversion of Ethylene into Ethyl Iodide.—This conversion is effected by direct addition of hydrogen iodide. This reaction is not as easy to carry out as is usually supposed. It can be done, however, in 90—95% yield, on about the 10-micromole scale by heating ethylene with saturated hydriodic acid in a tube similar to that used for the reduction of acetylene but rather smaller. The capacity of this tube is arranged so that the ethylene will exert an initial pressure at laboratory temperature of about 0·5 atm. Hydrogen iodide is objectionable in the main high-vacuum apparatus, so a small ancillary apparatus (Fig. 2) is used for evacuating the reaction vessel. 0·1 Ml. of saturated hydriodic acid is pipetted into *C* and frozen in liquid nitrogen. The tube is evacuated through T_2 and T_1, through a tube of calcium metal *A* ; T_1 and T_2 are closed, *A* is removed, T_2 reconnected through T_1 to the main high-vacuum apparatus without unfreezing, and the ethylene condensed into *C*, which is then sealed off at *B*. *C* is heated for one hour at 144° (boiling *o*-xylene), and the reaction is then nearly complete.

Recovery of the ethyl iodide vapour is carried out in another ancillary vacuum line (Fig. 3). The reaction vessel is attached below T_4, the space above it is evacuated, and T_4 closed. The break seal is then opened. With T_1 and T_2 closed, and T_3 and T_4 open, the entire contents of *C* are distilled into 0·5 ml. of 50% sodium hydroxide in *B*, *C* being warmed with a flame to make sure that nothing is left; T_3 is then closed, and the contents of *B* boiled and cooled to $-78°$. At this stage, most of the ethyl iodide and residual ethylene are in the vapour phase and can be removed by cooling *A* in liquid nitrogen and with T_1 and T_4 closed and T_2 and T_3 being opened for 3 minutes. T_3 is then closed, the contents of *B* boiled and again cooled to $-78°$, and the vapour phase again condensed for 3 minutes into *A*. After four such extractions, all the ethyl iodide is in *A*, together with some ethylene and a trace of water. The ethylene is removed by cooling *A* to $-183°$, and distilling the ethylene into a liquid-nitrogen cooled trap connected with the McLeod gauge, where it is subsequently measured. The ethyl iodide is then dried by leaving it in contact for 5 minutes with about 100 mg. of anhydrous magnesium perchlorate, after which it is measured as vapour in the McLeod gauge. Ethyl iodide can be checked for purity by cooling it in the McLeod gauge trap to $-78°$ whereat it exerts a known and easily measurable vapour pressure. It can then be distilled off, and if no volatile impurities are present, the residue will continue to exert the same saturation vapour pressure until not enough remains for this. The McLeod gauge reading should not then change appreciably, even if the trap is allowed to warm to room temperature as would be the case if any condensable impurities were present.

Pure dry ethyl iodide vapour can be manipulated for short periods with greased stopcocks, but if it is to be stored, it should be transferred to a trap shut off with a mercury ventil, as otherwise it will be lost into the vacuum greases at an initial rate of about 5% per hour.

Conversion of Ethyl Iodide into Ethylmagnesium Iodide.—Still on about the same scale, this conversion is carried out by reaction in ethereal solution with a magnesium mirror in a small vessel (Fig. 4). This vessel has a platinum filament on which is hung a small piece of magnesium ribbon. It is dried thoroughly by baking out at 300° under vacuum for a total of about 30 hours, and a mirror is then thrown upon the walls of the tube by heating the filament electrically. About 150 standard microlitres of dry ethyl iodide vapour are condensed in, followed by 10 standard millilitres of very carefully dried ethyl ether vapour, after which the vessel is removed from the apparatus by sealing off at *A*. The reaction vessel is incubated at 37° for 2 hours. It is then re-sealed to the apparatus at *B*, and if the yield of Grignard reagent is to be determined, the break seal *C* is opened, all volatile contents of the tube are distilled off, and the water vapour is condensed on to the dry residue. Ethane formed is purified by removal of all possible contaminants, identified by measurement of its vapour pressure at liquid-oxygen temperature, and measured in the McLeod gauge. Yields of Grignard reagent on this scale are rather low, the best being about 55%. The incubation time of 2 hours at 37° is an optimum.

FIG. 4.



Radioactivity Measurements.—All the syntheses mentioned have been repeated with labelled carbon dioxide as starting material. Radioactivity was measured at the various stages by using an internal β-counter of Brown and Miller type (*Rev. Sci. Instr.*, 1947, **18**, 496) with carbon dioxide–carbon disulphide filling. Measurements of specific activity only were required, and the absolute activity could be adjusted so that a convenient counting rate was produced by the 10—20 micromoles of gas without the taking of aliquots. In using the counter it was found unnecessary always to burn the radioactive sample to carbon dioxide before introducing it into the counter filling. In the case of radio-acetylene, radio-ethylene, and radio-ethane, the sample could be introduced directly, as the amounts of hydrocarbons were small and no counts were lost owing to adsorption on the walls of the counter. A slight disadvantage in this procedure is a slow increase in background count. Over a period of several months the background of one counter increased from 70 counts/minute to about 140, this being reduced only to 120 by heating out at 300° under vacuum. This effect may be due to a ^{14}C contamination produced by slight decomposition of radio-acetylene and radio-ethylene in the discharge of the counter with the formation of polymerised material or elementary carbon. In general, the gas counter was found to behave very satisfactorily.

Apart from a 5% loss in specific activity at the stage, carbon dioxide ⟶ acetylene, there was no evidence of exchange reactions during the synthesis. The specific activity of radio-ethane produced from radio-ethylmagnesium iodide was that which was to be expected from the measured specific activity of the radio-acetylene from which it had been produced. This implies that in the conditions of the experiment there is no exchange between the radioactive ethyl iodide or ethyl magnesium iodide and the inactive ethyl groups of the ethyl ether used as solvent.

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