bromo-3-phenylpropane; octene-1, 1,1,1-trichloro-3-bromononane; vinyl acetate, 1-bromo-3,3,3-trichloropropyl acetate; isobutylene 1,1,1-trichloro-3-bromo-3-methylbutane; propylene, 1,1,-1-trichloro-3-bromobutane. It is assumed that the ethylene addition product is 1,1,1-trichloro-2-bromopropane. It is regarded as highly probable that the allyl chloride addition product is 1,1,1,4-tetrachloro-3-bromobutane.

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The Synthesis of Organic Compounds Labelled with Isotopic Carbon[1]

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The use of isotopes of carbon such as C^{13} and C^{14} for biological studies is limited by the present availability of relatively small quantities of isotope. Another difficulty arises from the fact that the synthesis of organic compounds labelled with isotopic carbon may frequently involve condensations which give low yields. In order to conserve isotope we have devised new or modified synthetic methods for the preparation of such compounds on a small scale. To study the metabolism of acetate,[3a,b,c] acetoacetate,[3a,b,c] pyruvate and glycine, the preparation of these substances from isotopic cyanide[4] and methane[5] was investigated.

The synthesis of carboxyl-labelled sodium acetate (I) from isotopic carbon dioxide and cyanide has been reported by Olsen, Hemingway and Nier[6] and Weinhouse, Medes and Floyd,[7] respectively. By trapping carbon dioxide in a large evacuated bulb (equipped with a dropping funnel and reflux condenser for preparing Grignard reagent) significant losses of carbon dioxide were avoided during the reaction. Acetic acid was isolated in yields of approximately 90% while only 5% of the original carbon dioxide failed to react and could be recovered.

For the preparation of ethyl acetoacetate labelled with C^{13} in the carbonyl and carboxyl positions, sodium acetate labelled in the carboxyl group was converted in excellent yields to ethyl acetate by refluxing with diethyl sulfate followed by subsequent distillation of the ethyl acetate. The procedure of Hudson, Dick and Hauser[8] was employed to obtain ethyl acetoacetate in yields of 26%. Thirty-five per cent. of the initial sodium acetate was recovered. The yield based on the sodium acetate consumed was 40%. Sodium acetoacetate (II) was obtained by hydrolysis with alkali in 91% yield (based on the ethyl acetoacetate used).

Sodium acetoacetate (III) labelled in the carbonyl position only was prepared by a modification of the Röttinger and Wenzel reaction.[9] The procedure as originally described by these authors gave rise to non-isotopic acetoacetate when the Grignard reagent prepared from ethyl bromoacetate was condensed with isotopic methyl acetate. When the preparation of the Grignard reagent was carried out in the presence of isotopic methyl acetate, however, the resulting ethyl acetoacetate was found to contain C^{13} located solely in the carbonyl position.

For the preparation of sodium acetoacetate (IV) containing C^{13} in the carboxyl group, isotopic ethyl bromoacetate was synthesized in 86% yield from carboxyl-labelled sodium acetate by adapting the method of Auwers and Bernhardi.[10] The Grignard reaction was then carried out with non-isotopic methyl acetate as above described. Both samples of ethyl acetoacetate, after conversion to sodium acetoacetate, were degraded to carbon dioxide and acetone by standard procedures. Isotope analyses were performed on these materials to determine the location of the C^{13}.

Glycine (V) containing C^{13} in the carboxyl position was synthesized in two ways. By condensation of isotopic ethyl bromoacetate with potassium phthalimide followed by the hydrolytic procedure described by Schoenheimer and Ratner,[11] glycine was obtained in 69% yield. The over-all yield from barium carbonate was 55%. Olsen, Hemingway and Nier[6] have reported the synthesis of isotopic glycine from methane by way of acetic acid, acetyl chloride, bromoacetyl chloride and bromoacetic acid. The over-all yield was 34% (recalculated) based on the methane used.

A better synthesis of glycine involving condensation of N-chloromethylphthalimide (A) with isotopic sodium cyanide was developed. Starting

(1) Taken in part from report 1B-40 issued by the Isotopes Branch, Research Division, Manhattan District.

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(3) (a) Buchanan, Sakami, Gurin and Wilson, *J. Biol. Chem.*, **157**, 747 (1945); (b) Buchanan, Sakami, Gurin and Wilson, *ibid.*, **159**, 695 (1945); (c) Buchanan, Sakami, Gurin and Wilson, *Federation Proc.*, **5**, 126 (1946).

(4) The isotopic cyanide was generously supplied by Dr. H. C. Urey.

(5) Separated by thermal diffusion.

(6) Olsen, Hemingway and Nier, *J. Biol. Chem.*, **148**, 611 (1943).

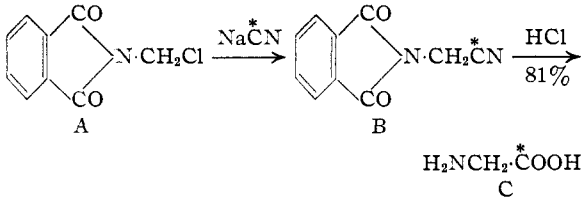
(7) Weinhouse, Medes and Floyd, *ibid.*, **158**, 411 (1945).

(8) Hudson, Dick and Hauser, THIS JOURNAL, **60**, 1960 (1938).

(9) Röttinger and Wenzel, *Monatsh.*, **34**, 1867 (1913).

(10) Auwers and Bernhardi, *Ber.*, **24**, 2219 (1891).

(11) Schoenheimer and Ratner, *J. Biol. Chem.*, **127**, 301 (1939).



* Designates carbons labelled with C^{13}.

with 0.5 g. of isotopic sodium cyanide, 0.81 g. of glycine was obtained representing a yield of 81% (based on the isotopic sodium cyanide employed).

For the synthesis of carboxyl-labelled sodium pyruvate (VI) lactic acid was prepared from acetaldehyde and isotopic sodium cyanide by the cyanohydrin reaction.[12] As a result of several modifications, a 95% yield of sodium lactate from sodium cyanide was obtained. The lactic acid was converted to butyl lactate, oxidized with aqueous acid permanganate to butyl pyruvate[13] and the resulting product hydrolyzed by titration with sodium hydroxide. Calculated on the basis of the $NaC^{13}N$ employed, the yield of sodium pyruvate was 50–55%.

Pyruvic acid (VII) labelled in the alpha and beta positions was synthesized in similar fashion from appropriately labelled lactic acid.

Experimental

Barium Carbonate.—The isotopic methane was forced through a micro burner fitted into a glass candle and burned in a continuous stream of oxygen. A kovar wire lead was sealed into the candle so that a continuous spark was obtained at the tip of the burner by means of a 5000-volt 60-cycle transformer thus assuring safe uninterrupted burning of the methane. The carbon dioxide evolved was absorbed in 10% aqueous sodium hydroxide and converted to barium carbonate by addition of aqueous barium chloride. The yield of barium carbonate from methane by this process ranged from 85–95%.[14]

Sodium Acetate (I).—A 3-liter glass bulb equipped with a dropping funnel and side-arm was employed in this synthesis. The generator for $*CO_2$ which consisted of a 50-ml. capacity test-tube equipped with a dropping funnel and side-arm was attached through a tube containing anhydrone to the side-arm of the bulb and the entire apparatus evacuated with an oil pump. The carbon dioxide was generated from isotopic barium carbonate by slowly dropping in 40% perchloric acid and the bulb then closed off from the generator by means of a stopcock. The methylmagnesium iodide was prepared in the funnel. Magnesium turnings, iodine and ether were introduced, the funnel fitted with a short West condenser and the methyl iodide introduced through the top. After the reagent had been prepared, the funnel was stoppered and the entire apparatus chilled in the refrigerator to −15°. The reagent was slowly admitted into the bulb and the reaction bulb shaken or rotated until the solution had congealed. The reaction mixture was left in the cold room overnight and worked up for acetic acid in the usual manner. The acetic acid was partitioned between water and ethyl ether (K_5, 82.2, theory, 82.1) and neutralized carefully with sodium hydroxide. From 10 g. of barium carbonate containing 3.05 atom per cent. excess C^{13}, 3.67 g. of fused sodium acetate was obtained assaying 1.50 atom per cent. excess C^{13}.

Sodium Acetoacetate (II).—Isotopic sodium acetate (3.88 atom per cent. excess C^{13}) prepared as described above was employed for the synthesis of acetoacetate. Fused sodium acetate (2.4 g.) was refluxed with 8 ml. of diethyl sulfate for twenty minutes and the ethyl acetate then distilled out (oil-bath at 100–150°). The yields were usually quantitative. The ethyl acetate was then redistilled directly into an ethereal solution of sodium triphenylmethane according to the procedure of Hudson, Dick and Hauser.[8] Ethyl acetoacetate was worked up by the usual procedures and fractionally distilled in a slow stream of nitrogen. Ethyl acetoacetate (0.55 g.) was obtained (29% yield based on the sodium acetate used). The product was hydrolyzed by standing for six hours with a slight excess of 1 *N* sodium hydroxide according to Ljunggren.[15] The excess alkali was neutralized and the preparation freed of ethanol by lyophilization. Analysis by the procedure of Lorber[16] indicated that the yield of sodium acetoacetate from ethyl acetoacetate was 91%. The acetoacetate was degraded to carbon dioxide and acetone.[8b] The carbon dioxide contained 7.60 atoms per cent. excess C^{13} while the acetone was found to contain 2.53 atoms per cent. excess. The isotope content of the acetoacetate was accordingly 3.80 atoms per cent. excess.

Sodium Acetoacetate (III).—Isotopic potassium acetate (6.5 g.) was used and converted to methyl acetate by heating with 15 ml. of dimethyl sulfate according to the procedure of Graebe.[17] The yield of redistilled methyl acetate was quantitative. The methyl acetate diluted with 15 ml. of anhydrous ether along with 3 g. of magnesium turnings was placed in a 50-ml. round-bottom flask equipped with a side-arm dropping funnel and reflux condenser. The stirrer extended through the bore of the condenser. Ethyl bromoacetate (7.4 ml.) was added dropwise with gentle warming to start the reaction, which was maintained at a gentle boil by controlling the rate of addition of the ethyl bromoacetate. The product was isolated in the usual manner. The ethyl acetoacetate weighed 0.80 g., and upon analysis by the procedure of Lorber,[16] was found to be 88% pure. Upon analysis for C^{13}, the carbon dioxide was found to contain no excess C^{13} while the acetone contained 0.40 atom per cent. excess (carbonyl = 0.4 × 3 = 1.20). Since the original acetate used in this synthesis contained 0.65 atom per cent. excess C^{13}, theory requires 1.30 atom per cent. excess C^{13} in the carbonyl group.

Sodium Acetoacetate (IV).—Isotopic sodium acetate was converted into ethyl bromoacetate by the procedure of Auwers and Bernhardi.[10] From 6 g. of sodium acetate, 10.5 g. of ethyl bromoacetate boiling at 172° was obtained (86% yield). The Grignard reagent was prepared from this substance in the presence of non-isotopic methyl acetate as described above. The isolated acetoacetate was degraded and analyzed for C^{13}. The resulting carbon dioxide contained 0.86 atom per cent. excess C^{13} while the acetone contained 0.02 atom per cent. excess.

Glycine (V).—N-Hydroxymethylphthalimide (22.5 g.) prepared by the procedure of Sachs[18] was allowed to stand for two hours with excess sulfurous oxychloride. The mixture was heated for thirty minutes on a boiling water-bath, excess sulfurous oxychloride removed by evaporation, and the residue recrystallized from toluene. N-Chloromethylphthalimide (A) (19 g.) melting at 133–134° was obtained (yield 77%). Recrystallization from hot dioxane raised the melting point to 134–135°.

To a solution of 0.50 g. of isotopic sodium cyanide (8.70 atom per cent. excess C^{13}) in 25 ml. of acetone-free methanol was added 2.0 g. of N-chloromethylphthalimide dissolved in 8 ml. of warm dioxane. After standing two hours the mixture was evaporated to dryness and the residue successively extracted with 15, 10 and 5-ml. portions

(12) Cramer and Kistiakowsky, *J. Biol. Chem.*, **137**, 549 (1941).

(13) Byk-Guldenwerke, *Chem. Fabrik*, German Patent 526,366, *Chem. Zentr.*, **102**, II, 768 (1931).

(14) Isotope analyses for C^{13} were carried out on a mass-spectrometer manufactured by the Consolidated Engineering Corporation. The error of the method is 1–2%.

(15) Ljunggren, *Biochem. Z.*, **145**, 425 (1924).

(16) Lorber, *ibid.*, **181**, 366 (1929).

(17) Graebe, *Ann.*, **340**, 244 (1905).

(18) Sachs, *Ber.*, **31**, 1225, 3230 (1898).

of warm dioxane. The extracts were combined and evaporated to dryness. The resulting material was hydrolyzed by heating under reflux for fifteen hours with a mixture of 11 ml. of acetic acid, 12 ml. of concentrated hydrochloric acid and 13 ml. of water. After chilling to 0°, phthalic acid was removed by filtration and the filtrate evaporated to dryness *in vacuo*. The residue was dissolved in water and chloride removed with excess silver carbonate. The combined filtrate and washes were evaporated to approximately 25 ml. and saturated with hydrogen sulfide. After removal of silver sulfide by filtration, the filtrate and washes were evaporated to dryness, the residue taken up in a few ml. of water and glycine precipitated by means of 95% ethanol. The product after washing with 95% ethanol was dried (m. p. 238–239°). The yield was 0.62 g. (81% yield based on the isotopic sodium cyanide used) of glycine containing 4.27 atom per cent. excess C^{13}. The molecular weight determined by perchloric acid titration was 75.2 (theory 75.1). A Nessler test indicated the presence of a trace of ammonia.

Sodium Pyruvate (VI).—One gram of isotopic sodium cyanide (8.87 atom per cent. excess C^{13}) was dissolved in 4 ml. of water. The solution was cooled to 0°, 10.3 ml. of ice-cold 2.15 *N* sulfuric acid were added, followed by 1.4 ml. of acetaldehyde and 2.0 ml. of 1.00 *N* sodium hydroxide solution. After thorough shaking, the mixture was allowed to stand at room temperature for twenty minutes. Forty ml. of concentrated hydrochloric acid (d. 1.18) were added, and the mixture rapidly shaken and boiled for six minutes. The product was diluted with 100 ml. of water, cooled and neutralized with saturated sodium hydroxide. An excess of sodium hydroxide was added, the solution distilled until the ammonia was completely removed, and the remaining solution exactly neutralized with sulfuric acid. The solution was evaporated until it started to bump. Fifty ml. of toluene was then added, and the remaining water was removed as the heterogeneous azeotrope by continuous distillation utilizing a water trap. The residue after evaporation of remaining toluene was extracted with three 50-ml. portions of absolute ethanol. The solvent was distilled off, and the residue extracted with 15 ml. of absolute alcohol. The solid material was removed by centrifugation and extracted with three 1-ml. portions of absolute alcohol. The solutions were combined and the solvent was removed by distillation; the last traces were removed by allowing to stand twenty-four hours in an evacuated desiccator. Based on the sodium cyanide employed, the yield of sodium lactate was 94–96% as determined by the method of Friedemann and Graeser.[19] The isotope content was 2.93 atom per cent. excess C^{13}.

For conversion to the butyl ester, the anhydrous salt was treated with 11 ml. of 2.5 *N* sulfuric acid in butyl alcohol followed by addition of benzene. The mixture was boiled for twelve hours using a water trap to collect water formed in the reaction. After removal of the benzene by distillation the excess sulfuric acid was neutralized with sodium *n*-butylate in *n*-butyl alcohol. The blue endpoint of benzene-azo-α-naphthylamine (0.1% in butyl alcohol) is satisfactory for this titration. The sodium sulfate was removed by centrifugation, washed with butyl alcohol and the combined solution distilled *in vacuo* from a small quantity of non-volatile gum. The distillate was fractionally distilled at atmospheric pressure through a small fractionating column equipped with an outer jacket which could be maintained at a temperature of approximately 120°. The yield of butyl lactate from sodium lactate is about 80%.

Butyl lactate (1.94 g.) was oxidized to pyruvate by addition to an ice-cold solution consisting of 26.14 ml. of 0.167 *M* potassium permanganate and 4.44 ml. of 1.96 *N* sulfuric acid. Thirty ml. of cold water were added with shaking and the solution allowed to stand for three hours at 18–20°. The solution was then chilled, made alkaline with 5 ml. of cold sodium carbonate solution and repeatedly extracted with ether. The ether was dried over calcium chloride, evaporated, and the residual oil distilled at a bath temperature of 190–205°. The yield of butyl pyruvate was 1.41 g. Analyzed by the method of Fromageot and Desneuille,[20] it was 95% pure. Sodium pyruvate was obtained by titrating butyl pyruvate with 1 *N* sodium hydroxide at room temperature under which condition rapid hydrolysis occurs. The butyl alcohol was extracted with ether and the aqueous solution freed of ether by aeration.

Sodium Pyruvate (VII).—Isotopic barium carbide was prepared essentially by the process of Cramer and Kistiakowsky.[12] Dry barium carbonate (5.70 atom per cent. excess C^{13}) and magnesium were intimately ground together and placed in a bomb[21] constructed of iron and fitted with an inlet and outlet tube by means of which it could be swept out with hydrogen. The bomb was heated over a Meeker burner to red heat for ten minutes, cooled and the barium carbide converted to acetylene. Isotopic acetylene was collected in an evacuated bulb (described above in the synthesis of acetic acid), converted to acetaldehyde and finally to lactic by the procedure described above. The lactic acid labelled in the alpha and beta positions with C^{13} was converted into pyruvic acid by the procedure employed with the carboxyl-labelled compound. Analysis for C^{13} indicated an excess of 3.60 atom per cent.

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Summary

Procedures have been developed for the small-scale production of the following substances containing an excess of the stable isotope of carbon C^{13}: CH_3C^*OOH, CH_3COC^*OOH, $^*CH_3C^*OCOOH$, $CH_3C^*OCH_2C^*OOH$, $CH_3C^*OCH_2COOH$ and $CH_3COCH_2C^*OOH$.

Isotopic glycine labelled in the carboxyl group has been prepared in 81% yield from isotopic sodium cyanide.

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(19) Friedemann and Graeser, *J. Biol. Chem.*, **100**, 291 (1933).

(20) Fromageot and Desneuille, *Biochem. Z.*, **279**, 174 (1935).

(21) We wish to thank Dr. H. G. Wood for suggesting the use of the bomb and for details concerning its construction.