[CONTRIBUTION FROM THE CHEMICAL RESEARCH LABORATORY OF TRACERLAB, INC.]

Syntheses of Benzene, Toluene and Benzoic Acid Labeled in the Ring with Isotopic Carbon

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A satisfactory synthesis is described for preparation of ring-labeled toluene, benzoic acid and benzene from isotopically-labeled barium carbonate.

In an earlier communication[2] we outlined an efficient procedure for the introduction of isotopic carbon into benzene and into a specific position in the ring of toluene and benzoic acid. In addition to the condensation of acetone-2-C^{14} to mesitylene-1,3,5-C_3^{14} described earlier,[3] the syntheses of ring-labeled benzene derivatives which have been described since our preliminary report include the preparation of toluene-1,3,5-C_3^{14} from pyruvic acid through uvitic acid-1,3,5-C_3^{14} [4] and of benzene-C^{14} by dehydrogenation of cyclohexane which was obtained by the rearrangement of cyclopentyl-(carbinol-C^{14}) and methylcyclopentane-1-C^{14}.[5]

The specific activity of the products from each of these routes is limited not by the specific activity of the carbonate supplied but by the total quantity of isotopic carbon available for the synthesis and by the degree of refinement attained in carrying out on a small scale the troublesome steps which are involved in each of the syntheses presently available.

In this communication we have described the details of the synthesis of ring-labeled intermediates by reaction of ethyl acetate-1-C^{14} with the di-Grignard reagent from pentamethylene dibromide. The yield of 1-methylcyclohexanol was substantially increased over that reported by Grignard and Vavon[6] by employing a considerable excess of the Grignard reagent. In addition to the *t*-carbinol there was formed a considerable quantity of high boiling residue from which it was convenient to remove the last traces of labeled methylcyclohexanol by addition of the inactive alcohol followed by distillation.

Dehydration of the resulting 1-methylcyclohexanol was followed by dehydrogenation of the 1-methylcyclohexene to toluene. Dehydrogenation of 1-methylcyclohexene over 30% platinum- or palladium-on-asbestos at 350° in the usual manner[7,8] led to an initial rapid evolution of hydrogen which ceased after about 65 to 80% of the theoretical quantity had been collected. Batches of catalyst prepared under a variety of conditions showed similar behavior and gave a product which decolorized permanganate, had a low refractive index and was presumably a mixture of toluene, methylcyclohexene and methylcyclohexane. The use of a base metal dehydrogenation catalyst at 450°, however, led to the evolution of the theoretical quantity of hydrogen and the production of pure toluene.

Benzoic-1-C^{14}-acid, from oxidation of the labeled toluene with permanganate, was converted to benzene by decarboxylation.

Experimental

Ethyl Acetate-1-C^{14}.—Sodium acetate-1-C^{14} was prepared in 95% yield by carbonation of 78.0 mM. of methylmagnesium iodide with 63.6 mM. of carbon dioxide containing 70.0 mc. of C^{14}. Reaction of the dry salt with triethyl phosphate according to the procedure of Ropp[9] afforded 57.2 mM. (95% yield) ethyl acetate-1-C^{14} with a specific activity of 1.10 mc./mM. This and the other radioassays reported were performed by combustion of a small aliquot of the product and measurement of the radiocarbon content in a gas counting apparatus of the type described by Janney and Moyer.[10] Calibration of the gas counting system was achieved by use of a National Bureau of Standards carbon-14 standard.

1-Methylcyclohexanol-1-C^{14}.—The Grignard reagent was prepared from 8.75 g. of magnesium turnings (360 mM.) and 39.4 g. (171 mM.) of redistilled pentamethylene dibromide in 215 ml. of anhydrous ether. The Grignard reagent, insoluble in ether, separates as an oil. The use of an ether-benzene mixture to dissolve the Grignard reagent appeared to offer no advantage since the yield of carbinol was not increased and its isolation was made somewhat more difficult.

To the vigorously stirred suspension of Grignard reagent cooled in an ice-bath was added slowly a solution of 5.04 g. (57.2 mM., 63.0 mc.) of ethyl acetate-1-C^{14} in 60 ml. of dry ether. The ice-bath was removed when the addition was complete and the reaction mixture was stirred overnight at room temperature and then refluxed for one hour.

The Grignard complex was decomposed with saturated ammonium chloride solution. After separation of the main ether layer, the aqueous phase was extracted several times with ether. The combined ether extracts, washed with water until neutral, were dried over calcium sulfate and distilled. Distillation of the residue afforded 5.27 g. (46.2 mM.) of 1-methylcyclohexanol-1-C^{14}, b.p. 62–66° (25 mm.), suitable for dehydration.

Since the quantity of methylcyclohexanol retained in the high boiling residue and in the distillation apparatus represents an appreciable fraction, particularly in the small scale preparation of high activity product, two additional batches of lower activity carbinol were distilled through the same apparatus. Finally the last traces of activity were removed by distillation of 10 g. of non-radioactive methylcyclohexanol through the apparatus. The various fractions were individually carried through the reaction sequence and a similar stripping technique was employed in the dehydration and dehydrogenation steps.

1-Methylcyclohexene-1-C^{14}.—1-Methylcyclohexanol-1-C^{14}, 5.27 g. (46.2 mM.) was transferred to a small flask attached to a 9″ Podbielniak column after addition of a small crystal of iodine.[11] The flask was immersed in an oil-bath maintained at 135–140°. Dehydration proceeded smoothly and a mixture of water and methylcyclohexene slowly distilled. After removal of the water layer, the olefin was carefully dried over calcium hydride. Distillation afforded 2.11 g. (22 mM., 38.5% over-all from ethyl acetate) of 1-methylcyclohexene-1-C^{14}, b.p. 103–106°. A refractive index of $n^{20}D$ 1.4495 was observed in a typical dummy run for the product obtained at this stage; reported $n^{20}D$ 1.4497.[12]

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[1] Electrochemicals Dept., E. I. du Pont de Nemours and Co., Niagara Falls, New York.

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Toluene-1-C^{14}.—The dehydrogenation apparatus, similar to that described by Ruzicka,[13] consisted of a pot, catalyst tube (90 cm. long attached to a side arm equipped with a stopcock so as to allow the product to be recycled), and a reflux condenser. A 3-l. gas buret connected to the condenser served to measure the volume of evolved hydrogen, which was collected over water.

The catalyst tube, charged with a 70-cm. column of pellets of dehydrogenation catalyst,[14] was heated slowly to 450° while a stream of dry hydrogen was passed slowly through the system. After 20 hours the hydrogen flow was interrupted and the system was evacuated to 0.1 mm. until no more water was evolved. Finally dry hydrogen was admitted to bring the system to atmospheric pressure.

1-Methylcyclohexene-1-C^{14}, 2.11 g. (22.0 mM.) was added to the pot and slowly distilled through the catalyst bed at 450°. Hydrogen evolution, rapid at first, practically ceased after the second or third cycle over the catalyst. In all 926 ml. (41.5 mM.) of dry hydrogen (S.T.P.) was collected; calculated 985 ml. (44.0 mM.); the pure toluene obtained in the dehydrogenation amounted to 1.91 g. (95% yield) including a small quantity isolated from the hot catalyst bed by evacuation at 0.1 mm.

The toluene isolated in a typical dummy run had a refractive index n^{29}D 1.4908 as compared with n^{29}D 1.4892 observed for an authentic sample.

Benzoic-1-C^{14} Acid.—Oxidation of 1.91 g. of toluene-1-C^{14} with 7.80 g. of potassium permanganate[15] in 96 ml. of distilled water and reduction of the manganese dioxide with sulfur dioxide in the usual manner afforded 1.87 g. (15.4 mM., 74% yield) of benzoic-1-C^{14} acid, m.p. 122.6–123.0° cor.; a sample of pure benzoic acid melted at 123.2–123.6°. The product had a specific activity of 1.07 mc./mM., in excellent agreement with the specific activity of the initial carbon dioxide.

In the small scale run the over-all radiochemical and weight yields of benzoic acid from carbon dioxide amounted to 24.2%; the actual yield is somewhat higher, however, since quantitative recovery of methylcyclohexanol and methylcyclohexene was not attempted here and the remaining active products were recovered from the distillation apparatus and residues by the carrier technique. In two other runs, 131 and 149 mmoles of carbon dioxide containing 53.0 and 68.0 mc. of C^{14} were converted to benzoic-1-C^{14} acid in 36.7 and 24.6% yields, resp., when the syntheses were performed without further dilution. In the three preparations the over-all yield of benzoic-1-C^{14} acid obtained from 191 mc. of carbon dioxide C^{14} amounted to 64.6 mc. (33.8% yield) including the benzoic acid of lower specific activity isolated by processing all of the stripping fractions.

Benzene-C^{14}.—Heated under nitrogen in a distilling flask with 20 ml. of freshly distilled quinoline with 4.0 g. of powdered copper oxide, 10.0 g. of benzoic-1-C^{14} acid underwent smooth decarboxylation at 260°. After purification by drying over phosphorus pentoxide and distillation there was obtained 5.5 g. of benzene, $n^{24.5}$D 1.4978; observed for an authentic sample, $n^{24.5}$D 1.4971. The product from a representative dummy run had a b.p. 80–81°.

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[13] L. Ruzicka and M. Stoll, *Helv. Chim. Acta*, **7**, 84 (1924).

[14] Universal Oil Products "Dehydrogenation Catalyst."

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[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF NORTHWESTERN UNIVERSITY]

Preparation of Peptides Containing Cysteine

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N-(N-Phenylacetyl-L-cysteinyl)-glycine and N-(N-phenylacetyl-L-cysteinyl)-D-valine were prepared. The procedures employed and some of the properties of the intermediates are described.

N-(N,N′-Dicarbobenzoxy-L-cystinyl)-diglycine (III) was prepared[1] and converted to N-(S-benzyl-L-cysteinyl)-glycine (IV).[2] IV could be crystallized from water as recommended by Loring and du Vigneaud[2] providing the temperature of the water was kept below 80°. Above 80° IV was converted rapidly to L-benzylmercaptomethyldiketopiperazine (V). Compound IV reacted with phenylacetyl chloride to give N-(N-phenylacetyl-S-benzylcysteinyl)-glycine (VI). On treatment of VI with sodium in liquid ammonia N-(N-phenylacetyl-L-cysteinyl)-glycine (I) was obtained. The homogeneity of I was established by analysis and mercapto group determination.[3]

In a similar series of reactions, N-(N-phenylacetyl-L-cysteinyl)-D-valine (II)[4,5] was prepared. Thus, N,N′-dicarbobenzoxy-L-cystinyl chloride[1] was converted to N-(N,N′-dicarbobenzoxy-L-cystinyl)-di-D-valine (VII). VII was converted to N-(S-benzyl-L-cysteinyl)-D-valine (VIII), and VIII was converted to N-(N-phenylacetyl-S-benzyl-L-cysteinyl)-D-valine. This latter substance was obtained only as a sirup, but the sirup on reduction with sodium and liquid ammonia gave crystalline II.

Attempts to prepare I or II directly from N-acylated cysteine or N,N′-diacylated cystine were not successful. The step involving formation of the acid chloride or azlactone always failed to give a characterizable product. Some of the difficulties involved are of general interest and are reported herein.

N-N′-Diphenylacetyl-L-cystine (IX) was prepared from phenylacetyl chloride and L-cystine by the method of Shiple and Sherwin.[6] When crystallized from water the product melted at 119–121°, in agreement with the report of Shiple and Sherwin.[6] However, when crystallized from ethyl acetate by addition of ligroin the product melted at 159–161°. Each form of the compound had a neutralization equivalent in agreement with that calculated for IX and the two forms had identical specific rotations.

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[3] J. W. Kimball, R. L. Kramer and E. E. Reid, THIS JOURNAL, **43**, 1199 (1921).

[4] It was of interest to use D-valine since penicillamine is of the D-series. See H. T. Clarke, J. R. Johnson and R. Robinson, "The Chemistry of Penicillin," Princeton University Press, Princeton, N. J., 1949, p. 6.

[5] The resolution of valine using the method of E. Fischer, *Ber.*, **39**, 2320 (1906), was improved by using toluene and distilling the azeotrope. With this technique the reaction time was reduced from 12 hours to three hours and 90% formic acid could be used.

[6] G. J. Shiple and C. P. Sherwin, *J. Biol. Chem.*, **55**, 671 (1923).