ily at 0° to give 1-phenyl-1-chloroethane and 2-chloro-2-methyl-1-phenylpropane, respectively. Since the addend thus disposes itself oppositely upon these two alkenes it is of interest to consider the addition rate and disposition of hydrogen chloride to propenylbenzene (III), which is intermediate in type between I and II.

The elements of hydrogen chloride have been added to propenylbenzene previously[3] but, since aluminum chloride was used in the reaction and the products were not completely accounted for (our repetition gave a 66% yield), it is not possible to compare this addition with that of styrene and 2-methyl-1-phenyl-1-propene. Consequently we have imposed an identical condition of reaction (0° and 18 hours) on all three alkenes and find that no addition to propenylbenzene occurs. Only when the system is sealed and allowed to remain at 25° for four days can 43% of the chloride be obtained, the remainder being unchanged propenylbenzene.

In view of the opposite disposition of hydrogen chloride to styrene and 2-methyl-1-phenyl-1-propene we have sought to ascertain whether the chloride from propenylbenzene is a mixture. Like the product reported by Shamshurin ours seems to be 1-chloro-1-phenylpropane. In order to make certain that it is not a mixture we have prepared a Grignard reagent from it and then have treated it with carbon dioxide. This process yields only 2-phenylbutanoic acid, and proves that a detectable amount of 2-chloro-1-phenylpropane is not present. It is necessary to employ this characterization *via* the Grignard reaction because the hydrolytic conditions by which 1-chloro-1-phenylethane and 2-chloro-2-methyl-1-phenylpropane may be converted to the alcohols cause partial dehydrohalogenation of 1-chloro-1-phenylpropane.

Experimental[4]

1-Chloro-1-phenylethane.—A solution of 6.40 g. (0.0615 mole) of styrene (b.p. 48° at 19 mm.) in 50 ml. of anhydrous peroxide-free diethyl ether at 0–1° was saturated with dry hydrogen chloride and then allowed to remain at 0° for 18 hours. Distillation yielded 7.30 g. (84%) of 1-chloro-1-phenylethane, b.p. 74–75° (14 mm.), $n^{20}D$ 1.5273, d^{20}_4 1.058. This halide was characterized by hydrolysis with 1% aqueous sodium hydroxide to an 85% yield of 1-phenylethanol; the crude urethan melts at 104–105° and thus is essentially pure.

Under comparable conditions the yield of 2-chloro-2-methyl-1-phenylpropane from 2-methyl-1-phenyl-1-propene was only 18% (62% after 40 hours) and no detectable amount of 1-chloro-1-phenylpropane was obtained from propenylbenzene during 40 hours.

1-Chloro-1-phenylpropane.—A solution of 23.6 g. (0.20 mole) of the equilibrium mixture (14:86) of *cis*- and *trans*-propenylbenzene (b.p. 59–61° at 14 mm., $n^{20}D$ 1.5494) in 140 ml. of anhydrous peroxide-free ether was saturated at 0° with dry hydrogen chloride and then sealed off. After four days at 25° the solution was distilled. The first fraction, b.p. 54–72° (10 mm.), $n^{20}D$ 1.5409, 13.5 g., represents 57% of impure recovered propenylbenzene. On redistillation, 11.9 g., b.p. 58–62° (11 mm.), $n^{20}D$ 1.5492, m.p. −38 to −34°, was obtained; a mixture melting point with the equilibrium mixture of *cis*- and *trans*-propenylbenzene was now lowered.

The second fraction from distillation of the reaction system boiled at 72–79° (10 mm.), $n^{20}D$ 1.5246, and weighed 13.2 g., and is thought to be a 43% yield of 1-chloro-1-phenylpropane.

2-Phenylbutanoic Acid.—A Grignard reagent was prepared during seven hours from 8.6 g. (0.35 atom) of magnesium, 250 ml. of ether and 11.0 g. (0.071 mole) of the 1-chloro-1-phenylpropane obtained as described above. After this reagent was saturated with carbon dioxide it was hydrolyzed with 200 ml. of 12% hydrochloric acid. After the etherous layer was extracted with alkali and then evaporated, the residue (1.4 g.) was halogen-free.

The chilled alkaline extract was acidified with hydrochloric acid and extracted thrice with 30-ml. portions of ether. The water-washed extract, dried with magnesium sulfate, was distilled, finally at 148–149° (10 mm.), 9.14 g. (78.4%), m.p. 39–42°. Crystallization from petroleum ether raised this m.p. of 2-phenylbutanoic acid to 42.0–43.0°. It was characterized by conversion to its amide (m.p. 83.5–84.8°) in 65% yield.

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(3) A. A. Shamshurin, *Trudy Uzbekskogo Gosudarst Univ. Sbornik Rabot Khim.*, **15**, 75 (1939); *C. A.*, **35**, 3984 (1941).

(4) Melting points have been corrected against reliable standards.

The Biosynthesis of Radioactive Senecioic Acid (β-Methylcrotonic Acid) in Particle-free Extracts of Rat Liver[1]

BY JOSEPH L. RABINOWITZ

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During a study of precursors in cholesterol biosynthesis to be published elsewhere,[2] it became apparent that senecioic acid (β-methylcrotonic acid) or closely related substances might be involved. Experiments were undertaken in which 1-C^{14}-acetate was added to aqueous, particle-free extracts of rat liver along with carrier non-radioactive senecioic acid. After incubation, senecioic acid was recovered and found to be radioactive.

The production of senecioic acid by rat liver may well be accounted for as a result of a decarboxylation and dehydration of β-hydroxy-β-methylglutaric acid[2] which has been shown recently to be formed by rat liver. The role of senecioic acid in the biosynthesis of cholesterol is as yet unknown. It has been frequently conjectured that senecioic acid may be a precursor of terpenes.[3–6]

The method of isolation, extraction and preparation of derivatives is described in the Experimental part. The results are presented in Table I.

TABLE I

INCORPORATION OF 1-C^{14}-ACETATE INTO CHOLESTEROL AND SENECIOIC ACID BY AQUEOUS EXTRACTS OF RAT LIVER

	Radioactivity recovered		
Expt.	Senecioic acid, c.p.m./mg. C	Cholesterol, c.p.m./mg. C	Dibromoisovaleric acid, c.p.m./mg. C[b]
1	437	137	473
2	784	238	lost
3[a]	856	112	884
4	234	122	297

[a] No carrier (senecioic acid) during incubation. [b] Corrected for dilution.

Experimental

Aqueous, particle-free extracts (7 ml.) of rat liver[2] were incubated with 1 mg. each of adenosine triphosphate, diphosphorpyridine nucleotide and 1-C^{14} potassium acetate

(1) The radioactive materials were obtained on allocation from the United States Atomic Energy Commission.

(2) J. L. Rabinowitz and S. J. Gurin, *Biol. Chem.*, in press (1954).

(3) R. E. Kremers, *J. Biol. Chem.*, **50**, 31 (1922).

(4) A. A. Prokofiev, *Bull. Acad. Sci. U. S. S. R.*, 908 (1939).

(5) A. Kuzin and N. Nelirajeva, *Biokhimia*, **6**, 261 (1941).

(6) J. Bonner and A. W. Galston, *Botanical Rev.*, **13**, 581 (1947).

(1.032 × 10^6 c.p.m./μmole). The gas phase was 95% O_2–5% CO_2, and the time of incubation was 3 hours at 34°. The conditions used were those found to be optimum for cholesterol biosynthesis.[2] To each preparation 5 mg. of senecioic acid was added prior to incubation. Following the incubation period, 15 mg. of senecioic acid and 0.5 mg. of cholesterol were added to each flask. Each specimen was saturated with KCl acidified to *p*H 2 with phosphoric acid, and extracted continuously with ether for 20–30 hours. The ether solution was evaporated to dryness and kept in a desiccator over KOH for 24 hours. The dark oily residue was extracted with a few ml. of 10% acetic acid and again extracted continuously with ether. The ether extract was afterwards evaporated to dryness. The second oily residue was transferred to a vacuum microsublimation apparatus and heated at 60° at 3 mm. for 48 hours. A colorless crystalline sublimate was obtained. The product (8–12 mg.) melted at 68–70°. A mixed melting point with authentic senecioic acid showed no depression. The sublimed acid was plated and assayed for radioactivity; the counts were corrected to infinite thinness. The acid was subsequently converted to α,β-dibromoisovaleric acid[7] and recrystallized (m.p. 105–107°). A mixed melting point with authentic dibromoisovaleric acid showed no change. Both the radioactive senecioic acid and α,β-dibromoisovaleric acid were diluted with carrier and recrystallized. Subsequent radioassay indicated the expected reduction in activity. Cholesterol was isolated as the digitonide and plated.[2] The slight discrepancy between the activities of the original senecioic acid and the dibromo derivative is within the range of counting errors. The average specific incorporation of acetate into senecioic acid was 3.35 × 10^{-4} μmole senecioic acid/μmole acetate/flask.

(7) W. Massot, *Ber.*, **27**, (1894).

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The Synthesis of δ-Aminovaleric Acid-δ-C^{14}[1]

BY MORTON ROTHSTEIN

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The preparation of δ-aminovaleric acid-δ-C^{14} (or any C^{14}-radioisomer of δ-aminovaleric acid) has not been reported previously. Since the δ-labeled compound was required in this Laboratory for metabolic studies, its preparation was carried out by a new synthesis based on the following sequence of reactions

$$KC^{14}N + Br(CH_2)_3COOC_2H_5 \longrightarrow$$

$$N^{14}C(CH_2)_3COOC_2H_5 \xrightarrow[Ac_2O]{PtO_2}$$

$$[AcNHC^{14}H(CH_2)_3COOC_2H_5] \xrightarrow{HCl}$$

$$HCl\cdot H_2NC^{14}H_2(CH_2)_3COOH \xrightarrow[IR\text{-}4]{Amberlite}$$

$$H_2NC^{14}H_2(CH_2)_3COOH$$

Because ethyl γ-cyanobutyrate is an oil, optimal conditions for the cyanation reaction were determined from the yield of glutaric acid obtained by hydrolysis of the ester. With this procedure it was found that by refluxing a slight excess of ethyl γ-bromobutyrate with potassium cyanide in 70% ethanol for 12 to 16 hours a 61% yield of glutaric acid was obtained based on the total amount of potassium cyanide added. Use of a 30% excess of bromo ester did not affect the yield.

During the radioactive preparation, gases produced by the cyanation reaction were bubbled through a solution of sodium hydroxide. It was found that by this procedure 36% of the cyanide originally present in the reaction mixture could be recovered. This is an amount similar to that recovered in the somewhat analogous preparation of lysine-6-C^{14},[2] and represents a considerable saving of radioisotope. The utilization of this technique should therefore be worthwhile in other cyanations involving isotopic cyanide.

In non-radioactive runs the isolated δ-aminovaleric acid hydrochloride contained small amounts of ammonium chloride, probably from the hydrolysis of unreduced nitrile. This impurity was successfully eliminated by treatment of the amino acid hydrochloride solution with the anion exchanger Amberlite IR-4, yielding a halogen free product.

Experimental

Ethyl γ-Cyanobutyrate-cyano-C^{14}.—A solution of 13.2 mg. (0.4 mc.) of $KC^{14}N$[3] in 2 ml. of water containing 8 mg. of potassium hydroxide was added to 2 ml. of water containing 790 mg. of inert potassium cyanide (95% minimum purity), making a total of 803 mg. (0.0124 mole) of potassium cyanide in 4 ml. of water. This solution was then added to a solution of 2.5 g. (0.0128 mole) of ethyl γ-bromobutyrate in 22 ml. of 95% ethanol. An additional 3 ml. of water in two portions was used to complete the transfer. The resulting solution was refluxed for 14 hours under a slow stream of nitrogen which finally was passed through 10 ml. of 10% sodium hydroxide. Argentimetric titration of this trapping solution indicated a recovery of 36% of the potassium cyanide from the reaction mixture.

The reaction mixture was distilled to dryness *in vacuo*. The distilling head was then washed down with a little absolute ethanol, the mixture once more taken to dryness, and the residual solid, presumably potassium bromide, extracted four times with a total of 40 ml. of dry ether. The combined extracts were filtered and evaporated in an air stream with gentle warming. The yellow oily residue was dissolved in 20 ml. of ethanol, boiled with a little Norite for 10 minutes, and then filtered. Evaporation of the solvent yielded a small amount of colorless oil, presumably ethyl γ-cyanobutyrate-cyano-C^{14}. In similar but non-radioactive runs, a 61% yield of glutaric acid (based on potassium cyanide) was obtained after hydrolysis of this material with hydrochloric acid.

δ-Aminovaleric Acid-δ-C^{14}.—The cyano ester was dissolved in 28 ml. of acetic anhydride and 250 mg. of platinum oxide was added. The mixture was hydrogenated in a Parr hydrogenator for 18 hours at 40° under a pressure of 50 lb./sq. in. The resulting mixture was filtered, distilled to dryness *in vacuo* and hydrolyzed for 5.5 hours with 20 ml. of concentrated hydrochloric acid. The solution was then distilled to dryness *in vacuo*, water added, and the distillation repeated. The residue was dissolved in 50 ml. of water and slowly passed through a column containing the anion-exchange resin IR-4 (carbonate form). The column was washed with distilled water until the solution emerging was both ninhydrin negative and free of radioactivity. The final volume was about 250 ml. This was distilled to dryness *in vacuo* and the residue which contained a yellow impurity from the resin was dissolved in ethanol, treated with Norite, filtered, and evaporated to dryness. The residue was dried thoroughly over phosphorus pentoxide and then treated with warm 95% ethanol to which acetone was added until the cloud point was reached. After standing overnight in a refrigerator, a faintly yellow solid was obtained. A small second crop was obtained by evaporation of the mother liquors to near dryness followed by the addition of 5 ml. of absolute ethanol to the residue. The total amount

(1) This research has been supported in part by the United States Atomic Energy Commission under contract with the University of Rochester, W-7401-eng-49, and in part by funds from the Office of the Surgeon General.

(2) M. Rothstein and C. J. Claus, THIS JOURNAL, **75**, 2981 (1953).

(3) Purchased from Nuclear Instrument and Chemical Corp., Chicago, Illinois.