Summary.—Ozonolysis of anhydrosarsasapogenoic acid gave 3(β)-hydroxy-16-keto-*bis-nor*-cholanic acid, thus establishing structure I and eliminating the formula earlier suggested by Fieser.

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Tracer Studies with Radioactive Carbon: The Oxidation of Propionic Acid

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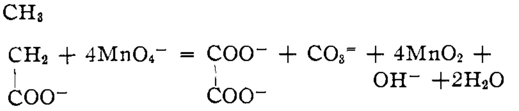
Using radioactive carbon,[1] C^{14}, as a tracer we have investigated the oxidation of propionic acid with (1) alkaline permanganate and (2) dichromate in acid solution. Propionate is converted to oxalate and carbonate[2] by alkaline permanganate. The reaction may be represented by the equation



$$\begin{array}{l}CH_3\\ CH_2\\ \vert\\ COO^-\end{array} + 4MnO_4^- = \begin{array}{l}COO^-\\ \vert\\ COO^-\end{array} + CO_3^= + 4MnO_2 + OH^- + 2H_2O \quad (1)$$

This reaction is followed by the slow oxidation of oxalate. (The ΔF^0_{298} for the oxidation of $C_2O_4^=$ by alkaline permanganate is ~ -250 kcal.[3]) However under certain conditions (temperature of $\sim 100°$ and $\sim 2\ M$ OH^-) the reaction rates are sufficiently different so that the oxalate/carbonate ratio is close to 1.

Employing short-lived radio-carbon, C^{11} (twenty-one minute half-life), reaction (1) was used[4] to determine the distribution of labelled carbon in the propionic acid formed from glycerol[5,6] by the propionic acid bacteria in the presence of $C^{11}O_2$. From 70 to 75% of the radiocarbon was found[4] in the oxalate fraction and 25 to 30% in the carbonate. Also in a single experiment the barium salt of the labelled propionate was thermally decarboxylated into (presumably) $(C_2H_5)_2CO$ and barium carbonate. The activity of the ketone was $\sim$5–7 times that found in the barium carbonate; however, this experiment was incomplete because the molal ratio of ketone/carbonate recovered was not measured. It was prematurely concluded[4] that the C^* was randomly distributed in the propionic acid. Further work[7,8] has shown this conclusion to be erroneous and the C^* to be present only in the carboxyl group. Thus it was considered of interest to study the mechanism of the propionate oxidation and in particular to answer the question: does the $CO_3^=$ come from the carboxyl group, the α- or β-carbon or from all positions of the propionic acid?

It is apparent that the use of labelled carbon affords a direct method of study and we have therefore synthesized propionic acid ($C_2H_5C^*OOH$) with radiocarbon in the carboxyl group by means of the reaction between C^*O_2 and C_2H_5MgBr. 1.58×10^{-3} mole of C^*O_2 was shaken with 6×10^{-3} mole of C_2H_5MgBr in ether for about thirty minutes at room temperature and the excess Grignard reagent hydrolyzed by careful addition of water. Excess solid silver sulfate was added to precipitate silver bromide and the propionic acid separated from this mixture by acidification with sulfuric acid and steam distillation. The propionic acid formed was subjected to a Duclaux distillation and the constants found to agree to within 1% with those obtained using reagent propionic acid. Over 90% of the C^*O_2 was converted into propionic acid. 9×10^{-4} mole of the $C_2H_5C^*OOH$ was treated with $\sim 4 \times 10^{-3}$ mole of sodium permanganate in 2 N sodium hydroxide at 100°. Under these conditions reaction (1) is complete within two hours The excess MnO_4^- was reduced in the cold with hydrogen peroxide and the manganese dioxide centrifuged off. The solution was acidified and the carbon dioxide swept out by a stream of nitrogen and absorbed in calcium hydroxide solution. The calcium carbonate was filtered, washed, dried, and weighed. The oxalate was precipitated as calcium oxalate in slightly acid solution. The radioactivity of these fractions was measured by means of a screen wall Geiger counter. The results are tabulated in Table I.

TABLE I

Moles	Moles[a]	Activity, counts/min.	
$CO_3^=$ obtained	$C_2O_4^=$ obtained	$CO_3^=$	$C_2O_4^=$
9×10^{-4}	9.7×10^{-4}	45 ± 3	136 ± 3

[a] The oxalate value is high, probably due to hydrate formation.

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(1) Ruben and Kamen, *Phys. Rev.*, **57**, 549 (1940); **59**, 349 (1941).

(2) Przewalsky, *J. prakt. Chem.*, **88**, 500 (1913).

(3) *Cf.* "The Oxidation States of the Elements and their Potentials in Aqueous Solutions," by W. M. Latimer, Prentice-Hall, Inc., New York, N. Y., 1938, p. 302.

(4) Carson, Foster, Ruben and Kamen, *Science*, **92**, 433 (1940).

(5) Carson and Ruben, *Proc. Nat. Acad. Sci.*, **26**, 422 (1940).

(6) Wood, Werkman, Hemingway and Nier, *J. Biol. Chem.*, **135**, 789 (1940).

(7) Carson, Foster, Ruben and Barker, *Proc. Nat. Acad. Sci.*, **27**, 229 (1941).

(8) Wood, Werkman, Hemingway and Nier, *Proc. Soc. Exptl. Biol. Med.*, **46**, 313 (1941).

The activities of the samples are corrected for self absorption of the soft β-radiation and are therefore comparable. These results indicate that under the above conditions only ~25% of the $CO_3^=$ comes from the carboxyl group.[9] It is apparent that the oxidation is complex and further work with the oxidation of various labelled derivatives of propionic acid (lactic, pyruvic, β-hydroxypropionic, etc.) under varying conditions is in progress so that the mechanism of these reactions can be understood more clearly.

The oxidation of propionic acid by $Cr_2O_7^=$ in concentrated sulfuric acid solution yields carbon dioxide and acetic acid.[10] The reaction can be represented by the equation

$$C_2H_5COOH + Cr_2O_7^= + 8H^+ = 2Cr^{+++} + CO_2 + CH_3COOH + 5H_2O$$

Control experiments showed that propionic acid in 18 *N* sulfuric acid is completely oxidized to CH_3COOH and carbon dioxide by $Cr_2O_7^=$ within three hours at 100°. No other products could be detected. $\sim 5 \times 10^{-4}$ mole of labelled propionic acid (containing 160 counts/min.[11]) was refluxed with 1.5×10^{-3} mole of $K_2Cr_2O_7$ in 18 *N* sulfuric acid in a stream of nitrogen. The evolved carbon dioxide was trapped and determined as above. The acetic acid was separated by steam distillation and a measurement of the Duclaux constants showed only CH_3COOH to be present. The radioactivities of the carbon dioxide and acetic acid were measured and the results are tabulated in Table II.

TABLE II

Moles	Moles	Activity	
CH_3COOH	CO_2	CH_3COOH	CO_2
$4.6 \cdot 10^{-4}$	$1.2 \cdot 10^{-3}$[a]	0 ± 2	158 ± 5

[a] The value for carbon dioxide production is much too high (mainly due to absorption of atmospheric carbon dioxide) and was caused by a mishap in the analytical determination.

These results show that carbon dioxide is produced exclusively by rupture of the bond between the carboxyl and α-carbon. The results of further experiments now in progress will be reported soon.

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[9] Wood, *et al.*, THIS JOURNAL, **63**, 2140 (1941), have found that in 0.1 *N* OH^-, 37% of the carbonate arises from the carboxyl group. It would seem that the OH^- concentration plays a role in determining whether the bond between the α- and β-carbons or the α and carboxyl shall be ruptured. In a single experiment using 0.1 *N* OH^- we have repeated the permanganate oxidation of propionate and the results appear to bear this out; however, we are investigating this point in detail.

[10] Polonovski and Lindenberg, *Compt. rend.*, **209**, 46 (1939).

[11] Prepared by the C^*O_2–C_2H_5MgBr method.

2'-Nitro-2-aminobiphenyl

BY DONALD PURDIE

In the course of a research project it became necessary to prepare 2'-nitro-2-aminobiphenyl; previous attempts at the preparation of this substance by Mascarelli and Gatti seem to have run into difficulties as it is described as a slowly crystallizing oil which could not be obtained in the pure state.[1] Nevertheless its formation was proved by the preparation of many derivatives.

A method of preparation for this substance, suitable for large scale work, is now described; starting from 2,2'-dinitrobiphenyl it differs from the method of Mascarelli in the use of aqueous alcoholic sodium polysulfide as a reducing agent in place of aqueous alcoholic ammonium sulfide. A pure product is obtained readily and the saving of time and manipulation is considerable. As a check on its identity the product was acetylated and the 2'-nitro-2-acetamidobiphenyl so formed was shown to have the same melting point as that observed by Mascarelli and co-workers; their observation that recrystallization from benzene is essential for a sharp-melting product was also confirmed.

Experimental

Ethanol (600 cc.) and 2,2'-dinitrobiphenyl (42 g.) were boiled under reflux condenser and a solution of sodium polysulfide (prepared by boiling 12 g. of sulfur with 48 g. of sodium sulfide crystals and 150 cc. of water until dissolved) slowly added. The solution turns green and then orange; boiling was continued for three hours and the whole allowed to stand overnight. Most of the ethanol was then removed by distillation and the residue poured into 1 liter of ice water. The product was extracted with ether and the ethereal solution washed with water and then dried over caustic soda pellets; this removes much impurity and is essential if a pure product is to be obtained. After removing most of the ether the rest was cooled and the 2'-nitro-2-aminobiphenyl then separated as indistinct crystals. Thrice recrystallized from ethanol the final product had m. p. 94–94.5° and formed orange needles easily soluble in ether or benzene, sparingly in cold ethanol or petroleum ether.

Anal. Calcd. for $C_{12}H_{10}N_2O_2$: N, 13.1. Found: N, 13.4.

The acetyl derivative was prepared in the usual way and after recrystallization from ethanol and then from benzene had m. p. 159–60° (Mascarelli gives 158°). Crystallization from ethanol alone always gave a low-melting product.

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[1] Mascarelli and Gatti, *Atti accad. Lincei*, **10**, 441 (1929); *Gazz. chim. ital.*, **59**, 861 (1929); Mascarelli, Gatti and Pirona, *ibid.*, **61**, 786 (1931).