The remaining two enzymes of the cycle have been recently characterized. One of them, which may be referred to as crotonase (Reaction 3), is the subject of a preceding note.[6] The other enzyme, ethylene reductase, catalyzes Reaction 4. Our method of replacing the naturally occurring CoA compounds by the readily synthesized analogs of N-acetylthioethanolamine again proved useful in this case. We found that in place of crotonyl-S-CoA the simpler compound S-crotonyl-N-acetyl thioethanolamine is reduced through the action of ethylene reductase.

S-Crotonyl-N-acetylthioethanolamine was obtained through reaction of crotonyl chloride with the lead salt of N-acetyl thioethanolamine, m.p. 61.5–62°. In aqueous solution it shows two characteristic absorption bands with peaks at 224 mμ (ϵ = 11500) and 262 mμ (ϵ = 6750). The method used by Fischer and Eysenbach[7] to study fumarate reductase, namely, the oxidation of a leuco dye, such as leucosafranin, was used to assay ethylene reductase as shown in Reaction 4.

In this reaction crotonic acid cannot replace the thioester derivative. The enzyme assay, in which the appearance of color from the leuco dye is followed, is illustrated in Fig. 1. By the use of this assay ethylene reductase was purified about 50-fold from sheep liver extracts through steps involving acetone fractionation, adsorption and elution from calcium phosphate gel and ammonium sulfate fractionation. The solution of the purified enzyme is yellow. A colorless, almost inactive protein can be precipitated from the above solution with ammonium sulfate at *p*H 3.6. Activity of this protein can be partially restored by addition of yeast Kochsaft or crude preparations of FAD. This suggests that, like fumarate reductase, ethylene reductase may be a flavoprotein. The two enzymes, however, are not identical. DPNH or TPNH cannot substitute for the leuco dye.

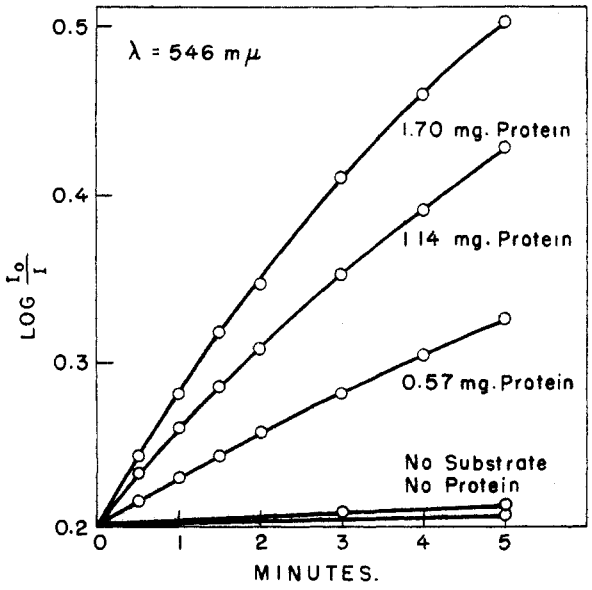

Fig. 1.—2.6 μM. S-crotonyl-N-acetylthioethanolamine and 0.5 μM. leucosafranin T in 2.1 ml. of 0.066 *M* phosphate buffer, *p*H 7.1; enzyme as indicated; temp. 17° (*d*, 0.5 cm.).

With cruder preparations of the enzyme the crotonyl-thioethanolamine derivative can be replaced by β-hydroxybutyryl-S-CoA (prepared either enzymatically[2] or synthetically[8]) indicating that the preparations also contain crotonase,[6] the enzyme catalyzing Reaction 3. These observations prove that ethylene reductase reacts with crotonyl CoA.

(6) J. R. Stern and A. del Campillo, THIS JOURNAL, **75**, 2277 (1953). Joint work on this enzyme is being carried out in the New York University and Munich laboratories.

(7) F. G. Fischer and H. Eysenbach, *Ann. Chem.*, **530**, 99 (1953).

(8) T. Wieland and L. Rueff, *Angew. Chem.*, in press.

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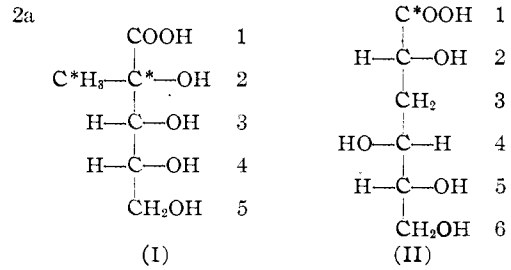
CONCERNING THE MECHANISM OF FORMATION OF SACCHARINIC ACIDS

Sir:

Since the discovery of the saccharinic acids some seventy years ago, the mechanism by which they arise through the action of alkali on reducing sugars has remained obscure. Nef[1] first suggested as the crucial step in their formation an intramolecular isomerization and hydration similar to the benzilic acid rearrangement. This proposal was later modified and modernized by Isbell,[2] who interpreted the reaction sequence in terms of consecutive electron displacements. An alternative mechanism, involving the intermolecular recombination of fragments of the original sugar has been largely disregarded on account of the failure to observe formation of higher-carbon saccharinic acids from the action of alkali on lower-carbon sugars.

We now have examined, by means of C^{14}-labeling experiments, the formation of two saccharinic acids, "D-glucosaccharinic" acid (I) and "D-galacto-α-metasaccharinic" acid (II). Our results indicate that the branched-chain and the straight-chain acid studied are formed by *different* general mechanisms.



1-C^{14}-D-Mannose[3] was converted by the action of saturated lime-water at room temperature[4] to C^{14}-"D-glucosaccharinic acid." The latter was degraded, by oxidation with sodium metaperiodate, to carbon dioxide (C-1), acetic acid (C-2a, C-2), formic acid (C-3, C-4) and formaldehyde (C-5). Over 95% of the original radioactivity was found in the acetic acid fragment and degradation of the latter showed that the labeling was distributed approximately in the ratio C-2a:C-2, 2:3.

(1) J. U. Nef, *Ann.*, **357**, 294 (1907); **376**, 1 (1910).

(2) H. S. Isbell, *J. Research Natl. Bur. Standards*, **32**, 45 (1944).

(3) J. C. Sowden, *J. Biol. Chem.*, **180**, 55 (1949).

(4) H. Kiliani, *Ber.*, **15**, 701, 2953 (1882).

To confirm this result, the branched-chain saccharin was condensed with *o*-phenylenediamine to give an anhydro-saccharin benzimidazole (m.p. 240–241°; C, 61.3, H, 6.00) and the latter was oxidized to benzimidazole carboxylic acid (C-1, C-2). Decarboxylation of the benzimidazole carboxylic acid confirmed that nearly 60% of the original radioactivity had been located in the tertiary carbon (C-2). This result is incompatible with the benzilic acid rearrangement mechanism as postulated by Nef and Isbell, but is compatible with a recombination mechanism involving sugar fragments whose identity is not yet known with certainty.

1-C^{14}-D-Galactose[5] was converted by the action of saturated lime-water at room temperature to C^{14}-"D-galacto-α-metasaccharinic acid."[6] A Ruff degradation of the latter[6] and radio-assay of the resulting D-*threo*-2-deoxypentose as the benzylphenylhydrazone[7] showed that less than 5% of the original radioactivity was located in this 5-carbon fragment (C-2, C-3, C-4, C-5, C-6). To confirm this result, the straight-chain saccharin was converted to its benzimidazole derivative (m.p. 186–187°; C, 57.1, H, 6.53) and the latter converted, by oxidation followed by decarboxylation, to benzimidazole (C-1). Approximately 95% of the original radioactivity was found in this derivative. This result is compatible with the benzilic acid rearrangement mechanism of Nef and Isbell.

Further work is in progress with other saccharinic acids to generalize the results reported here and to clarify the mechanism of formation of the branched-chain "D-glucosaccharin."

We wish to thank Anheuser–Busch, Inc., St. Louis, Mo., for their generous support during the course of this work.

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(5) Obtained from the National Bureau of Standards through the courtesy of Dr. H. S. Isbell.

(6) H. Kiliani and H. Naegell, *Ber.*, **35**, 3528 (1902).

(7) P. A. Levene and T. Mori, *J. Biol. Chem.*, **83**, 803 (1929).

BOOK REVIEWS

Charbons Actives (Adsorption des Gaz et des Vapeurs). By C. COURTY, Professeur de Chimie Physique, à la Faculté des Sciences de Lyon. Gauthier-Villars, 55 Quai des Grands-Augustins, Paris (6e), France. 1952. ix + 534 pp. 16.5 × 25 cm. Price, 4,500 fr.

This rather long book is essentially an expansion of some notes that related to war-time work on gas masks. The theoretical parts have been expanded to the limit, but the experimental data that are used as examples remain confined to charcoal; even carbon black is excluded. Now as Professor Duclaux points out in the preface, most adsorption theories have little to do with the chemical nature of the adsorbent, and so in the reviewer's opinion the scheme of the book is unsound.

Most of the theoretical material presented is rather old; for example the 1943 paper of Emmett and de Witt is a high water mark for the work of this school. However, the 563 references, and the exhaustive treatment of semi-practical rate problems will make this book very interesting to people working directly in the field of active carbons.

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SEATTLE 5, WASHINGTON GEORGE D. HALSEY, JR.

Die Physik der Hochpolymeren. Volume I. Die Struktur des Freien Molekuls—Allgemeine Physikalische Methoden zur Bestimmung der Struktur von Molekulen und ihre Wichtigsten Ergebnisse. By H. A. STUART, Früher O. Professor der Physik an der Technischen Hochschule Dresden, Z. 'Zt. Hannover. Springer-Verlag, Reichpietschufer 20, Berlin W 35, Germany. 1952. xxi + 609 pp. 16.5 × 23.5 cm. Price, DM 69.

This volume should be very useful as a reference work to everyone, such as the writer, who is interested in the structure of molecules and the methods of molecular structure investigation, as well as to the high polymer chemist. It is the most comprehensive, critical and up-to-date review of this subject with which this writer is acquainted.

According to the preface it was undertaken because of the dependence of structural investigations of polymers on purely physical methods. Consequently, Prof. Stuart has undertaken to collect the available structural information which these methods have yielded on small molecules and to examine critically the available methods for studying the structure of molecules. Various sections of the book have been written by G. Scheibe, W. Maier and J. Juilfs. The extent of the survey is indicated by the chapter headings: I. Valence and Molecular Forces, (79 pp.); II. The Size and Shape of Molecules, (23 pp.); III. The Nuclear Framework of Molecules, (80 pp.); IV. The Internal Mobility of Molecules and Their Statistical Shape, (63 pp.); V. Dielectric Constants, Electric Moment and Molecular Structure, (89 pp.); VI. Light Scattering, Polarizability, and Molecular Structure, (78 pp.); VII. Electrical Double-Refraction, Optical Anisotropy and Molecular Structure, (49 pp.); VIII. Characteristic Vibrations of the Nuclear Framework, (109 pp.); IX. Light Absorption and Constitution, (13 pp.).

The thoroughness with which the literature on these numerous topics is covered is impressive; in general the references seem quite complete up to about 1950 and some are as recent as 1951. For example, there is an excellent short summary of the results obtained in the very current field, microwave spectroscopy. The results are conveniently compiled in 129 tables, many of them available elsewhere, but some of them unique. In the section entitled "Stability of Internuclear Distances and Valence Angles," for example, the energy necessary to deform molecules is tabulated for a number of characteristic linkages. These energies are calculated straightforwardly from force constants (also given), to be sure, but this reviewer found it most enlightening to realize that the energy necessary to deform many bond angles by 5.73° was less than 500 cal./mole.

Naturally, as in any such compilation, occasional minor errors have crept in. (Rollefson's determinations of the dipole moments of HCl and DCl from infrared dispersion are referred to as Stark-Effect measurements in Table 57.) The number of such errors appears to be remarkably small; this,