That is

$$2NH_3 + 2NO_2 + H_2O \longrightarrow NH_4NO_3 + NH_4NO_2$$
$$NH_4NO_2 + NO_2 \longrightarrow NH_4NO_3 + NO$$

Hydrazine has not been found by us and must therefore be produced in an amount less than 0.1% of total nitrogen, if at all. The above stoichiometry was shown to exist at 25 and at 100° by standard chemical and physical techniques. In the range of 150 to 200° the pressure change agreed, to within a few per cent., with the change predicted.

The kinetics of this reaction has been studied between 150 and 200° by following the pressure change with time. Pressures up to 100 mm. of each gas were used and the ratio NO_2:NH_3 was varied from 4:1 to 1:4. An examination of initial rates showed a third order reaction—first with respect to ammonia and second with respect to nitrogen dioxide. The initial third order rate law was not followed throughout the entire course of the reaction but, instead, an acceleration took over. A large negative temperature coefficient was found—*e.g.*, at 151° the specific third order rate constant was shown to be 1.1×10^{10} cc.^{2} mole^{-2} sec.$^{-1}$, whereas, at 205° it has dropped to 2.2×10^9 cc.^{2} mole^{-2} sec.$^{-1}$. A plot of the log of the rate constant as a function of the reciprocal of the absolute temperature gave an excellent straight line over the 50° interval studied and led to a "negative activation energy" of -12.5 ± 1 kc.

The negative temperature coefficient and the "activation energy" of -12.5 ± 1 kc. leads to the proposition that N_2O_4 and not NO_2 is the reactive species since the ΔH of dissociation of N_2O_4 to NO_2 is just about that value—*i.e.*, 13.6 kc. at 25°.[5] Another possibility involves inhibition by NO since the dissociation of NO_2 to NO also has a ΔH of this order of magnitude.

The effect of NO on this reaction is now being considered in order to clear up this last point. In addition work is in progress to determine what effect, if any, excess O_2 and H_2O might have. Finally, a survey of rates from room temperature to 400° is being carried out.

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ACTION OF INTESTINAL EXTRACTS ON "BRANCHED" OLIGOSACCHARIDES[1]

Sir:

α-Amylotic hydrolysis of amylopectin produces maltose, glucose and a mixture of "branched" oligosaccharides.[2,3,4] In gastrointestinal digestion maltose is further hydrolyzed to glucose by maltase (α-glucosidase)[5] and the glucose absorbed. The fate of the branched oligosaccharides is unknown. We have isolated from small intestinal mucosa a new enzyme, which hydrolyzes the α-1,6-linkages of isomaltose,[6] panose[7] and the mixture of branched oligosaccharides remaining after extensive α-amylolysis of amylopectin. This new enzyme allows essentially complete digestion of starch to occur in the gastrointestinal tract. The name oligo-1,6-glucosidase[8] is accordingly proposed.

With isomaltose as substrate (0.0004 *M*), a quantitative activity assay has been set up measuring the time rate of reduction of TPN[9] in the presence of excess hexokinase,[10] Zwischenferment[11] and ATP. The system is buffered at *p*H 7.4 with 0.083 *M* glycylglycine in a final volume of 3.0 ml. When substrate is added there is an initial time lag of about one minute, after which the reaction proceeds linearly for at least 4–6 minutes provided TPN is present in excess. Rate of TPN reduction is proportional to extract added over a tenfold range of dilution. A unit of enzyme activity has been defined as that amount of enzyme which effects an increase in optical density at 340 mμ in the Beckman model DU spectrophotometer of 0.001 per minute under the stated conditions.[12] Table I lists specific activities (units/mg. protein) of extracts from four species. In the case of freshly prepared extracts of rabbit intestine, jejunum and ileum show much lower activities than extracts of duodenum.[13]

Oligo-1,6-glucosidase is differentiated from amylo-1,6-glucosidase[14] by the inability of the latter to hydrolyze (microenzymatic test) isomaltose, panose or the mixture of branched oligosaccharides.[15] Furthermore, although crude intestinal extracts rapidly liberate glucose from the phosphorylase limit dextrin (or glycogen), this activity is lost during subsequent purification. Starch-treated extracts[14] no longer liberate glucose from the phosphorylase limit dextrin (or glycogen) while hydrolysis of isomaltose continues. α-Amylase rather than amylo-1,6-glucosidase would therefore seem responsible for glucose production in the untreated extracts.[16]

α-1,6-Glucosidases have been described from

(1) Supported in part by a grant from the Graduate College, University of Illinois, Urbana.

(2) W. J. Whelan, *Biochem. Soc. Symposia* No. 11, p. 17 (1953).

(3) P. Nordin, G. Wild and D. French, Abstracts of 124th Meeting American Chemical Society, p. 53C.

(4) K. H. Meyer and W. F. Gonon, *Helv. Chim. Acta*, **34**, 308 (1951).

(5) J. P. Peters and D. D. Van Slyke "Quantitative Clinical Chemistry," Vol. I, Second Edition, Williams and Wilkins Co., Inc., Baltimore, Md., p. 103.

(6) We are indebted to Dr. Allene Jeanes, Northern Regional Research Laboratory, Peoria, for the sample of isomaltose, 6-(α-D-glucopyranosyl)-D-glucose.

(7) We are indebted to Dr. S. C. Pan, Squibb Institute for Medical Research, New Brunswick, N. J., for the sample of panose, 4-(α-isomaltosyl)-D-glucose. During intermediate stages of panose hydrolysis, maltose accumulates which is hydrolyzed by maltase (α-glucosidase) present to free glucose.

(8) If more than one α-1,6 splitting enzyme is present, the name would then apply to the class of enzymes.

(9) Abbreviations: triphosphopyridine nucleotide, TPN; adenosine triphosphate, ATP.

(10) Purified from yeast by an unpublished method of C. R. Park.

(11) A. Kornberg, *J. Biol. Chem.*, **182**, 805 (1950).

(12) The over-all reaction has been followed also by increase in reducing power, and by paper chromatography.

(13) Bacterial contamination as a major source of activity has been ruled out by filtration through a bacteriological filter (sintered glass), centrifugation at top speed of Sorvall centrifuge, Model SS-1 for 30 minutes, and direct bacteriological counts. We are indebted to J. R. Stamer and I. C. Gunsalus, of the Department of Bacteriology, for performing these counts.

(14) G. T. Cori and J. Larner, *J. Biol. Chem.*, **188**, 805 (1950).

(15) Unpublished experiments, J. Larner and L. H. Schlisefeld.

(16) Glucose production in the untreated extracts could occur by α-amylase action alone as well as by the action of oligo-1,6-glucosidase on the "branched" dextrins remaining after α-amylase action. Starch-treated extracts still contain maltase (α-glucosidase).

other sources. Oligo-1,6-glucosidase differs from R-enzyme (plants) in that the latter does not hydrolyze the terminal α-1,6-linked glucose residues of isomaltose or panose (except possibly when acting with α-amylase).[2] A limit dextrinase from *Aspergillus oryzae* culture filtrate has been described[17] which would seem to have an activity similar to oligo-1,6-glucosidase on "branched" oligosaccharides.

(17) L. A. Underkofler and D. K. Roy, *Cereal Chem.*, **28**, 18 (1951).

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THE SYNTHESIS OF (+)-α-LIPOIC ACID AND ITS OPTICAL ANTIPODE

Sir:

The racemic form of a compound active as a coenzyme in the oxidative decarboxylation of pyruvate has been synthesized.[1,2] This racemate has been designated DL-α-lipoic acid[1] and 6-thioctic acid.[2] The synthesis of the naturally occurring biologically active isomer, (+)-α-lipoic acid, has not been reported. We wish to report a new synthesis which has made possible the preparation of (+)-, (−)- and DL-α-lipoic acid.

The addition of thioacetic acid to 7-carboethoxy-2-heptenoic acid (I)[3] yielded 7-carboethoxy-3-acetylthioheptanoic acid (II) which was converted to 7-carboethoxy-3-acetylthioheptanoyl chloride (III). The reduction of III with sodium borohydride yielded a mixture of ethyl 6-acetylthio-8-hydroxyoctanoate (IV) and ethyl 6-thiol-8-hydroxyoctanoate (V). The mixture was converted by alkaline hydrolysis to 6-thiol-8-hydroxyoctanoic acid (VI), $n^{23.5}$D 1.4989. Iodine oxidation of VI produced bis-[3-(1-hydroxy-7-carboxyheptyl)] disulfide (VII). The introduction of the thiol group into the 8-position of both VI and VII was carried out by refluxing with thiourea in aqueous hydrobromic acid followed by alkaline hydrolysis. Following the introduction of sulfur into VII, the product was reduced with sodium borohydride and reoxidized to yield DL-α-lipoic acid (VIII), m.p. 59.5–61.0° (micro-block); $\lambda_{max}^{CH_3OH}$ 333 mμ (ϵ 150); *anal.* Calcd. for $C_8H_{14}O_2S_2$ (206.2): C, 46.60; H, 6.84; S, 31.05. Found: C, 46.90; H, 6.91; S, 31.34; mol. wt. (ebull.), 212 ± 7; neut. equiv., 206.

For the preparation of (+)- and (−)-α-lipoic acid, DL-7-carboethoxy-3-acetylthioheptanoic acid (II) was resolved. Treatment of II with *l*-ephedrine yielded the crystalline salt of the levorotatory form, m.p. 130.0–134.5°. The dextrorotatory isomer was isolated from the residue by precipitation in the form of its benzhydrylamine salt, m.p. 92–96°.

(+)-7-Carboethoxy-3-acetylthioheptanoic acid, $[\alpha]^{24}$D + 7.1° (*c*, 6.93; CH_3OH), when used in the above sequence yielded (+)-α-lipoic acid, m.p. 46.0–48.0° (micro-block); $[\alpha]^{23}$D +104° (*c*, 0.88; C_6H_6), $\lambda_{max}^{CH_3OH}$ 333 mμ (ϵ 150). *Anal.* Calcd. for $C_8H_{14}O_2S_2$ (206.2): C, 46.60; H, 6.84; S, 31.05. Found: C, 46.95; H, 6.85; S, 31.00; mol. wt. (ebull.), 194 ± 2; neut. equiv., 208. In a similar manner (−)-7-carboethoxy-3-acetylthioheptanoic acid, $[\alpha]^{24}$D −7.2 (*c*, 6.91; CH_3OH), yielded (−)-α-lipoic acid, m.p. 45.5–47.5° (micro-block); $[\alpha]^{23}$D −113° (*c*, 1.88; C_6H_6); $\lambda_{max}^{CH_3OH}$ 333 mμ (ϵ 140). Found: C, 46.65; H, 6.66; S, 31.32; mol. wt. (ebull.), 212 ± 3; neut. equiv. 208. When equal amounts of (+)- and (−)-α-lipoic acid were mixed and recrystallized from cyclohexane, the racemic compound, DL-α-lipoic acid, m.p. 60–61° (micro-block), was obtained.

In the enzymatic POF assay,[4] the activity of synthetic (+)-α-lipoic acid was double that of DL-α-lipoic acid. The activity of (−)-α-lipoic acid was essentially zero, *ca.* 1% that of DL-α-lipoic acid. The properties listed above substantiate the identity of our synthetic (+)-α-lipoic acid and the natural α-lipoic acid.[5,6,7] This lends additional support to the structural conclusions advanced[5,6] previously.

(1) C. S. Hornberger, Jr., R. F. Heitmiller, I. C. Gunsalus, G. H. F. Schnakenberg and L. J. Reed, THIS JOURNAL, **75**, 1273 (1953).

(2) M. W. Bullock, J. A. Brockman, Jr., E. L. Patterson, J. V. Pierce, M. H. von Saltza, F. Sanders and E. L. R. Stokstad, *ibid.*, **76**, 1828 (1954).

(3) G. B. Brown, M. D. Armstrong, A. W. Moyer, W. P. Anslow, Jr., B. R. Baker, M. V. Querry, S. Bernstein and S. R. Safir, *J. Org. Chem.*, **12**, 160 (1947).

(4) I. C. Gunsalus, M. I. Dolin and L. Struglia, *J. Biol. Chem.*, **194**, 849 (1952).

(5) L. J. Reed, I. C. Gunsalus, G. H. F. Schnakenberg, Q. F. Soper, H. E. Boaz, S. F. Kern and T. V. Parke, THIS JOURNAL, **75**, 1267 (1953).

(6) E. L. Patterson, J. V. Pierce, E. L. R. Stokstad, C. E. Hoffmann, J. A. Brockman, Jr., F. P. Day, M. E. Macchi and T. H. Jukes, *ibid.*, **76**, 1823 (1954).

(7) M. Calvin and J. A. Barltrop, *ibid.*, **74**, 6153 (1952).

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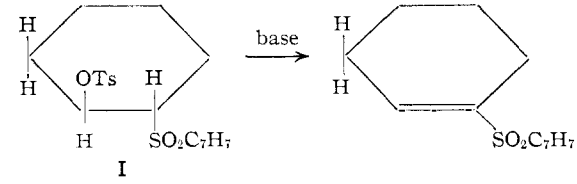
E2 ELIMINATION REACTIONS IN THE CYCLOHEXANE AND CYCLOPENTANE SERIES[1]

Sir:

A study of base-catalyzed E2 elimination reactions of *trans*-2-(*p*-tolylsulfonyl)-cyclohexyl *p*-toluenesulfonate (I), its *cis* isomer (II), the corresponding *trans* and *cis* isomers (III and IV) in the cyclopentane series, and an open-chain analog, 1-(*p*-tolylsulfonyl)-2-propyl *p*-toluenesulfonate, $C_7H_7SO_2CH_2CH(OTs)CH_3$ (V), with trimethylamine, triethylamine and hydroxide ion has revealed the information reported below.



I

(1) Each of these reactions gives an α,β-unsaturated sulfone. For I and III this corresponds to elimination of a hydrogen *cis* to the tosylate group in preference to a hydrogen *trans* to the tosylate

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