

our ester with the one prepared by him from digoxigenin. He reported [private communication] that the melting points were identical and that the melting point of a mixture was not depressed. He has also supplied the specific rotation of his ester,  $[\alpha]^{24}_{D} + 45.6 \pm 3^\circ$ ;  $[\alpha]^{24}_D + 38.9 \pm 3^\circ$  (1.183% in methanol).

The esters of three acids derived from digoxigenin have now been compared with the corresponding esters of known structure. The results show that digoxigenin has a hydroxyl group at C-12, the steric arrangement of which is opposite to that of the corresponding hydroxyl group of desoxycholic acid. A similar steric arrangement of the hydroxyl group at C-12 is present in the  $\alpha$ -lagodesoxycholic acid described by Kishi [*Z. physiol. Chem.*, **238**, 210 (1936)].

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RECEIVED MAY 18, 1939

#### THE ACTION OF PERIODIC ACID ON $\alpha$ -AMINO ALCOHOLS

Sir:

Periodic acid readily splits [Malaprade, *Bull. soc. chim.*, (5) **1**, 833 (1934)] substances carrying the grouping  $\begin{array}{c} \text{R} \quad \text{R}' \\ | \quad | \\ \text{---C---C---} \\ | \quad | \\ \text{OH} \quad \text{OH} \end{array}$  (in which R or R' may

be H) to the ketones or aldehydes  $\text{---C(=O)R}$  and  $\text{---C(=O)R}'$ . This reaction recently has been applied very effectively to glucoside derivatives [Jackson and Hudson, *THIS JOURNAL*, **59**, 994, 2049 (1937); **60**, 989 (1938)]. We now find that this reaction may be extended to cases in which hydroxyl is replaced by  $\text{---NH}_2$  or by  $\text{---NHR}$ , and are actively engaged in trying to determine the range of its applicability.

Specifically, serine ( $\text{HOCH}_2\text{CH}(\text{NH}_2)\text{CO}_2\text{H}$ ) is rapidly and quantitatively split, and the dimedon derivative of  $\text{H}_2\text{CO}$  can be isolated in 95% yield. The progress of  $\text{HIO}_4$  consumption with time is entirely consistent with the assumption that the other direct products from serine are (as would be expected) ammonia and glyoxylic acid. The latter is further oxidized, over a period of a day or two, to formic acid and carbon dioxide, according to the established reaction [Fleury and Bon-Bernatets, *J. pharm. chim.*, **23**, 85 (1936)]. Threonine reacts like serine, producing acetaldehyde, which has not as yet been quantitatively determined.

Of the naturally occurring amino acids which

do not have a  $\beta$ -hydroxy group, tryptophan reacts rapidly with much more than one mole of periodic acid to form an insoluble product. Methionine and cystine are also somewhat rapidly attacked, but, we believe, chiefly through oxidation of their sulfur. Glycine, alanine, tyrosine, histidine, aspartic acid, asparagine, and glutamic acid reduce periodic acid at somewhat varying rates, which are estimated to be at most  $1/1000$  as fast as the reaction with serine. The nature of these reactions has not yet been established, but they do not seem likely to offer any insurmountable obstacle to the use of periodic acid for the quantitative study of serine, threonine, and the somewhat hypothetical hydroxy-glutamic acid in protein hydrolyzates, which we are undertaking.

As a secondary amine, diethanolamine ( $\text{NH}(\text{CH}_2\text{CH}_2\text{OH})_2$ ) reacts very rapidly to liberate 4 moles of formic acid. In contrast with this, diethylaminoethanol ( $(\text{C}_2\text{H}_5)_2\text{NCH}_2\text{CH}_2\text{OH}$ ) shows practically no reaction. This behavior is probably typical of tertiary amines, and suggests that the fourth hydrogen of an ammonium ion ( $\text{R}_3\text{NH}^+$ ) is not sufficient to permit the desired reaction.

Preliminary results with an acylated derivative of serine indicate an extremely slow attack, the course of which is not yet definitely determined. Since, however, this last reaction could, if successful, be of even more interest in the study of protein chemistry than those already noted, our interest in it is being continued.

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RECEIVED APRIL 29, 1939

#### PANTOTHENIC ACID AS A FACTOR IN RAT NUTRITION

Sir:

In the course of experiments designed to isolate from liver extracts a substance necessary for rat growth, it became apparent that the substance was unstable in the presence of acid and alkali, and that it could be concentrated by procedures many of which previously had been used for the isolation of pantothenic acid. Starting with 95% alcoholic liver extract, the following methods were employed: (1) extraction from acid aqueous solution by amyl alcohol and return into dilute aqueous alkali; (2) adsorption on norite and

elution with hot 60% ethanol solution; (3) continuous extraction by diethyl ether from acid aqueous solution; (4) partition of the brucine salt between chloroform and water, the activity appearing in the aqueous phase; (5) conversion of the brucine salt into the calcium salt; (6) fractionation of the latter by the procedures of Williams and co-workers [(THIS JOURNAL, 60, 2719 (1938))].

By these means 510 mg. of white, varnish-like calcium salt (corresponding to Williams' fraction "C") was obtained from an extract derived from 160 kg. of liver. This material was fed in amounts averaging 8 mg. per week to each of six albino rats receiving a vitamin B-free diet supplemented by thiamin, flavin and the alkali-hydrolyzed eluate from fuller's earth adsorbate of liver extract. The average gain in weight for each week was as follows: (1) 13.4 g., (2) 19.1 g., (3) 18.8 g. The animals of a control group receiving the same basal diet and supplements, but without the calcium salt preparation, gained on the average as follows: (1) 6.5 g., (2) 4.5 g., (3) 4.2 g. The calcium salt preparation therefore actively stimulates rat growth.

Through the kindness of Dr. Leo Rane the calcium salt preparations were tested for their ability to stimulate the growth of *Streptococcus hemolyticus* and the diphtheria bacillus. They were found to behave like pantothenic acid preparations in stimulating the growth of both microorganisms. For these reasons it appears likely that pantothenic acid is one of the substances, in liver extracts, which are necessary for rat growth.

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RECEIVED APRIL 24, 1939

#### PANTOTHENIC ACID AS A GROWTH FACTOR FOR THE DOCHEZ NY5 STRAIN OF HEMOLYTIC STREPTOCOCCUS

Sir:

A medium composed of gelatin hydrolyzate, amino acids, inorganic salts, glucose plus such accessory factors as glutathione, thiochrome, nicotinic acid, betaine, flavin, and glucosamine in the presence of a calcium-alcoholic precipitate of a highly purified liver extract provides almost optimum conditions for the growth of the Dochez

NY5 strain of hemolytic streptococcus [L. Rane, and Y. Subbarow, *Proc. Soc. Exp. Biol. Med.*, **38**, 837-839 (1938)]. We have found that the further addition of uracil, guanylic acid, xanthine, hypoxanthine, nicotinic acid amide in place of nicotinic acid, and a fraction of liver extract as yet unidentified may also be of significance in the growth of this strain of hemolytic streptococcus.

Certain similarities in the isolation and properties of the unknown factor in the liver extract and pantothenic acid suggested the possibility of substitution. Pantothenic acid "U-6000, ca. 50%," kindly supplied by Dr. R. J. Williams, has been tried. Pantothenic acid is active in the growth of the Dochez NY5 strain of hemolytic streptococcus, as indicated in the table. The amount of growth was equal to that obtained with the calcium-alcoholic precipitate of liver extract as described in our previous publication.

Pantothenic acid per 10 cc. basal medium, $\gamma$	100	50	25	10	5	2.5	1	0.5
Nephelometer reading (cf. L. Rane, and Y. Subbarow, — <i>loc. cit.</i> )	2.9	2.9	2.8	2.8	2.8	2.9	3.5	>4.7
Control, growth of organism in meat infusion broth	2.3							

It is of additional interest that a product synthesized in collaboration with G. H. Hitchings of the Harvard School of Public Health is able to replace pantothenic acid in an otherwise chemically-defined medium. The compound was made by the conjugation of  $\beta$ -alanine ethyl ester with the acyl chloride of acetylated  $\alpha,\delta$ -dihydroxyvaleric acid. The dihydroxyvaleric acid was obtained by the deaminization of *d*-ornithine. However, the material so prepared was needed in larger amounts than was pantothenic acid.

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LEO RANE

RECEIVED APRIL 24, 1939

#### REACTION OF NEOPENTYL CHLORIDE WITH SODIUM

Sir:

We have isolated from the reaction of one mole of neopentyl chloride and sodium, a 13% yield of 2,2,5,5-tetramethylhexane, b. p. 135° at 736 mm.,  $n_D^{20}$  1.4049, a 36% yield of neopentane, f. p. -19 to -20°, b. p. 8.3° at 720 mm., and 17.6 g. of a