min K in a pure or nearly pure form. The vitamin was said to give a characteristic color reaction with sodium ethylate in alcoholic solution. We hoped that this reaction would be useful in the isolation of vitamin K from alfalfa, especially because the typical color changes described by the European workers were noticed with relatively crude concentrates. However, it was found that the color reaction is not a criterion for the presence of the vitamin as illustrated by the following experiment.

One gram of a vitamin K concentrate which gave the color reaction very strongly and had a potency of 1 unit [*J. Nutrition*, **17**, 303 (1939)] in 15 γ was dissolved in petroleum ether and chromatographically adsorbed on a slightly heat-activated calcium sulfate. Washing with petroleum ether was continued until the lowest yellow zone had passed into the filtrate. The adsorbed substance was then eluted with ether and we obtained 0.3 g. of a material with a very intense color reaction but with no vitamin K activity at 15 γ. The yellow filtrate contained 0.6 g. of an oil which did not give the typical color reaction; only a slight darkening occurred with the ethylate. However, it was fully active in a dose of 8 γ, containing the entire potency of the initial preparation.

Upon further purification by several chromatographic adsorptions on a more highly activated calcium sulfate, a concentrate was obtained which behaved like a single substance chromatographically. Its potency was comparable to that of the vitamin K_1 of McKee, *et al.* [THIS JOURNAL, **61**, 1295 (1939)], assuming that the potency of their product was not 100 but 1000 units per mg., as stated in their earlier paper [*Proc. Soc. Exptl. Biol. Med.*, **40**, 482 (1939)]. It was a light yellow oil which darkened on standing even in the refrigerator and which gradually lost potency. It did not give derivatives with reagents for keto groups or for hydroxyl groups, and in spite of its high degree of unsaturation failed to react with maleic anhydride in boiling benzene. Exposure to bright sunlight caused an almost instantaneous formation of a pink coloration fading in a few minutes. During the last steps of the isolation process the sodium ethylate color reaction became again positive, although it never reached the intensity of the previously separated inactive fraction. It may be that the blue coloration is given by readily formed decomposition products of vitamin K.

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RECEIVED MAY 19, 1939

THE RELATION BETWEEN METHYL *etio*-DESOXYCHOLATE AND THE METHYL DIHYDROXY-*etio*-CHOLANATE DERIVED FROM DIGOXIGENIN; METHYL 12-*epi*-*etio*-DESOXYCHOLATE

Sir:

Steiger and Reichstein [*Helv. Chim. Acta*, **21**, 828 (1938)] have degraded digoxigenin to a dihydroxy- and a diketo-*etio*-cholanic acid and a diketo-*etio*-cholenic acid. We have recently shown [THIS JOURNAL, **60**, 2824 (1938)] that these diketo acids are identical with the corresponding acids derived from desoxycholic acid. The dihydroxy acid, however, was found to be different from *etio*-desoxycholic acid in that its methyl ester melted at 180–183° while methyl *etio*-desoxycholate melted at 145–146°. Since both acids have the α configuration at C-3, epimerism at C-12 appeared to be the only plausible explanation of the difference. However, it became important to test this point in order to eliminate the possibility of a flaw in the other comparisons. The evidence now at hand confirms this explanation and furnishes additional proof for the positions of the oxygen atoms in the acids derived from digoxigenin.

Reduction of methyl 3,12-diketo-*etio*-cholanate in alcohol with platinum oxide catalyst resulted in a mixture of esters. The esters with the β configuration at C-3 were removed by precipitation with digitonin. The esters with the α configuration were separated by adsorption analysis on a column of alumina. Repeated crystallization of the fraction with higher melting point gave an ester (methyl 12-*epi*-*etio*-desoxycholate) which melted at 176–178°; $[\alpha]^{25}_{5461}$ + 49.4 ± 2.4° (0.203% in alcohol). Analysis of the ester was not entirely satisfactory because of the presence of ash (Calcd. for $C_{21}H_{34}O_4$: C, 71.96; H, 9.59. Found [corrected for ash]: C, 71.69; H, 9.81), but the 3-monobenzoate of the ester gave satisfactory values (Calcd. for $C_{28}H_{38}O_5$: C, 73.96; H, 8.42. Found: C, 74.06; H, 8.62). The monobenzoate melts at 136–138°; $[\alpha]^{25}_{5461}$ + 62 ± 3°.

Professor Reichstein very kindly compared

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, $[\alpha]^{24}_{5461}$ + 45.6 ± 3°; $[\alpha]^{24}D$ + 38.9 ± 3° (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 α-lagodesoxycholic acid described by Kishi [*Z. physiol. Chem.*, **238**, 210 (1936)].

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ROCHESTER, MINNESOTA W. M. HOEHN

RECEIVED MAY 18, 1939

THE ACTION OF PERIODIC ACID ON α-AMINO ALCOHOLS

Sir:

Periodic acid readily splits [Malaprade, *Bull. soc. chim.*, (5) **1**, 833 (1934)] substances carrying the grouping $-\overset{R}{\underset{OH}{C}}-\overset{R'}{\underset{OH}{C}}-$ (in which R or R′ may be H) to the ketones or aldehydes —C(=O)R and —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 $-NH_2$ or by $-NHR$, and are actively engaged in trying to determine the range of its applicability.

Specifically, serine ($HOCH_2CH(NH_2)CO_2H$) is rapidly and quantitatively split, and the dimedon derivative of H_2CO can be isolated in 95% yield. The progress of 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 β-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 hydroxyglutamic acid in protein hydrolyzates, which we are undertaking.

As a secondary amine, diethanolamine ($NH(CH_2CH_2OH)_2$) reacts very rapidly to liberate 4 moles of formic acid. In contrast with this, diethylaminoethanol ($(C_2H_5)_2NCH_2CH_2OH$) shows practically no reaction. This behavior is probably typical of tertiary amines, and suggests that the fourth hydrogen of an ammonium ion $(R_3NH)^+$ 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