formation is the conversion of kryptogenin (I) to diosgenin (II). The subsequent methylation by methanolic hydrochloric acid is completely analogous to the transformation of glucose to α-methyl glucoside under like treatment. In addition, the formula (VI) is in complete accord with the facts in having a reactive hydroxyl group, a methoxyl group, and two inert oxygens. Furthermore, formulation of bethogenin as VI accounts for the lack of typical carbonyl absorption. It is likely that treatment with hydrogen bromide in acetic acid regenerates the carbonyl groups of kryptogenin with the loss of methanol, accounting for the typical ketone absorption of the product. Such a reaction occurs when bethogenin is treated with hydroxylamine in pyridine. In this case the methoxyl group is lost and a dioxime is formed, melting 250–253° dec., which is identical with the dioxime prepared from kryptogenin. *Anal.* Calcd. for $C_{27}H_{44}O_4N_2$: C, 70.4; H, 9.6; N, 6.1. Found: C, 70.6; H, 9.7; N, 6.0. The formulation of bethogenin as IV is doubtful since it should then readily show ketone absorption.

Our experiments indicate that bethogenin obtained by Noller is formed by the reaction of kryptogenin with methanol present in the acid mixture used for the hydrolysis of the saponins and is, therefore, not the aglucone of a naturally occurring glycoside.

We thank Parke, Davis and Company.

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RECEIVED JULY 19, 1943

EXISTENCE OF AN ALKALI-STABLE DERIVATIVE OF PANTOTHENIC ACID IN BIOLOGICAL MATERIALS

Sir:

Although alkalies readily destroy pure pantothenic acid, alkaline hydrolysis of fresh tissues does not always reduce the pantothenic acid activity—as measured by the microbiological method—completely to zero.[1] A sample of pork liver after autoclaving for one and one-half hours at 15 pounds pressure with six volumes of 2 *N* sodium hydroxide still showed pantothenic acid activity equivalent to 1.5 μg. per g. of the original tissue.[1b] The active substance was removed from the hydrolyzate by adjusting to *p*H 1 and adsorbing on charcoal, and was eluted from the charcoal with a pyridine:alcohol:water mixture. Eluate A, Table I, is a typical preparation. Such eluates showed no further loss of activity when autoclaved in 0.5 *N* sodium hydroxide.

Preparations containing the alkali-stable substance were similarly obtained from 1:20 liver powder.[2] Eluate B, Table I, was obtained from liver powder which had not been treated with alkali, and eluate C is the same preparation after being autoclaved with 0.5 *N* sodium hydroxide and further purified.

TABLE I

PANTOTHENIC ACID CONTENT OF LIVER FRACTIONS

Expt.	Material	Treatment	Pantothenic acid content,[a] μg. per g.
1	Eluate A	.....	0.88
2	Eluate A	Digested with clarase 48 hr., 37°, *p*H 4.8	9.0
3	Solution from expt. 2	Autoclaved 1 hr. with 1/5 vol. of *N* NaOH	<0.067
4	Liver powder	.....	400
5	Liver powder	5 g., 100 cc. 0.5 *N* NaOH 48 hr. at room temp.	11.6
6	Eluate B	.....	120
7	Eluate C	.....	6.5

[a] Assays were carried out by the method of Neal and Strong.[1b] Results are expr ssed as calcium pantothenate and are calculated back to the original starting material in each case.

Table I shows the results of microbiological assays on these and other preparations. The fact that somewhat similar potencies were observed after alkali treatment either of the starting material or of an eluate prepared from it (expts. 4, 5, 6, and 7) makes it appear unlikely that the residual activity is due to an artefact arising from the action of sodium hydroxide on some component of the original tissue. It seems more reasonable to assume that the activity is due to a preëxisting derivative of pantothenic acid substituted in such a way as to protect the amide linkage from alkaline hydrolysis. This view harmonizes nicely with the results of expts. 1, 2, and 3. Apparently, the derivative is hydrolyzed by clarase into pantothenic acid itself, which then shows normal susceptibility to alkaline attack.

A chick assay carried out on eluate 3 showed that this preparation is able to replace pantothenic acid in the diet of the chick and indicated a

(1) (a) Strong, Feeney and Earle, *Ind. Eng. Chem., Anal. Ed.*, **13**, 566 (1941); (b) Neal and Strong, *ibid.*, in press.

(2) Obtained from Wilson Laboratories, Chicago, Ill.

potency of approximately 27 μg. per g. of the original liver powder. The chick thus appears to utilize the substance about four times as efficiently as *L. casei*, probably because of partial hydrolysis in the intestinal tract.

The substance is readily adsorbed by charcoal at *p*H 1 but not at *p*H 9 and only partially at *p*H 4.5. It is slowly extractable by ether from strongly acid solutions and from preliminary tests appears to be precipitated by lead acetate. It is stable at *p*H 1 for at least forty-eight hours at 37°, but a sample lost 77% of its potency on autoclaving in 0.5 *N* hydrochloric acid for one hour at 15 pounds pressure. It has been found not only in liver, but also in yeast, vitab, cheese and eggs. The amount present varies from a trace to more than half of the original total pantothenic acid activity. Attempts to isolate the substance are in progress.

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CRYSTALLINE NATURAL α- AND γ-TOCOPHEROLS

Sir:

In a recent paper the purification of natural α-, β-, and γ-tocopherols was described.[1] Further investigation has shown that natural α- and γ-tocopherols thus purified can be crystallized. Synthetic *d,l*-α-tocopherol (Merck) was also converted to a solid but amorphous state. This note describes the method of crystallization and certain properties of the crystalline products.

α-Tocopherol (0.5 g.) in methyl alcohol (20 cc.) was cooled to −35° and scratched occasionally with a glass rod. After eight to ten days the tocopherol crystallized in transparent needles, m. p. 2.5–3.5°. The extinction coefficient of the crystals in ethyl alcohol ($E^{1\%}_{1\,cm.}$ 292 mμ = 71) was slightly but not significantly lower than that of uncrystallized α-tocopherol ($E^{1\%}_{1\,cm.}$ 292 mμ = 73.7).[1] This provides additional evidence of the purity of the latter.

Crystalline γ-tocopherol, obtained by the same procedure, consisted of clumps of transparent needles which melted at −3 to −2° and had $E^{1\%}_{1\,cm.}$ 298 mμ = 93.2. Uncrystallized γ-tocopherol had $E^{1\%}_{1\,cm.}$ 298 mμ = 92.8.[1] Therefore, the extinction coefficient of γ-tocopherol was also unchanged significantly by crystallization.

Synthetic α-tocopherol (Merck) was obtained as a white amorphous solid when cooled to −35° in methyl alcohol solution and seeded with natural α-tocopherol crystals. It melted to a light straw colored oil at about 0° and had $E^{1\%}_{1\,cm.}$ 292 mμ = 70.

Attempts to crystallize natural β-tocopherol were unsuccessful.

(1) Baxter, Robeson, Taylor and Lehman, THIS JOURNAL, **65**, 918 (1943).

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NEW BOOKS

Dictionary of Biochemistry and Related Subjects. Editor-in-Chief, WILLIAM MARIAS MALISOFF, Professor of Biochemistry at the Polytechnic Institute of Brooklyn. Published by Philosophical Library, Inc., 15 East 40th St., New York, N. Y., 1943. 579 pp. 15.5 × 23.5 cm. Price, $7.50.

The intention of the Editor-in-Chief in compiling this work is expressed in the preface as follows: "The Dictionary of Biochemistry is a pioneering effort in an entirely new field. There have been no previous dictionaries of this kind. Furthermore, the concept of a "dictionary" has been changing from that of a mere alphabetical glossary to something resembling an encyclopedia. . . . The dictionary contains a great deal of glossary material and also a great deal of fairly lengthy authoritative discussion. It tries to maintain a balance between obsolescent, established, and newly explored material. It is designed for readers of biochemical literature who might want the definitions of terms used more than a decade ago as well as of terms just coined. There was no intention of replacing textbooks or abstract or review journals, except insofar as certain items are greatly neglected or are not easily available."

One will find in this volume about ten thousand definitions of biochemical terms. In addition, there are short articles on various topics by forty-six collaborators. Among these collaborators, one will find the names of many leading investigators in the medical sciences. Some of the