COMMUNICATIONS TO THE EDITOR

STEROLS. CLIX. SAPOGENINS. LXXI. BETHOGENIN

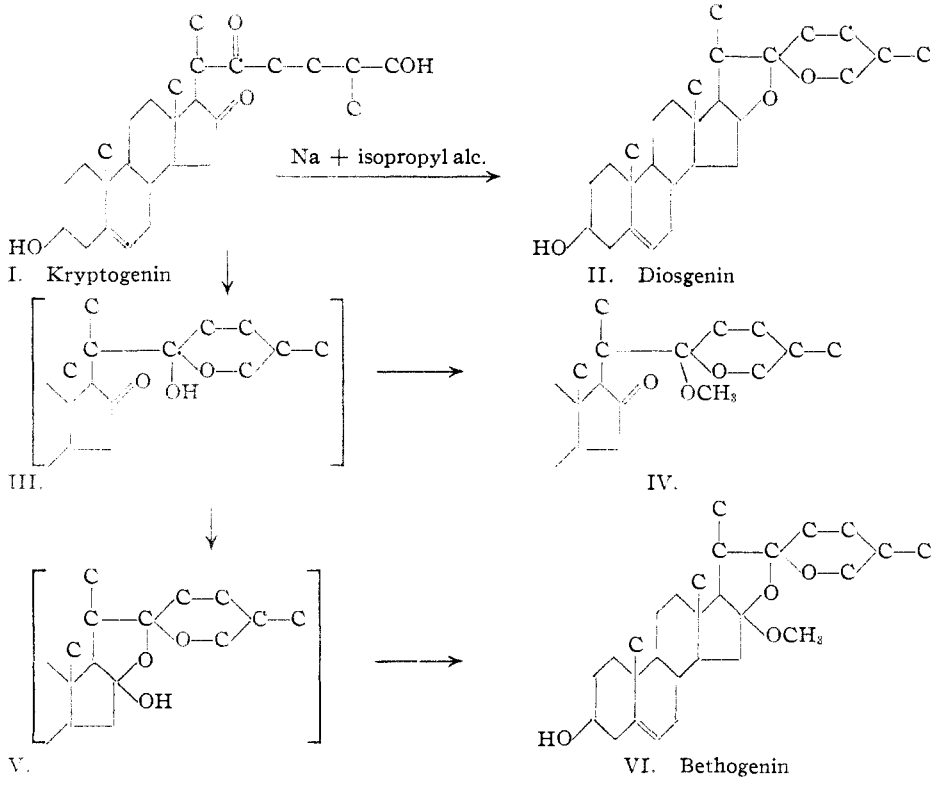
Sir:

Noller and co-workers [THIS JOURNAL, **64,** 2581 (1942)] reported the isolation of diosgenin and a new steroidal sapogenin, bethogenin, in equal quantities from *Beth* root (obtained from S. B. Penick and Co.). For this new substance they initially proposed the formula $C_{27}H_{40}O_4$, but recently [*ibid.*, **65,** 1435 (1943)] revised this to $C_{28}H_{44}O_4$. Two of the oxygen atoms are present as hydroxyl and methoxyl groups, while the other two inert oxygens are assigned to the side-chain characteristic of the steroidal sapogenins.

In the course of our investigations of plant sources for sapogenins, we have processed over ten thousand pounds of *Beth* root from twenty sources (including several lots from S. B. Penick and Co.) and have failed to find this substance. In all cases diosgenin (II), kryptogenin (I), pennogenin, nologenin, fesogenin and kappogenin [*ibid.*, **65,** 1199 (1943)] accounted for over 85% of the total crystalline products.

On the basis of these plant studies, we long suspected that bethogenin was formed from kryptogenin. That this assumption is correct is shown by the fact that kryptogenin readily gives bethogenin. Thus, treatment of the former (I) with methanol containing a small amount of hydrochloric acid, followed by crystallization of the product from 2% methanolic potassium hydroxide gives the latter (VI) in almost quantitative yields. Bethogenin: m. p., 193–194°; calcd. for $C_{28}H_{44}O_4$: C, 75.6; H, 10.0. Found: C, 75.6; H, 10.1. Acetate: m. p., 230°; calcd. for $C_{30}H_{46}O_5$: C, 74.0; H, 9.5. Found: C, 73.9; H, 9.5. Benzoate: m. p., 220°; calcd. for $C_{35}H_{48}O_5$: C, 76.6; H, 8.8. Found: C, 76.8; H, 8.7. On the other hand, similar treatment of kryptogenin (I) with ethanol free from methanol gives only unchanged material even under more vigorous conditions than above.

Kryptogenin (I) shows strong absorption in the ultraviolet region characteristic of the ketone grouping, $\lambda_{max.} = 2850$, $\log \epsilon = 1.81$. Bethogenin does not show this typical carbonyl absorption. Noller reports, however, that bethogenin after treatment with hydrogen bromide in acetic acid gives a product showing almost identical absorption as above, $\lambda_{max.} = 2850$, $\log \epsilon = 1.77$.



In view of the method of formation of bethogenin from kryptogenin, we suggest its structure to be VI. This reaction probably proceeds through two hemi-ketalization products (III) and (V) followed by methylation. A pertinent illustration of the ketal

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.

RUSSELL E. MARKER
R. B. WAGNER
SCHOOL OF CHEMISTRY AND PHYSICS CLARENCE H. RUOF
THE PENNSYLVANIA STATE COLLEGE PAUL R. ULSHAFER
STATE COLLEGE, PENNA. DALE P. J. GOLDSMITH

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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.