THE RELATIVELY INERT OXYGEN ATOM OF DIGOXIGENIN, SARMENTOGENIN AND THE STEROID COMPOUNDS OF THE ADRENAL CORTEX

Sir:

We have recently described [THIS JOURNAL, **60,** 1493, 2566 (1938)] a series of *etio*-cholanic acids and esters with oxygen atoms at C-3 and C-12. Steiger and Reichstein [*Helv. Chim. Acta,* **21,** 828 (1938)] have prepared from digoxigenin a similar series of acids which were thought to have oxygen atoms at C-3 and C-11. Since the 3,*x*-diketo-4-*etio*-cholenic acids derived from digoxigenin and from corticosterone proved to be different, they considered it doubtful that an oxygen atom occupied C-11 in the acids derived from digoxigenin. Through the courtesy of Professor Reichstein we have been able to compare directly the methyl diketo-*etio*-cholanates and the methyl diketo-4-*etio*-cholenates from the two sources. The results given in the table show that the properties of the esters from the two sources are identical. We conclude that Reichstein's acids are identical with those derived from desoxycholic acid and that the inert oxygen atom of digoxigenin, which was assigned to C-11 by Tschesche and Bohle [*Ber.* **69,** 793 (1936)], is actually at C-12.

		From digoxigenin		From desoxycholic acid	
	Acid or ester	M. p., °C.	$[\alpha]^{19}_D$	M. p., °C.	$[\alpha]^{25}_D$
1	Methyl diketo-*etio*-cholanate	171–172[a]	138.3 ± 2	169–170[a]	142 ± 3
2	Methyl diketo-4-*etio*-cholenate	236–238[b]	185 ± 2	236–238[b]	190 ± 6
3	Diketo-*etio*-cholanic acid	170		178	
4	Dihydroxy-*etio*-cholanic acid	280–286		283–286	
5	Methyl dihydroxy-*etio*-cholanate	180–183		145–146	

[a] Mixtures melted at 169–170°.

[b] Mixtures melted at 236–237°.

The other comparisons of the table are less satisfactory since the specific rotations of these compounds of Reichstein were not known and specimens were not available for mixed melting point determinations. The melting points of the diketo-*etio*-cholanic acids are in reasonable agreement, since we have found, as has Reichstein, that the acid is very difficult to purify. The methyl dihydroxy-*etio*-cholanates appear to be different since the melting point of our ester was sharp and could not be raised by distillation or recrystallization. Accepting this difference, epimerism at C-12 is indicated since the configuration of both esters at C-3 is α (no precipitation with digitonin). This difference is not certain, however, since the corresponding acids melt at the same point.

It has been generally accepted since the work of Tschesche and Bohle [*Ber.* **69,** 2497 (1936)] that digoxigenin and sarmentogenin differ only in the configuration at C-9. Inasmuch as some of the adrenal steroids contain an oxygen atom which corresponds in almost all respects with the inert oxygen atom of sarmentogenin, it has been assumed [Marker, THIS JOURNAL, **60,** 1725 (1938)] that they had a configuration at C-9 like that of sarmentogenin which was supposed to have a *cis* linkage of rings B and C instead of the normal or *trans* linkage. Marker [THIS JOURNAL, **60,** 1061 (1938)] has made the same assumption for his urane compounds. There is now no need to assume such a configuration at C-9 for sarmentogenin to explain its difference from digoxigenin, although such a possibility is by no means excluded. Consequently, such an assumption for the adrenal compounds has no basis. Indeed, all the evidence [Reichstein, *Helv. Chim. Acta,* **19,** 402 (1936); **20,** 978 (1937); **21,** 161 (1938)] points to a normal steroid skeleton with an oxygen atom at C-11.

DEPARTMENT OF BIOCHEMISTRY H. L. MASON
THE MAYO FOUNDATION W. M. HOEHN
ROCHESTER, MINNESOTA

RECEIVED OCTOBER 22, 1938

ABSENCE OF COMBINED FATTY ACID IN CEREAL STARCHES

Sir:

The common cereal starches contain fatty material which cannot be extracted by solvents such as petroleum ether, ethyl ether or carbon tetrachloride. From this behavior, T. C. Taylor and his co-workers assumed that the fatty acid must be present as an ester of the carbohydrate substance. His later work indicated that the α-amylose fraction of the starch was characterized by—and owed its insolubility to—this esterification with fatty acid.

Recent investigations in these Laboratories show that the fatty acids in corn, wheat and rice starches can be removed completely by Soxhlet extraction with certain water-miscible fat solvents, particularly methanol, the cellosolves, and 80% dioxane. The de-fatted starch retains its characteristic granule structure and optical birefringence, and gelatinizes in hot water to give a paste of normal high viscosity. By treatment with alcoholic oleic acid, such de-fatted starch can be reimpregnated with fatty acid, and this added fat cannot be extracted by hydrocarbon type solvents.

Hence, it appears that free fatty acid is distributed throughout the starch granule as an extraneous impurity. To explain selective extraction, it is suggested that only those fat solvents bearing hydrophilic groups can penetrate into the granule, hydrocarbon types merely washing the surface. This investigation is being continued, and final results will be reported in detail at an early date.

CORN PRODUCTS REFINING COMPANY
EDGEWATER, N. J. THOMAS JOHN SCHOCH

RECEIVED SEPTEMBER 23, 1938

PANTOTHENIC AND NICOTINIC ACIDS AS GROWTH FACTORS FOR LACTIC ACID BACTERIA

Sir:

In a previous publication the preparation of highly active concentrates (1 unit in 0.5 γ) of an essential growth factor for lactic acid bacteria has been described [Snell, Strong and Peterson, *Biochem. J.*, **31,** 1789 (1937)]. Considerable difficulty has been encountered in attempts to further purify this factor, and a long series of fractionation procedures resulted in only about a two-fold increase in activity (1 unit in 0.20 γ).

Recently, information became available [R. J. Williams, papers presented before the American Chemical Society at Milwaukee, Wisconsin, Sept. 5–9, 1938, and private communications] which revealed a striking similarity between the properties of the above growth factor and pantothenic acid [Williams, *et al.*, THIS JOURNAL, **55,** 2912 (1933)]. Both are water-soluble, nitrogenous, organic, hydroxy acids, easily destroyed by aqueous acids or alkalies, but relatively resistant to the action of light, nitrous acid, bromine, and mild oxidizing agents. Through the kindness of Dr. R. J. Williams it has now been possible to subject two samples of pantothenic acid to our bacterial test. One preparation, A, contained approximately 40%, and the other, B, 83% calcium pantothenate. The following results were obtained on *Lactobacillus casei* as the test organism:

Supplement	γ Added per 10 cc. medium a	b	c	d	Cc. 0.1 *N* acid produced per 10 cc. medium a	b	c	d
Sample A	0.0	0.05	0.1	0.5	0.5	1.4	3.5	7.0
Sample B	.0	.02	.05	.3	.5	1.3	3.3	7.8

These and other tests indicated that sample A contained one unit in approximately 0.13 γ, and that B possessed about twice this activity.

Sample B was further tested on *Bacillus lactis acidi*, *Lactobacillus arabinosus*, *Lactobacillus pentosus*, *Lactobacillus delbrückii*, *Bacillus brassicae*, *Streptococcus lactis*, *Leuconostoc mesenteroides*, and *Propionibacterium pentosaceum*, and proved to be highly active for each organism. All of these organisms had previously been shown to require the growth factor. It appears certain that the active substance in our concentrates is identical with pantothenic acid, and that our best preparation contained approximately 26% pantothenic acid.

In addition to pantothenic acid some lactic acid bacteria seem to require nicotinic acid as well. Six species tested were found to grow very poorly or not at all in an acid-hydrolyzed casein medium containing both riboflavin and pantothenic acid. When small amounts of nicotinic acid were added to this medium, a marked improvement in growth and acid production occurred in some cases.

Organism	γ Nicotinic acid added per 10 cc. medium a	b	c	d	Cc. 0.1 *N* acid produced per 10 cc. medium a	b	c	d
L. casei	0.0	0.1	0.3	0.5	2.2	4.1	5.4	6.6
L. arabinosus	.0	.1	.3	.5	1.7	4.7	...	5.2

From the above data it appears that nicotinic acid is also an essential growth factor for at least two species of lactic acid bacteria.

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RECEIVED SEPTEMBER 24, 1938

ORIENTATION OF HIGH MOLECULAR WEIGHT LINEAR POLYMERS IN UNSTRETCHED FILMS

Sir:

Recent electron diffraction studies by Storks[1] furnish definite evidence that unstretched films of polyethylene sebacate consist of relatively small crystallites which are oriented with their long axes approximately in the plane of the film, and with limited rotation around this axis, "probably due in part to the presence of carbonyl groups along the chain." Further, Storks points out that such films will probably show a preferred orientation until the film thickness exceeds the average chain length; only beyond this point do high molecular weight linear polymers exhibit truly amorphous Debye–Scherrer ring patterns.

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[1] Storks, THIS JOURNAL, **60,** 1753 (1938).