[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF THE UNIVERSITY OF TEXAS]

Pantothenic Acid. IX. The Biological Activity of Hydroxypantothenic Acid

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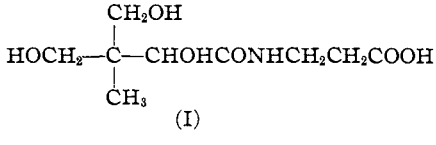
The structure of pantothenic acid as reported[1,2] is thought to be that of a single specific substance responsible for the observed physiological activity of tissue extracts under conditions used in testing for this substance.

Subsequent to the report of Williams, *et al.*,[3] that pantothenic acid consisted of a condensation product between β-alanine and an unidentified dihydroxy acid, and before the correct structure was established, several related condensation products of lactones and β-alanine were prepared in our and other laboratories.[4,5,6,7] Several of these products were found to possess slight physiological activity when tested on organisms such as the lactic acid bacteria[8] which do not respond to β-alanine alone.

The compounds tested included in addition to those previously mentioned,[4] N-(α,δ-dihydroxyvaleryl)-β-alanine and N-(α,ε-dihydroxycaproyl)-β-alanine. These were prepared from ornithine and lysine, respectively, followed by coupling with β-alanine. Of these two compounds the former has been shown by two independent groups of workers[6,9] to support the growth of certain hemolytic streptococci in synthetic media, when furnished in place of the naturally occurring pantothenic acid. None of the above compounds, however, possessed more than a fraction of 1% of the activity of pure pantothenic acid.

It occurred to one of us (H. K. M.) to prepare the compound with the structure (I)



(I)

on the supposition that it might possess considerable physiological activity. This proved to be the case.

(1) R. J. Williams and R. T. Major, *Science*, **91**, 246 (1940).
(2) Stiller, *et al.*, THIS JOURNAL, **62**, 1779 (1940).
(3) Williams, Weinstock and Mitchell, Abstracts Division of Organic Chemistry, Amer. Chem. Soc., Milwaukee (1938).
(4) Mitchell, Weinstock, Snell, Stanbery and Williams, THIS JOURNAL, **62**, 1776 (1940).
(5) Snell, Woolley and Strong, unpublished data (1939).
(6) Subbarow and Rane, THIS JOURNAL, **61**, 1616 (1939).
(7) T. Reichstein, private communication (1940).
(8) E. E. Snell, F. M. Strong and W. H. Peterson, *J. Bact.*, **38**, 293 (1939).
(9) Woolley and Hutchings, *ibid.*, **39**, 287 (1940).

Experimental

Preparation of "Hydroxypantothenic Acid" (I).— Sixteen grams of the aldehyde $CH_3C(CH_2OH)_2CHO$ was prepared by condensing formaldehyde with *n*-propionaldehyde according to the procedure of Koch and Zerner.[10] The viscous aldehyde obtained was dissolved in 20 ml. of ether and kept cool in an ice-bath while 10 ml. of liquid hydrogen cyanide and 1 drop of trimethylamine were added. After standing overnight the mixture was hydrolyzed by treating with 50 ml. of 6 *N* hydrochloric acid on the steam-bath for four hours. It was then made strongly alkaline with sodium hydroxide, and extracted twice with ether. The aqueous residue was made to *p*H 1.0 with concentrated hydrochloric acid and heated on the steam-bath for five hours. The resulting product was extracted twice with ether. The extracts contained very little lactone and were discarded. The solution was then extracted continuously with ether. Evaporation of the ether yielded about 3 g. of material which proved to have an equivalent weight of 143 (theoretical 146), an oxidation equivalent[11] of 1.49 (theoretical 1.42) and to be mostly in the form of a lactone. Attempts to further purify the material by distillation were unsuccessful.

The lactone was coupled with β-alanine salt according to the procedure of Williams, *et al.*[12] The product was purified by precipitation from alcoholic solution by the addition of five volumes of petroleum ether. From 235 mg. of β-alanine sodium salt and 344 mg. of crude lactone there was obtained 201 mg. of sodium hydroxypantothenate.

The product thus obtained was tested, by techniques previously described, for its activity in promoting the growth of yeast and of various lactic acid bacteria.[8] Its activity for these organisms was compared by parallel tests using synthetic dextrorotatory calcium pantothenate (Merck Laboratories[13]) as a standard, with a concentrate of known potency from liver. The results are given in Table I.

Discussion

It is evident that the availability of hydroxypantothenic acid for growth varies not only with the organism, but with the conditions under which it is grown (such as incubation time and the medium used). Indeed, in many cases the growth curves obtained were entirely dissimilar, showing high activity at low dosage levels and progressively lower activity with increasing concentration. Under different testing conditions hydroxypantothenic acid might show activity materially

(10) H. Koch and Th. Zerner, *Monatsh.*, **22**, 443 (1901).
(11) R. J. Williams, THIS JOURNAL, **59**, 288 (1937).
(12) R. J. Williams, H. K. Mitchell and E. E. Snell, *ibid.*, **62**, 1784 (1940).
(13) Stiller, Harris, Finkelstein, Keresztesy and Folkers, *ibid.*, **62**, 1785 (1940).

TABLE I

COMPARATIVE PHYSIOLOGICAL ACTIVITY OF HYDROXYPANTOTHENIC ACID

Organism	Incubation period, hr.	Synthetic (+) calcium pantothenate (standard)	Liver concentrate "potency" 3300	Sodium hydroxypantothenate (racemic value × 2)
Saccharomyces cerevisiae (GM)	14	100	24.6	5.2
Streptococcus lactis (R)	20	100	27.2	5.3-2.4[b]
Streptococcus lactis (R)	38	100	24.7	23.0-2.4
Lactobacillus casei e	17	100	27.3	1.5-1.7
Lactobacillus casei e	23	100	25.3	1.5-1.8
Lactobacillus casei e	24[a]		27.0	20.2-12.7
Lactobacillus arabinosus (17-5)	20	100	24.2	1.5
Bacillus brassicae (6-26)	38	100	26.9	1.7
Propionibacterium pentosaceum (P-11)	38	100	25.6	3.0-1.3

[a] This culture was grown in the improved medium used for the assay of pantothenic acid in crude tissue extracts (Pennington, Snell and Williams, to be published); other bacterial determinations were made in the pantothenic acid-free medium B of Snell, *et al.*[8] [b] The values given indicate those obtained in a single assay from low to high dosage levels within the assay range.

higher than has been observed. On the contrary, the concentrates from natural sources behaved exactly as synthetic calcium pantothenate, thus indicating that the "pantothenic acid" activity of natural extracts is due to a single substance as originally postulated,[14] and not to a mixture of related substances. Incidentally, the "potency" of pure synthetic calcium pantothenate appears to be very close to what would be predicted from results obtained before the synthesis[15] was accomplished.

Tests on the physiological activity of hydroxypantothenic acid for animals have not yet been made.

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Summary

"Hydroxypantothenic acid" (N-(α-hydroxy-β,-β'-dimethylolbutyryl)-β-alanine) has been synthesized and shown to possess striking biological activity. Its effectiveness varies with the microorganisms and the testing conditions.

From the variable results obtained with this compound and the concordant results obtained when concentrates from natural sources are tested for pantothenic acid, it is concluded that natural pantothenic acid is probably a single substance and that hydroxypantothenic acid probably does not occur in such concentrates.

(14) Williams, Lyman, Goodyear, Truesdail and Holaday, THIS JOURNAL, **55**, 2912 (1933).

(15) Williams, Truesdail, Weinstock, Rohrmann, Lyman and McBurney, *ibid.*, **60**, 2719 (1938).

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Oxidation of Alginic Acid by Periodic Acid

BY H. J. LUCAS AND W. T. STEWART[1]

Evidence in regard to the structure and mode of linkage of the mannuronic acid units in alginic acid[2] has been obtained recently by Hirst, Jones and Jones.[3] They subjected alginic acid to partial degradative methanolysis by means of methanolic hydrogen chloride and completely methylated the partially degraded alginic acid. This under drastic treatment with methanolic hydrogen chloride gave the methyl ester of 2,3-dimethylmethyl-*d*-mannuronide which was then hydrolyzed to 2,3-dimethyl-*d*-mannuronic acid. The last was oxidized to 2,3-dimethyl-*d*-mannosaccharic acid. From this and other evidence they concluded that in the mannuronic units of alginic acid hydroxyl groups are attached to C_2 and C_3, while bridge and ring linkages are attached to C_4 and C_5. Although the evidence did not permit a decision between pyranose and furanose structures, the former was favored in view of the resistance of alginic acid toward hydrolysis, and its large negative rotation.

Independent evidence from the oxidation of alginic acid is desirable. Periodic acid would be expected to convert the mannuronic units of al-

(1) Kelco Company Fellow, 1938-1939.

(2) Nelson and Cretcher, THIS JOURNAL, **51**, 1914 (1929); **52**, 2130 (1930); **54**, 3409 (1932); Bird and Haas, *Biochem. J.*, **25**, 403 (1931); Schoeffel and Link, *J. Biol. Chem.*, **100**, 397 (1933).

(3) Hirst, Jones and Jones, *Nature*, **143**, 857 (1939); *J. Chem. Soc.*, 1880 (1939).