whence $x = x_0e^{-k_at}$, and $y = y_0e^{-k_bt}$. Now

$$\mathrm{d}q/\mathrm{d}t = k_1x + k_2y$$
$$= k_1x_0\, e^{-k_at} + k_2y_0\, e^{-k_bt}$$

or

$$q = \frac{k_1x_0}{k_a}(1 - e^{-k_at}) + \frac{k_2y_0}{k_b}(1 - e^{-k_bt})$$

or

$$= \left(\frac{k_1x_0}{k_a} + \frac{k_2y_0}{k^b}\right) - \frac{k_1x_0}{k_a}e^{-k_at} - \frac{k_2y_0}{k_b}e^{-k_at}$$

If Equation 17 is used, one obtains with the above assumptions

$$\frac{\mathrm{d}q}{\mathrm{d}t} = \frac{a}{b}(x_0e^{-k_at} + y_0e^{-k_bt})$$

or

$$q = \frac{a}{b}\frac{x_0}{k_a}(1 - e^{-k_at}) + \frac{a}{b}\frac{y_0}{k_b}(1 - e^{-k_bt})$$

Hence by comparison, the use of Equation 17 has made $a/b = k_1 = k_2$. Thus it would appear from this that the specific rates of action for the two active groups are the same. I believe it then follows by comparison with equation 20 that $x_0/y_0 = 4.8$ whereas $ka = 2.0/b$; and $kb = 1/3b$ as before." Although there is recent evidence[28] that the enzyme tyrosinase exists as a single protein, rather than a mixture of enzyme proteins, it is conceivable that two different catecholase, activity centers of different inactivation rates, might exist on the single protein at the start of the reaction. No experimental data are at hand, therefore, to allow for a choice between the interpretation of Dr. Lineweaver and that presented by the authors.

that such a model is not inconsistent with previously known facts concerning the inactivation properties of different types of tyrosinase preparations. A general equation derived from this kinetic model is presented, and it is suggested that the chronometric equation is merely an empirical simplification thereof.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, OREGON STATE COLLEGE]

Pantothenic Acid Studies. VII. N-Methylpantothenic Acid[1]

BY REX D. LINDSAY AND VERNON H. CHELDELIN

Pantothenic acid has been shown to exhibit a high degree of structural specificity for physiological action. The unnatural optical antipode, (−)pantothenic acid, is devoid of activity for bacteria[2] or rats,[3] and with the exception of "hydroxypantothenic acid,"[4] all structural analogs which have been prepared show dramatic reduction in activity. In addition, many of these analogs have been shown to competitively inhibit the action of the vitamin.

The comparative actions of various analogs of pantoic acid and pantothenic acid have been studied in microörganisms in an effort to locate possible points of attachment of the vitamin within the cell.[5] It has been suggested that this attachment normally takes place through the pantoic acid portion, since changes in this portion usually produce inert or slightly active analogs,[5] whereas alterations in the β-alanine moiety give uniformly good inhibitors for organisms requiring the preformed vitamin.[6–11] However, the function of the amide nitrogen has never been elucidated. The present study was undertaken to prepare N-methylpantothenic acid and to determine its growth promoting activity for different organisms.

The synthesis of N-methyl-β-alanine presumably could be accomplished readily by treating β-bromopropionic acid with methylamine.[12] However, this reaction invariably led to the simultaneous formation of amides, which in our experience could not be removed satisfactorily. Another possibility appeared through the addition of methylamine to acrylonitrile, but hydrolysis to the acid was unsuccessful. Alcoholic acid hydrolysis gave the (ethyl) ester of N-methyl-β-alanine, but in low yields. The desired compound was finally prepared as the ester in 35% yield through addition of methylamine to methyl acrylate.[13]

Syntheses of N-methylpantothenic acid (α,γ-dihydroxy-β,β-dimethylbutyryl-β′-N-methylalanide) were thus performed by condensing pantolactone with the methyl or ethyl ester of N-methyl-β-alanine, followed by saponification of the ester with barium hydroxide and removal of barium with sulfate. The product after purification was obtained as a viscous oil, which was best characterized as the brucine salt.

Experimental

Preparation of β-Cyanoethylmethylamine.—Acrylonitrile (21 g.) was slowly added with stirring and cooling to methylamine (15 g.) in methanol (65 g.).[14] Removal of the solvent and distillation gave the product (25 g.), b. p. 73° (16 mm.).

Ethyl β-Methylaminopropionate.—Hydrolysis of β-cyanoethylmethylamine (18 g.) with sulfuric acid (45 ml.) and ethyl alcohol (53 ml.) for five hours, gave 5 g. (18% yield) of the product, b. p. 66° (15 mm.).

(1) This material is taken from the thesis presented by R. D. L. for the degree of Master of Science, Oregon State College, 1949. The investigation was supported by research grants from the Division of Research Grants and Fellowships of the National Institute of Health, U. S. Public Health Service and the Nutrition Foundation, Inc. Published with the Approval of the Monographs Publications Committee, Oregon State College, Research Paper No. 139, School of Science, Department of Chemistry. A preliminary report was presented before the Pacific Coast convention of Phi Lambda Upsilon, Corvallis, Oregon, February 18-19, 1949.

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TABLE I

THE GROWTH RESPONSE OF VARIOUS ORGANISMS TO N-METHYLPANTOTHENIC ACID AND N-METHYL-β-ALANINE

		Organisms — Apparent PA activity					
		L. arabinosus		*A. suboxydans*		LM yeast	
Material	γ/10 ml.	γ/10 ml.	% act.	γ/10 ml.	% activ.	γ/10 ml.	% activ.
$N-CH_3PA$[a]	0.1	0	0			0	0
	1	0.0015	0.15				
	2			0.3	15.0		
	5	.002	.040	0.85	17.4		
	10	.0035	.035	1.86	18.6		
	50	.0038	.076	2.95	5.9	0.02	0.04
	200					.22	.11
$N-CH_3PA$ + PA[b]	50+0.03	.065	.070				
	100+0.06	.10	.080				
	50+0.3					.33	.06
	100+0.6					.60	.00
$N-CH_3B$[c]	1000					.18	.018

[a] N-Methylpantothenic acid. [b] N-Methylpantothenic acid plus pantothenic acid, the latter being added as the calcium salt. [c] N-Methyl-β-alanine.

Preparation of Methyl β-Methylaminopropionate.—Eighty-six grams of methyl acrylate was added to 35 g. of methylamine dissolved in absolute ethyl alcohol and the mixture was allowed to react at room temperature for two days. Removal of the solvent and distillation gave 46 g. (35% yield) of the desired product,[13] b. p. 50° (11 mm.).

Preparation of DL-N-Methylpantothenic Acid.—Eight grams of methyl β-methylaminopropionate (or an equivalent quantity of the ethyl ester) was treated with 8 g. of DL-pantoyl lactone at 75° for four and one-half hours. The viscous material was converted to the free acid by saponification with 300 ml. of 0.45 *N* barium hydroxide. The excess barium ion was removed quantitatively with sulfuric acid, the *p*H adjusted to 6.0 with pyridine and the solution evaporated to dryness *in vacuo*. A slightly yellow, viscous oil was obtained. The carbon content was found to be 95% of the theoretical calculated for N-methylpantothenic acid. The brucine salt was prepared to purify the compound further.

The Brucine Salt of N-Methylpantothenic Acid.—N-Methylpantothenic acid in 50% (v/v) aqueous methanol was treated with a nearly saturated solution of brucine in the same solvent. On cooling, crystals formed and were washed on a sintered glass filter with 50% aqueous methanol and with 95% ethyl alcohol several times to remove excess brucine. The crystals were dried over phosphorus pentoxide for analysis.

Anal. Calcd. for $C_{33}H_{45}O_9N_3$: C, 63.20; H, 7.03; N, 6.71. Found: C, 63.60; H, 6.75; N, 6.24.

Organisms and Testing.—The organisms used for testing were *Lactobacillus arabinosus* 17-5; *Acetobacter suboxydans*, A. T. C. C. No. 621; and *Saccharomyces cerevisiae*, Lash Miller (LM) strain. All tests were performed by previously published methods.[15,16,17] β-Alanine was omitted from the *A. suboxydans* medium.

Results

The growth promoting effect of N-methylpantothenic acid was tested upon *L. arabinosus*, which requires the intact vitamin, and upon *A. suboxydans* and LM yeast, which require only pantoic acid and β-alanine, respectively. The effect of N-methyl-β-alanine upon yeast was also studied. The results are summarized in Table I. It may be seen that for *L. arabinosus* and yeast the analog possesses less than 0.1% of the activity of the vitamin, either alone or in the presence of sub-optimal concentrations of the vitamin. No inhibition of growth of either organism was noted by amounts up to 5 mg. of the analog per tube. N-Methyl-β-alanine is even less stimulatory for yeast than is N-methylpantothenic acid, although the relative activity of the two compounds is in approximately the same ratio as that of β-alanine and pantothenic acid for this organism.

N-Methylpantothenic acid possesses significant activity for *A. suboxydans*, varying from approximately 5 to 18% that of the vitamin. This recalls the similar activity of pantoyltaurine and other amides of pantoic acid for this organism.[5] It is probably due to hydrolysis of the analog by the organism to yield pantoic acid, which is as active as the intact vitamin.

Discussion

In attempting to apply the Woods–Fildes theory to the action of pantothenic acid analogs, it was pointed out previously[5] that attachment to the usual cellular enzymes is a prerequisite for both growth promoting activity and inhibition. Inert compounds must therefore be regarded as incapable of attachment, at least in the normal manner. Since N-methylpantothenic acid was virtually inactive for *L. arabinosus* and yeast under all conditions tested, it would appear that this analog does not combine with the apoenzyme. The amide group may therefore be considered as a point of attachment, either directly to the apoenzyme or through some other group which does not involve the functional metabolic role of the vitamin. The latter possibility should be considered in view of the rather large molecular weight (800) of coenzyme

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A[18] and perhaps larger weight of PAC, based upon its relative non-dialyzability.[19]

It has been pointed out[5] that since other inert analogs of pantothenic acid have in general differed from the vitamin in the hydroxy acid moiety, an attachment through the moiety is also required for the complete coupling of the vitamin molecule to its apoenzyme. Analogs which differ in the β-alanine portion of the molecule, on the other hand, are almost without exception competitive inhibitors of pantothenic acid, and it has been assumed that this moiety is involved (presumably through the carboxyl group) in metabolic reactions. This view has been strengthened by the findings[20,21] that coenzyme A and the pantothenic acid conjugate (PAC) described in this Laboratory are hydrolyzed by the combined action of a pigeon liver enzyme preparation and a phosphodiesterase, thus releasing the free vitamin for *L. arabinosus* activity. This relatively stable attachment to phosphorus would presumably occur through hydroxyl groups. Thus, although it is still not possible to obtain a detailed picture of the combination of the vitamin within the cell, on the basis of present evidence it seems plausible to suppose that connections between the vitamin and other units in the respective coenzyme molecules are normally made through hydroxy and amide nitrogen linkages.

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Summary

A new analog of pantothenic acid (α,γ-dihydroxy-β,β-dimethylbutyryl-β′-N-methyl alanide) has been prepared and tested for growth effects upon *A. suboxydans*, *L. arabinosus* and LM yeast. The compound has no activity for *L. arabinosus* or LM yeast and from 5–18% activity for *A. suboxydans*. On the basis of evidence obtained it appears that N-methylpantothenic acid does not combine with cellular enzymes in *L. arabinosus*. The amide group may therefore be considered a point of attachment, either directly to the apoenzyme or through some other group which does not involve the functional metabolic role of the vitamin.

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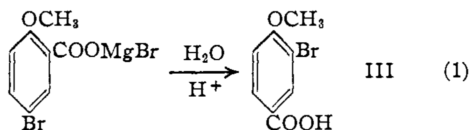
[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF NORTHWESTERN UNIVERSITY]

The Reaction of Magnesium with 2,4-Dibromoanisole

BY ALLEN S. HUSSEY AND I. J. WILK

It has been reported that the reaction of magnesium with 2,4-dibromoanisole (I) in equimolar quantities involves primarily the bromine atom ortho to the methoxyl group.[1] The isolation of 65% of 4-bromoanisole on hydrolysis and 30% of 4-bromo-2-hydroxyanisole on oxidation of the magnesium derivative of I was cited as evidence, although carbonation did not give the expected 5-bromo-2-methoxybenzoic acid (II), but gave instead 3-bromo-4-methoxybenzoic acid (III) in 60% yield.[1b–d] This unexpected result was explained to arise from a rearrangement of the bromomagnesium salt of II on treatment with dilute acid, as shown below



As proof for (1), synthetic II was reported to rearrange to III, and 5-bromosalicylic acid (IV) to rearrange to 3-bromo-4-hydroxybenzoic acid (V), when treated with ethylmagnesium bromide followed by dilute acid.[1b–e] The magnesium derivative of 2-bromo-4-chloroanisole (VI) was reported to give the rearranged acid, 3-chloro-4-methoxybenzoic acid (VII) by an analogous mechanism.[1d]

We have investigated these reactions and our results lead to an entirely different interpretation. The monomagnesium derivative of I is actually a mixture in which the isomer para to the methoxyl group is the major one; thus no rearrangement need be postulated to explain the formation of III on carbonation and acidification.

We have found that the monobromoanisole product obtained on hydrolysis consists of a mixture of 68–72% 2-bromoanisole and 28–32% 4-bromoanisole as indicated by ultraviolet absorption and refractive index. Furthermore, we have not been able to rearrange II to III, nor IV to V, although we varied conditions widely in attempts to do so. In addition, when the magnesium derivative of I was added to a large excess of ethyl carbonate[2] we were able to isolate only III in 55–60% yield (either as the free acid or its ethyl ester).

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