Abstract

A new analogue of pantothenic acid ($\alpha, \gamma$-dihydroxy $\beta, \beta$-dimethyl butyryl $\beta'$-N-methyl alanide) has been prepared and tested for growth effects upon A. suboxydans, L. arabinosus, and L. M. yeast. The compound has no activity for L. arabinosus or L. M. yeast and from 5-18% activity for A. suboxydans. On the basis of evidence obtained it appears that N-methyl pantothenic 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.

Other new analogues: Calcium ($\alpha, \gamma$-dihydroxy butyryl $\beta$-alanide; (6) sodium ($\alpha$-hydroxy isovaleryl) $\beta$-alanide; $\beta$-amino $\gamma$-hydroxy butyryl $\beta$-alanide hydrobromide have been prepared and tested on L. arabinosus.
and *A. suboxydans*. The first of these was found to be a good inhibitor for *L. arabinosus*. The other compounds were practically inert for both organisms. The behavior of these compounds appears to emphasize the importance of both hydroxy groups for attachment of the vitamin to cellular enzymes in lactic acid bacteria, and to the additional influence of the $\beta$-methyl groups in *Acetobacter*. The new inhibitor above is only the second such analogue recorded of pantothenic acid in which the pantoic moiety is altered.

Three analogues of pantoic acid have been prepared and tested on the same organisms. The results again point to the importance of the methyl groups for vitamin activity. It seems probable that they exert an influence upon the spatial arrangement, particularly of the hydroxy groups. These pantoic acid analogues were also tested in the presence of methionine to determine their possible methylation to vitamin-active materials. None of the analogues showed increased activity.
THE SYNTHESIS AND TESTING
OF SOME ANALOGUES
OF PANTOIC ACID AND \( \beta \)-ALANINE

by

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THE SYNTHESIS AND TESTING OF SOME
ANALOGUES OF PANTOIC ACID AND \( \beta \)-ALANINE

INTRODUCTION

Since the discovery of pantothenic acid by Williams in 1933 (42) and the elucidation of its structure in 1940 (43), this vitamin has been the subject of a large number of studies relating to the effect of alteration of structure upon its physiological behavior. As a result of these studies, it has been shown to possess a high degree of structural specificity for its vitamin action. Many analogs have been prepared in an effort to produce compounds of similar biological activity (1,26,32,40,41), but with the exception of "hydroxypantothenic acid" (26) all structural analogs which have been produced show dramatic reduction in potency. In addition, many of these analogs competitively inhibit the action of the vitamin. Many in vitro inhibitors have been found which are effective over a wide concentration range.

In general, inhibitors have arisen from alterations of the amino acid moiety of the molecule. Replacement of the carboxyl group by sulfonic acid (2,18,38), ketone (44) and alcohol (39) groups have produced the most
successful inhibitors. Structural isomers of either the β-alanine or the pantoyl moiety have in general produced inert or very slightly stimulatory compounds.

The comparative actions of various analogs of pantoic acid and pantothenic acid have been studied in microorganisms 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, whereas alterations in the β-alanine portion give uniformly good inhibitors for organisms requiring the performed vitamin (38,2,29,36,44,39). However the function of the amide nitrogen has never been elucidated. One phase of the present study was to prepare N-methyl pantothenic acid (α,γ-dihydroxyβ,β'-dimethyl butyryl β'-N-methyl alanine) and to determine its growth promoting activity for different organisms.

Inhibition studies by King et al (17) have pointed to methionine as a possible precursor of pantothenic acid. Since methionine is widely utilized in biological methylation, it was thought possible that the vitamin might arise by this means, through methylation of a compound related to pantoic acid but lacking the β-methyl
groups. To test this hypothesis $\alpha,\gamma$-dihydroxy butyric acid, $\alpha$-amino $\gamma$-hydroxy butyric acid, and $\alpha$-hydroxy isovaleric acid were prepared and tested in the presence of methionine on an organism which requires pantoic acid. These compounds were also coupled with $\beta$-alanine and tested with organisms requiring pantoic acid and pantothenic acid.

Glutamic acid has a stimulatory effect when tested on certain yeast in the presence of pantothenic acid; also the pantothenic acid conjugate described in this laboratory (16) has been shown to contain significant amounts of glutamic acid (19). From this information it was thought that a peptide of glutamic acid and $\beta$-alanine might possess even greater activity in the presence of pantoic acid and an organism which can couple the moieties. Schink (35) prepared ($\alpha$) glutamyl $\beta$-alanine and $\beta$-alanylglutamic acid and found no growth promoting activity. The significance of peptides in nature is well known and because of their importance the preparation of ($\gamma$) glutamyl $\beta$-alanine was attempted.
EXPERIMENTAL

N-METHYL $\beta$-ALANINE; N-METHYL PANTOTHENIC ACID

Attempted preparation from $\beta$-bromopropanoic acid and methyl amine. — $\beta$-Bromopropanoic acid was prepared according to "Organic Synthesis" (coll. vol. 1, p.131) from ethylene cyanohydrin and 48% hydrogen bromide. Five grams of the product were heated at 120° for six hours in a sealed tube with 15 ml. of a 33% solution of methyl amine in water (25). Attempted isolation by the procedure given led to low yields and an impurity of a hygroscopic amide.

Preparation of $\beta$-cyanoethylmethylamine. — Acrylonitrile (21 g.) was slowly added with stirring and cooling to methyl amine (15 g.) in methanol (65 g.). Removal of the solvent and distillation gave the product (25 g.). The fraction boiling at 73°/16 mm. (6) was collected. Yield 76%.

Ethyl $\beta$-methylaminopropionate. — Hydrolysis of $\beta$-cyanoethylmethylamine (18 g.) with sulfuric acid (45 ml.) and ethyl alcohol (53 ml.) for five hours, gave 5 g. of the product boiling at 66°/15 mm. (6). Yield 18%.

Methyl $\beta$-methylyaminopropionate. — Eighty-six grams of methyl acrylate were added to methyl amine
(35 g.) dissolved in absolute alcohol, the mixture was allowed to react at room temperature for two days. Removal of the solvent and distillation gave 46 g. of the desired product boiling at 50° /11 mm. (27). Yield 35%.

**Preparation of DL-N-methyl pantothenic acid.**

Eight grams (0.077 mole) of methyl p-methylamino propionate (or an equivalent quantity of the ethyl ester) were treated with 8 g. (0.077 mole) of DL pantoyl lactone at 75° for four and five tenths 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 pH adjusted to 6.0 with pyridine and the solution evaporated to dryness in vacuo (40). A slightly yellow, viscous oil was obtained. A carbon and hydrogen analysis showed the compound to be fairly pure; a salt was prepared to purify the compound further.

**The brucine salt of N-methyl pantothenic acid.**

N-methyl pantothenic acid in 50 per cent (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 \( \text{P}_2\text{O}_5 \) for analysis. Analysis calculated for \( \text{C}_{33}\text{H}_{45}\text{O}_9\text{N}_3 \):
C, 63.2; H, 7.03; N, 6.71. Found: C, 63.6; H, 6.75; N, 6.24.
HYDROXY AND AMINO ACIDS RELATED TO PANTOIC ACID

**Attempted preparation of \( \alpha, \gamma \)-dihydroxy butyric acid from \( \beta \)-hydroxy-propionaldehyde.** -- Acrolein (400 g.) was introduced into a steel bomb with four volumes of water and heated (28). The solution was then distilled according to Glattfield (9), however repeated trials failed to give the expected \( \beta \)-hydroxypropionaldehyde. The water solution was therefore saturated with sodium sulfate and extracted with ether in a continuous extractor for 24 hours. The ether extract was subjected to vacuum distillation and the fraction boiling at 83°/17 mm. was collected. Yield 100 grams (19%).

Twenty one grams (0.36 mole) of the above product and 37 grams (0.36 mole) of sodium bisulfite were dissolved in a minimum amount of water. The solution was cooled to 10° and a concentrated (95%) solution of sodium cyanide (18 g.) (0.36 mole) added slowly. The solution was extracted with ether, however no product was obtained. Other methods used for water soluble nitriles were tried without success (28).

**\( \beta \)-chloropropionaldehyde acetal.** -- Acrolein (112 g.) was treated with hydrogen chloride gas dissolved in ethyl alcohol according to "Organic Synthesis" (coll. vol. II, p. 137), and the product distilled. The fraction
boiling at 60-64°/9 mm. was collected. Yield 122 g. (35%).

**Sodium bisulfite addition product.** — The reaction was carried out according to Crawford (7). One mole of the above acetal was decomposed in one mole of warm water, cooled quickly and shaken with 0.25 mole sodium bisulfite in a saturated water solution. The layers were separated and the procedure repeated three times, each time with a fresh sample of 0.25 mole bisulfite solution. In this manner all of the acetal reacted. The use of four separate fractions of sodium bisulfite appeared essential, for yields were very low when fewer extractions were made.

**α-Hydroxy γ-chloropropionitrile.** — The bisulfite addition product from 120 g. of the acetal was treated with a cold aqueous solution of potassium cyanide (7). The crude nitrile was extracted with ether. Yield, 50 g. (58%).

**α-Hydroxy γ-chlorobutyric acid.** — When the nitrile above was hydrolyzed under reflux conditions according to the method of Raske (31), a black residue was obtained. However, the crude acid was obtained successfully by warming 25 g. of the nitrile in a water bath for forty-five minutes with concentrated HCl, diluting
the solution and evaporating to dryness. No further purification was attempted since it was assumed that the chloro acid may have undergone partial hydrolysis during this treatment.

**Calcium (α, γ-dihydroxy) butyrate.** — The chloro acid from the above step was hydrolyzed in water and extracted with ethyl acetate and ether according to the method of Raske (31). After removal of the solvent, the residue was dissolved in water and warmed with excess calcium carbonate. The insoluble portion was filtered off and the solution evaporated to dryness. The remaining wax was dried over P₂O₅ and ground under alcohol to a pale yellow powder. Yield 5 g. (9%). Analysis calculated for C₈H₁₄O₈Ca: Ca, 14.6. Found: Ca, 14.4.

Although the yield from this reaction represented a great improvement over that of Raske (31), it was still so low and the preparation so difficult, that γ-butyrolactone was employed for an alternate synthesis of the dihydroxy acid.

**α-Hydroxy γ-butyrolactone.** — γ-butyrolactone (200 g.) was heated with bromine (355 g.) and PBr₃ (5 g.) according to the method of Livak (22). The product was distilled and the fraction boiling at 130-133°/12 mm. collected. Yield 250 g. (65%).
Fifty grams (0.303 mole) of the α-bromo γ-butyrolactone were refluxed with 41.8 g. (0.303 mole) of potassium carbonate in 250 ml. of water for four hours. At the end of that period the solution gave a rapid test for halide with silver nitrate, whereas the starting bromo compound reacted rather slowly. The mixture was evaporated to dryness in vacuo and the residue acidified with 50% sulfuric acid in an ice bath. The solution was again evaporated to dryness and extracted with absolute alcohol and filtered. The filtrate was distilled under vacuum and the fraction boiling at 104-106/3 mm.* was collected. Yield 20 g. (65%). Calculated neutral equivalent, 102. Found, 103 ± 1.

Calcium (α, γ-dihydroxy butyryl) β-alanide. —

This compound was prepared according to the method of Snell (38) by fusing 5.47 g. (0.054 mole) of α-hydroxy γ-butyrolactone and 4.75 g. (0.054 mole) of β-alanine at 100 for two hours. The product was purified by dissolving it in absolute alcohol and filtering. The alcohol was removed and the residue shaken with calcium carbonate in water. The solution was filtered and the water removed in vacuo. For analysis the compound was dried over P₂O₅. Yield 9.6 g. (85%). Analysis

*Glatterfield et al (9) prepared this compound in a different manner and reported 104°/4 mm. as the boiling point.
calculated for C_{14}H_{24}O_{10}N_{2}Ca: N, 6.67; Ca, 9.51.
Found: N, 6.53; Ca, 9.30.

**\( \alpha \)-Amino \( \gamma \)-butyrolactone hydrobromide.** —
This compound was synthesized according to the method of Fischer and Blumenthal (8) from ethylene dibromide, the only modifications being the preparation of phenoxy-ethyl bromide as given by Marvel (24), and ethyl phenoxy-ethylmalonate as described by Leuchs (23).

Phenoxyethylbromide was prepared according to Marvel (24) and reacted with malonic ester to obtain \( \gamma \)-phenoxy-ethylmalonic ester. The ester was saponified to the corresponding acid and brominated to \( \alpha \)-bromo \( \gamma \)-phenoxy-ethylmalonic acid. This compound was decarboxylated by heating above its melting point. The bromo acid was treated with ammonia and the \( \alpha \)-amino \( \gamma \)-phenoxybutyric acid obtained decomposed with 48% HBr to give the desired \( \alpha \)-amino \( \gamma \)-butyrolactone hydrobromide. Yield 40%.

**\( \alpha \)-Amino \( \gamma \)-hydroxy butyric acid.** — \( \alpha \)-Bromo \( \gamma \)-butyrolactone (165 g.) was added to 990 ml. of 28% ammonium hydroxide and isolated according to Livak (22). Yield 43 g. (36%) m.p. 184.*

**\( \alpha \)-Amino \( \gamma \)-butyrolactone hydrobromide.** — This compound was prepared from \( \alpha \)-bromobutyrolactone as described by Livak (22) and the same yield obtained.

Fischer (8) reported the melting point of this compound as 187°.
α-Amino γ-hydroxy butyryl β-alanide hydrobromide.—
Three grams (0.012 mole) of α-amino γ-butyrolactone hydrobromide were heated with 1.08 g. (0.012 mole) of β-alanine for two hours at 100. The resulting light red oil was dissolved in methanol and decolorized by activated charcoal. The light yellow oil was then dried in a vacuum desiccator over P₂O₅ for several days. The product was ground to a powder and analyzed. Yield 2.6 g. (87%). Analysis calculated for C₇H₁₅O₄N₂Br: N, 10.3; Br, 29.5. Found: N, 10.1; Br, 29.7.

α-Hydroxy isolvaleric acid.—Bisulfite addition to butyraldehyde (72 g.) (1 mole) was effected in the usual manner with 104 g. (1 mole) of sodium bisulfite. The crystals obtained were washed with alcohol and dissolved in 200 ml. of water. The solution was placed in an ice bath and potassium cyanide (65 g.) (1.3 mole) added slowly with stirring. After the addition was complete the stirring was continued for 30 minutes, whereupon two layers separated. The solution was extracted with ether and the ether extract dried over sodium sulfate.

The nitrile was dissolved in three volumes of concentrated HCl and refluxed for 45 minutes as prescribed by Lipp (21). The solution was evaporated to dryness and extracted with ether. Since the solubility
was unexpectedly low, the material was transferred to a Soxhlet apparatus and ether extracted for eight hours. Twenty grams of a solid were obtained which melted at 100° (α-hydroxy isovaleramide = 100°) (11), and contained nitrogen but no carboxyl group. This compound was then refluxed in concentrated HCl for three hours and evaporated to dryness. The residue was shaken with ether, and on cooling crystals appeared. Yield 20 g. (17%). m.p. = 82.

Sodium (α-hydroxy isovaleryl) β-alanide. — To 9 ml. of refluxing isopropyl alcohol was added 0.26 g. (0.011 mole) of sodium through the condenser. After all the sodium had gone into solution, 1 g. (0.011 mole) of β-alanine was added and refluxed for 15 minutes. α-hydroxy isovaleric acid (1.35 g.) (0.011 mole) was added and the mixture refluxed for 2 hours. On cooling a solid separated, which was filtered. Yield 2 g. (81%). Analysis calculated for C_8H_{13}O_4Na: N, 6.68. Found: N, 6.45.
A PEPTIDE OF GLUTAMIC ACID

N-Carbobenzyo L-glutamic anhydride. — This compound was prepared by the general method of Bergmann (3) (4) as modified by Schink (35). Carbobenzyo chloride was made by adding freshly distilled benzyl alcohol to a 20% phosgene solution in toluene.

The carbobenzyo chloride (60 g.) was reacted with glutamic acid (26 g.) by shaking a cold aqueous suspension of the two materials in the presence of magnesium oxide (29 g.). It was found that the yield of this reaction could be materially increased by adding a few drops of 2N sodium hydroxide. The N-carbobenzyo glutamic acid was warmed with acetic anhydride at 100° for five minutes and the solvent removed in vacuo.

α-Benzyl N-carboznoxy L-glutamate. — Thirty grams of the anhydride were treated with 15 g. of freshly distilled benzyl alcohol in a sealed tube for six hours at 100°. The resulting oil was dissolved in ether and extracted with a 5% solution of sodium bicarbonate several times. The oil that separated from the acidification of the bicarbonate solution was extracted with ether and dried over sodium sulfate. Removal of the solvent gave a waxy solid that softened and cleared at 110°. Yield
16 g. (42%). Analysis calculated for C$_{20}$H$_{20}$O$_6$N: N, 3.83. Found: N, 3.81.

**Attempted preparation of α-benzyl N-carbobenzoxy γ-glutamyl chloride.** — Three and six tenths grams of PCl$_3$ were added portionwise to 6 g. of the above ester in ether. The phosphorus oxychloride formed was filtered off and the ether evaporated to dryness in the absence of air. The resulting oil was washed quickly with petroleum ether and dried. Analysis calculated for C$_{20}$H$_{21}$O$_5$N: N, 5.9. Found: N, 11.0. Carbobenzoxy amino acid chlorides are unstable (decomposing to the N-carboxyamino acid anhydrides) (37), and the majority of them have been prepared only as oils.

**Phthalylglutamic anhydride.** — Phthalic anhydride (15 g., 0.1 mole) and glutamic acid (15 g., 0.1 mole) were boiled together in pyridine (125 ml.) under reflux for two hours or until all the solid material had gone into solution (13). The solvent was removed in vacuo on a water bath.

The residue was heated with an excess of acetic anhydride (100 ml.) for two hours. The solution was then evaporated in vacuo until crystals appeared, and stored overnight at 0°. After filtration and air drying, the solid was washed with ether to remove acetic anhydride (13). Yield 20 g. (67%) The compound melted at 205-207°.
The anhydride was decomposed to the acid by warming it in water at 50-60° until solution was complete, cooling the solution and filtering. m.p. 190 C., N. E. 138; theory, 138.

Phthalyl \( \gamma \)-glutamyl \( \beta \)-alanine. — Eight and five tenths grams of the anhydride (0.03 mole) and 5 g. (0.06 mole) of \( \beta \)-alanine were heated together at 140° for one hour. The residue was removed by breaking the flask. After grinding the coupled product, it was dissolved by shaking 50 ml. of water at room temperature and filtering off the unreacted insoluble anhydride. The excess \( \beta \)-alanine employed in the coupling reaction was removed by shaking it in water with an ion exchange resin (Amberlite IR-100). The solution was then evaporated to dryness and dried over \( P_2O_5 \). The resulting hygroscopic oil was difficult to purify or analyze, however it appeared to be relatively pure on the basis of neutral equivalent and nitrogen analysis. Calculated for \( C_{16}H_{17}O_7N_2 \): N, 8.02%; N. E., 172. Found: N, 7.78%; N. E., 179.

Attempted removal of the phthalyl group. — Five grams (0.014 mole) of the tripeptide above and 0.85 grams (0.014 mole) of hydrazine hydrate in 500 ml. alcohol was refluxed for one and one-half hours. The alcohol was
removed by distillation and the residue warmed for seven minutes with 50 ml. of 2N hydrochloric acid. The solution was filtered and the filtrate evaporated to dryness in vacuo (41). The remaining oil was dried over P₂O₅ and then over sodium hydroxide in an Abderhalden drying apparatus. Chloride analysis of various preparations were erratic varying from 10-20%. The correct value is 14.02%. The oil had a very sharp acid odor. A nitrogen determination was run and a value of 7.78% was found, which indicates that the starting material was obtained.
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 (12, 33, 34). β-Alanine was omitted from the A. suboxydans medium.

RESULTS AND DISCUSSION

The growth promoting effect of N-methyl pantothenic 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 per cent of the activity of the vitamin, either alone or in the presence of suboptimal concentrations of the vitamin. No inhibition of growth of either organism was noted up to five mg. of the analog per tube. N-methyl- β-alanine is even less stimulatory for yeast than is N-methyl pantothenic acid,
<table>
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<th>Organisms</th>
<th>L. Arabinosus</th>
<th>A. Suboxydans</th>
<th>L. M. Yeast</th>
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<tr>
<td>Apparent PA activity</td>
<td>r/10 ml.</td>
<td>r/10 ml.</td>
<td>r/10 ml.</td>
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<tr>
<td>N-CH$_3$PA*</td>
<td>0</td>
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</tr>
<tr>
<td>1</td>
<td>.0015</td>
<td>.0015</td>
<td>.0015</td>
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<td>.015</td>
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<td>N-CH$_3$PA + PA</td>
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<td>.0650</td>
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<td>100</td>
<td>.10</td>
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<tr>
<td>N-CH$_3$-B</td>
<td>1 mg.</td>
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<td>.020</td>
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* N-methylpantothenic acid.
N-methylpantothenic acid plus pantothenic acid, the latter being added as the calcium salt.
N-methyl-$\beta$-alanine.
although the relative activity of the two compounds is in approximately the same ratio as that of $\ell$-alanine and pantothenic acid for this organism.

N-methyl pantothenic acid possesses significant activity for \textit{A. suboxydans}, varying from approximately three to fifteen per cent that of the vitamin. This recalls the similar activity of pantoyltaurine and other amides of pantolic acid for this organism (5). It is probably due to hydrolysis of the analog by the organism to yield pantolic acid, which is as active as the intact vitamin.

In attempting to apply the Woods-Fildes theory to the action of pantolic 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 inert analogs have in general differed from the vitamin in the hydroxy acid moiety, it was concluded that one or both of the hydroxy groups is required for complete attachment of the pantolic acid molecule to its apoenzyme (5). Analogos which differ in the $\ell$-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 that coenzyme A (14) and the pantothenic acid conjugate (PAC) (20) 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 \textit{L. arabinosus} activity. Such attachment to phosphorus would presumably occur through the hydroxy groups.

Since N-methyl pantothenic acid was virtually inactive for \textit{L. arabinosus} and yeast under all conditions tested, it would appear on the basis of the foregoing discussion that this analog also does not combine with the apoenzyme. The amide group may therefore be considered also 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 A (30) and perhaps larger weight of PAC, based upon its relative non-dialyzability (15).

The inhibitory actions of three analogs of pantoic acid were tested upon \textit{A. suboxydans}, which requires the
pantoic acid moiety. The analogs were tested for inhibitory effects in the presence of pantoic acid, and also in the presence of pantothenic acid. The results are summarized in Table II. In Table III are shown the effects of the $\beta$-alanides of these analogs upon \textit{L. arabinosus} in the presence of the vitamin, and on \textit{A. suboxydans} with pantoic acid, and also with pantothenic acid. Finally, these analogs were tested in the presence of methionine to determine their possible methylation to produce vitamin-active materials, but none of them showed increased activity.

As has been observed for pantothenic acid analogs in general, all of the compounds tested (Tables II and III) showed marked reduction in biological activity. Compound 6 shows competitive inhibition of pantothenic acid in \textit{L. arabinosus}. This compound, together with Drell and Dunn's "$\omega$-methyl pantothenic acid" (7a) are the only ones recorded involving changes in the pantoic moiety, which inhibit growth in organisms requiring the intact vitamin. Since both of these, as well as all inhibitors which are altered in the $\beta$-alanine structure, possess the $\alpha,\gamma$-dihydroxy configuration, it would appear that both hydroxy groups are necessary for attachment of pantothenic acid to cellular enzymes in lactic acid bacteria.
In contrast to the lactics, in which the \( \alpha, \gamma \)-dihydroxy structure appears to be of prime importance, growth in Acetobacter is also influenced by removal of the methyl groups of the pantoic acid moiety. The importance of the methyl groups is emphasized in the behavior of compounds 3 and 5, together with Nos. 1 and 2 of a previous paper (5).
### TABLE II

**EFFECT OF PANTOIC ACID ANALOGUES UPON A. SUBOXYDANS**

**RATIO = ANALOGUE AT 50% INHIBITION / GROWTH FACTOR**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Formula</th>
<th>Pantothentic Acid</th>
<th>Pantoic Acid</th>
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<tr>
<td>3.</td>
<td>CH₂OH–CH₂–CH(OH)–COOH</td>
<td>Inert</td>
<td>Inert</td>
</tr>
<tr>
<td>4.</td>
<td>CH₂OH–CH₂–CH(NH₂)–COOH</td>
<td>40,000</td>
<td>30,000</td>
</tr>
<tr>
<td>5.</td>
<td>CH₃CH(CH₃)–CH(OH)–COOH</td>
<td>20,000</td>
<td>20,000</td>
</tr>
<tr>
<td></td>
<td>CH₂OH–C(CH₃)₂–CH₂–COOH*</td>
<td>300</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>CH₃–C(CH₃)₂–CH(OH)–COOH*</td>
<td>not given</td>
<td>1,000</td>
</tr>
</tbody>
</table>

*Compounds and their activities (Nos. 1 and 2) taken from Cheldelin and Schink (5).*
TABLE III

EFFECT OF PANTOTHENIC ACID ANALOGUES UPON TWO ORGANISMS

\[ \text{RATIO} = \frac{\text{ANALOGUE}}{\text{GROWTH FACTOR}} \text{ AT 50% INHIBITION} \]

<table>
<thead>
<tr>
<th>Compound</th>
<th>Formula</th>
<th>Lactobacillus arabinosus 17-5</th>
<th>Acetobacter suboxydans</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>GROWTH FACTOR</td>
<td>Pantothenic Acid</td>
</tr>
<tr>
<td>(6)</td>
<td>(\text{CH}_2\text{OH-CH}_2\text{-CHOH-CO-NH-CH}_2\text{-CH}_2\text{-COOH})</td>
<td>2,000</td>
<td>Inert</td>
</tr>
<tr>
<td>(7)</td>
<td>(\text{CH}_2\text{OH-CH}_2\text{-CHNH}_2\text{-CO-NH-CH}_2\text{-CH}_2\text{-COOH})</td>
<td>Stimulatory (0.0005%)</td>
<td>Inert</td>
</tr>
<tr>
<td>(8)</td>
<td>(\text{CH}_3\text{CH(CH}_3\text{)-CHOH-CO-NH-CH}_2\text{-CH}_2\text{-COOH})</td>
<td>Inert</td>
<td>11,000</td>
</tr>
</tbody>
</table>
Compound (5) is only slightly inhibitory, whereas the analog with an additional \(\beta\)-methyl group possesses an antibacterial index (at 50\% inhibition) of 1000 and the isomer of the latter with the hydroxy group in the \(\gamma\)-position had a corresponding index of 10 (5). On the other hand, compound 3, which has no methyl groups is completely inert even though it is otherwise identical with pantoic acid. Since the methyl groups could hardly function directly in the formation of the vitamin or its coenzyme, it seems probable that they exert an influence upon spatial arrangement, particularly of the hydroxy groups.

Compound 7, which has an \(\alpha\)-amino group and is inert toward both test organisms, is evidently incapable of attachment. However, the corresponding analog of pantoic acid (compound 4) is somewhat inhibitory. The explanation for the latter behavior is not apparent.
SUMMARY

Some new analogues of pantoic acid and pantothenic acid have been prepared. These are: Calcium (α,γ-dihydroxy butyryl) β-alanide; sodium (α-hydroxy isovaleryl) β-alanide; α-amino γ-hydroxy butyryl β-alanide hydrobromide; and α, γ-dihydroxy β,β-dimethyl butyryl β′-N-methyl alanide. The above compounds, together with several others previously described, were tested for their growth effects upon A. suboxydans and L. arabinosus.

On the basis of evidence obtained it appears that N-methyl pantothenic 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.

The first compound above was found to be a good inhibitor for L. arabinosus, whereas the remainder were practically inert. The study points to the essentiality of configuration of the hydroxy groups for activity in lactic acid bacteria, and to the additional influence of the β-methyl groups in Acetobacter.
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