of 5,6-dimethoxy-8-nitroquinoline prepared by the Skraup reaction on 4-amino-5-nitroveratrole.[15]

8-Amino-5,6-dimethoxyquinoline.—The above 5,6-dimethoxy-8-nitroquinoline was reduced to the amine with iron and hydrochloric acid (70–80%), m. p. 145–147°. Recrystallization from ether gave a product melting at 147.5°, reported previously, 148°,[11] 148–149°.[12d]

8-(3-Aminopropylamino)-5,6-dimethoxyquinoline.—This was made by the method of Robinson and Tomlinson[4] with some slight modifications.[5] The 8-amino-5,6-dimethoxyquinoline, 13.5 g., was coupled with 3-bromopropylphthalimide by refluxing (125°) for four hours in methyl Cellosolve. The 5,6-dimethoxy-8-(3-phthalimidopropylamino)-quinoline was obtained by diluting the reaction mixture with water, neutralizing with sodium hydroxide, saturating with sodium carbonate, extracting with ether, and converting to the hydrochloride. This was purified by crystallization from methanol; m. p. 170–171°, 6.1 g. (22%). The use of *n*-propanol solvent or the absence of solvent failed to materially alter the purified yield.

Anal. Calcd. for $C_{22}H_{21}O_4N_3$: N, 10.77. Found: N, 10.98.

The phthalimide group was removed by refluxing the purified base with one equivalent of hydrazine hydrate in absolute ethanol for two hours, removing the solvent under vacuum, and shaking the residue in ether with excess 50% sodium hydroxide. The product was obtained as the hydrochloride by treating the ether extracts with Norit, drying with potassium carbonate and bubbling in dry hydrogen chloride; m. p. 205–206°. This was further purified by recrystallizing from methanol, m. p 207°, 66% yield.

Anal. Calcd. for $C_{14}H_{19}O_2N_3.2HCl$: N, 12.57. Found: N, 12.46.

(15) We are greatly indebted to Dr. R. B. Taylor of this Laboratory for the purified sample of 5,6-dimethoxy-6-nitroquinoline made by this procedure.

Summary

1. Five new derivatives of 8-(3-aminopropylamino)-6-methoxyquinoline have been prepared by reactions involving the terminal amino group.

2. The antimalarial activities of all of these derivatives were less than that of the parent compound and in general the toxicities were proportional to the per cent. of the parent compound in combination.

3. The corresponding 8-(3-aminopropylamino)-5,6-dimethoxyquinoline was also prepared.

STATE COLLEGE, PENNA. RECEIVED[16] JUNE 9, 1947

(16) Original manuscript received October 21, 1946.

[CONTRIBUTION FROM THE CHEMICAL LABORATORIES, GENERAL MILLS, INC.]

Investigation of the Reserve Carbohydrates of Leguminous Seeds. I. Periodate Oxidation[1]

BY OWEN A. MOE, SIDNEY E. MILLER AND MARJORIE H. IWEN

The results of the Malaprade[1a] reaction have been successfully employed by various investigators in the elucidation of the linkages present in polymeric carbohydrates. Oxidation by periodate has been applied to naturally occurring polysaccharides, and their derivatives, such as starch,[2a,b] cellulose[3,4,5] and alginic acid.[6] Recently, Lew and Gortner discussed the periodate oxidation of the reserve carbohydrates (so called gums) from the carob bean[7] (*Ceratonia silique*, L.) and the honey locust bean[8] (*Gleditschia tricanthos*, L). The present report concerns an investigation of the periodate oxidation of the reserve polysaccharide from guar seed (*Cyamposis tetragonalaba* (*psoralioides*)). In connection with this work it became necessary to repeat certain parts of the work of Lew and Gortner. Our results lead to conclusions about the structural linkages of these polymeric carbohydrates which are at considerable variance from the conclusions of Lew and Gortner.

These reserve carbohydrates, found in the endosperms of leguminous seeds, are mannogalactans (or galactomannans) since they are composed principally of mannose and galactose units. The ratios of mannose to galactose in the mannogalactans from carob, honey locust and guar are 3.0–4.0:1,[7] 4.4:1[8] and 2:1,[8a] respectively. Lew and Gortner reported the polysaccharide from the carob bean consumed approximately one mole of periodate per hexose unit. Hydrolysis of the polymeric dialdehyde (cleavage product) yielded two aldehydo compounds which were considered by them to be glyceric aldehyde and tartron dialdehyde. These results were construed as indicative of the presence of 1,2 linkages between the anhydro sugar units.

Lew[8] observed that the polysaccharide from the honey locust bean required 2–2.5 moles of periodate per hexose unit. Hydrolysis of the cleavage product (dialdehydo) yielded glyoxal which was identified as the phenylosazone. These results were interpreted by him as indicative either

(1) Paper No. 80, Journal Series, General Mills, Inc., Research Department. Presented at the 111th meeting of the American Chemical Society, Atlantic City, N. J., April 14–18, 1947.

(1a) M. L. Malaprade, *Bull. soc. chim.*, (4) **43**, 683 (1928); *Compt. rend.*, **186**, 382 (1928).

(2) (a) E. L. Jackson and C. S. Hudson, THIS JOURNAL, **59**, 2049 (1937); (b) C. G. Caldwell and R. M. Hixon, *J. Biol. Chem.*, **123**, 595 (1938).

(3) E. L. Jackson and C. S. Hudson, THIS JOURNAL, **60**, 989 (1938).

(4) G. Jayme, M. Saetre and S. Maris, *Naturwissenschaften*, **29**, 768 (1941).

(5) D. H. Grangaard, E. K. Gladding and C. B. Purves, *Paper Trade J.*, **115**, 41 (1942).

(6) H. J. Lucas and W. T. Stewart, THIS JOURNAL, **62**, 1792 (1940).

(7) B. W. Lew and R. A. Gortner, *Arch. Biochem.*, **1**, 325 (1943).

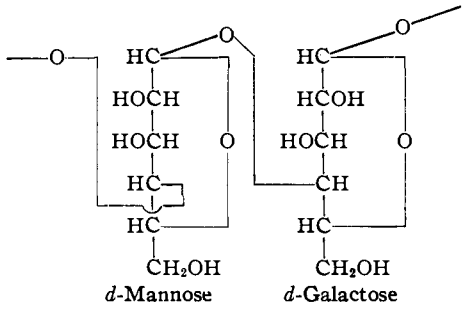
(8) B. W. Lew, Ph. D. Thesis, University of Minneosta, October, 1941.

(8a) Unpublished results of Dr. P. E. Ramstad of this Laboratory.

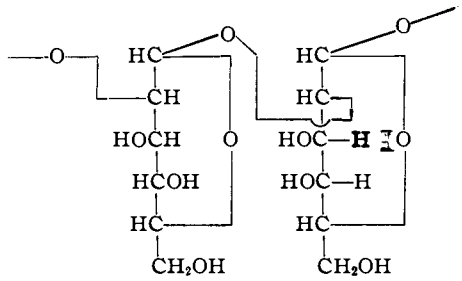
of 1,6 linkages between the anhydro sugar units or of the presence of side chains.

In the present study it was found that the polysaccharides from carob and honey locust beans consumed only one mole of periodate per anhydro sugar unit. Hydrolysis of the cleavage products yielded glyoxal in both cases. Likewise, the mannogalactan from guar seed consumed one mole of oxidant per hexose unit and on hydrolysis yielded glyoxal.

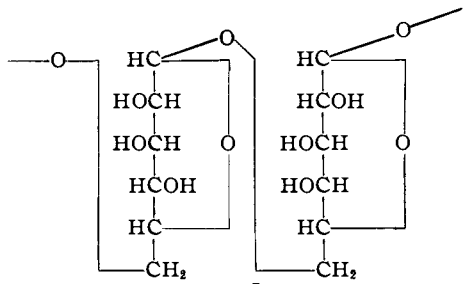
In general, consideration of the most probable linkages involved may be represented schematically by formulas Ia, Ib, Ic and Id indicating 1,4–1,2–1,6 and 1,3 linkages, respectively.



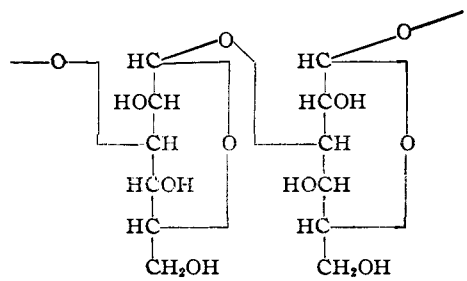
Ia



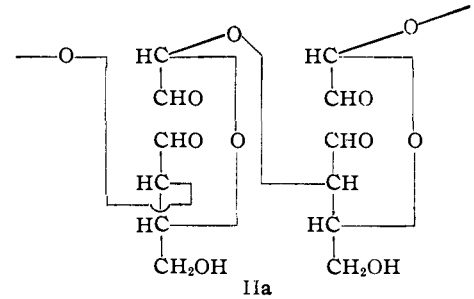
Ib



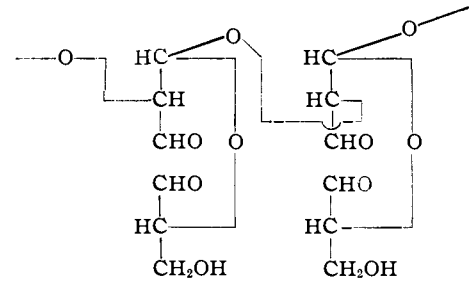
Ic



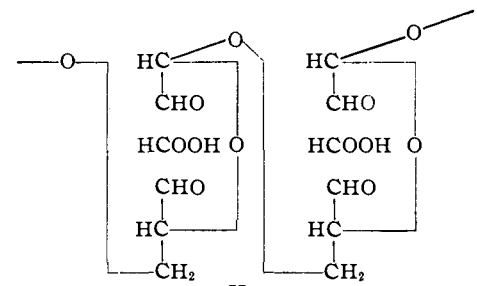
Id



IIa



IIb



IIc

Periodate oxidation of compounds containing structures Ia, Ib and Ic would yield polymeric dialdehydes IIa, IIb and IIc, respectively. A substance containing a 1,3 linkage (Id) obviously would not be attacked by periodate because of the absence of vicinal hydroxyl groups.[9] The formation of IIa and IIb from Ia and Ib, respectively, would require one mole of oxidant per hexose unit. Formation of IIc from Ic would involve the elimination of carbon atom number 3 as formic acid. Hence, this oxidation would require two moles of periodate per hexose unit. Only the polymeric dialdehydes IIa and IIc would yield glyoxal on hydrolysis. Therefore, on the basis of the results reported above, the presence of 1,4 linkages in the reserve carbohydrates from carob, honey locust and guar seeds is indicated.

Grangaard, Gladding and Purves[5] found from rate studies on the oxidation of starch by aqueous periodate that the reaction was selective only under controlled conditions (below 20° and within *p*H range of 2–5). Lew[8] apparently failed to recognize these factors since he carried out his oxidations in unbuffered media at room temperature in the summer time. The periodate oxidations

(9) V. C. Barry, T. Dillon and W. McGettrick, *J. Chem. Soc.*, 183 (1942), and subsequent papers.

reported in the present work were carried out at 16–17° and at *p*H 4.2–4.3 and the results obtained for the mannogalactans from the leguminous seeds of honey locust and guar are shown graphically in Fig. 1.

The combined effect of the temperature and the *p*H may be noted from Table I. This is particularly apparent if the twenty-four-hour readings are followed throughout. The buffered oxidations were carried out using 0.05 molar aqueous periodate (prepared from trisodium paraperiodate and acetic acid) and the unbuffered oxidations were carried out using 0.5 molar aqueous periodic acid in order to be identical with the work of previous investigators.[8]

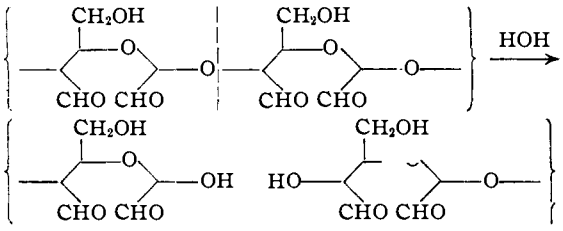
TABLE I

PERIODATE OXIDATIONS OF THE MANNOGALACTAN FROM HONEY LOCUST BEAN

Time, hr.	Moles of oxidant per hexose unit: Buffered at 16°[a]	Buffered at 31°	Unbuffered at 16°	Unbuffered at 31°
4	0.83	0.79	0.99	1.13
8	.90	..	..	..
24	.96	0.98	1.06	1.24
36	.96	..	..	..
48	.97	1.04	1.09	..
120		..	..	1.59
144		1.14	1.29[b]	..
312				2.53

[a] Product in finely divided state. [b] During last forty-eight-hour period temperature increased to 21.5°.

When the oxidant is buffered at *p*H 4.2, the temperature has but a small effect. However, when the unbuffered oxidant is used, the temperature effect is very appreciable. It may also be noted that the unbuffered oxidations possess a more rapid initial rate. The high value obtained when the periodate oxidation is carried out in an unbuffered medium at 31° is possibly due to the hydrolysis of the original polysaccharide or of its oxidation products.



In either case, this ultimately leads to α-hydroxyaldehydes which in turn are readily attacked by periodate.

In the case of the polysaccharides from carob and honey locust beans, there appeared to be a small increase ("drift") in the consumption of periodate after forty-eight hours when the buffered oxidations were carried out at 31°. This effect was not observed in the case of the carbohydrate from guar seed as noted in Table II.

The results that we have obtained show clearly

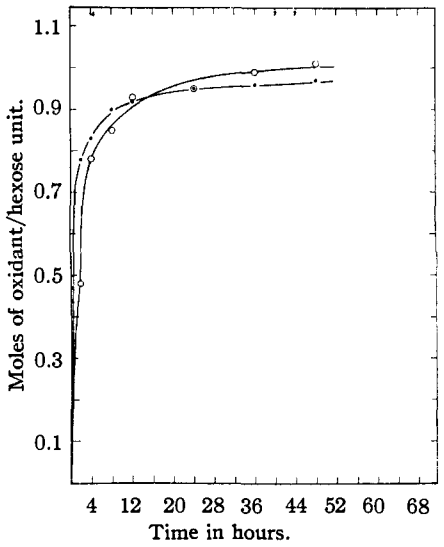


Fig. 1.—Periodate oxidation of the reserve carbohydrates (mannogalactans) from honey locust (●) and guar (○) seeds in buffered media at 16°.

that care must be exercised in conducting the Malaprade reaction.

TABLE II

OXIDATIONS IN BUFFERED MEDIA AT 31° OF THE MANNOGALACTANS FROM:

Time, hr.	Moles of oxidant per hexose unit: Carob	Honey locust	Guar
4	0.79	0.79	0.63
24	.94	.98	1.03
48	.98	1.04	1.06
144	1.09	1.14	1.06

Jayme, Saetre and Maris[4] found that the oxidation of polysaccharides such as cellulose with buffered periodate resulted in substantially higher yields of glyoxal than those obtained by use of unbuffered oxidant as originally employed by Jackson and Hudson.[3] In a later paper Jayme and Maris[10] reported that they obtained up to 53.5% of glyoxal (as phenylosazone) by hydrolysis of the cleavage product of cellulose when the carbohydrate was oxidized in a buffered medium. On the other hand, they obtained only 15.6% of glyoxal by the same procedure when an unbuffered oxidation medium was used. It is difficult to determine from the work of Jayme and Maris whether their yields were based on crude or pure glyoxalphenylosazones.

We were unable to find any substantial difference between the amounts of glyoxal (14–23%) obtained as the pure phenylosazone from the unbuffered oxidations and from the buffered oxidations on these reserve carbohydrates from leguminous seeds. In the present work, aliquot portions of the hydrolysis mixtures were treated with phenylhydrazine in the usual manner and the crude phenylhydrazones, which were obtained in

(10) G. Jayme and S. Maris, *Ber.*, **77**, 383 (1944).

high yields, usually melted in the range of 130–145°. The crude products were dissolved in hot benzene and the crystalline products deposited from the benzene solutions usually melted in the range of 160–166°. After recrystallization from aqueous alcohol, the pure glyoxal phenylosazones melted within the range of 167–170° and the melting points were not depressed when the phenylosazones were mixed with an authentic sample.

TABLE III

YIELD OF GLYOXAL (AS PHENYLOSAZONE) AT DIFFERENT STAGES OF PURITY

Source of mannogalactan	Oxidation medium	Yield (%) of phenylosazone from benzene	Yield (%) of phenylosazone from alcohol
Carob seed	Buffered	30	22
Carob seed	Unbuffered	28	22
Honey Locust seed	Buffered	27	18
Honey Locust seed	Unbuffered	30	14
Guar seed	Buffered	31	23
Guar seed	Unbuffered	26	19

The percentage yields of the glyoxal phenylosazones at different stages of purity are given in Table III.

Although our results are indicative of the presence of 1,4 linkages, they do not exclude the presence of branched chains. If branching of the chain does occur, it would most likely be found at the C_6 position. The possibility of a combination of 1,3 and 1,6 linkages has not been eliminated. It is not possible to draw any conclusions from our present results with regard to either α or β configuration at carbon atom 1. Further work leading to the elucidation of the structural linkages involved in these reserve carbohydrates of the leguminous seeds is under way at the present time.

These mannogalactans from the leguminous seeds are very sensitive to the action of borates, forming clear, irreversible gels as was observed by Williams[11] and Hart[12] in the case of the mannogalactan from carob bean. Böeseken[13] has clearly demonstrated the ability of boric acid to form cyclic complexes with *cis* glycols. The inability of boric acid to form cyclic complexes with most *trans* glycols may be due to the small size of the central boron atom (1.7 Å. diameter).[14] From a consideration of formulas Ia, Ib and Ic, it is apparent that in the case of 1,2 linkages the *cis* glycol grouping is located at the carbon atoms 3 and 4 of the galactose units. In case of 1,6 linkages, the *cis* glycol system is found in both the mannose and galactose units, whereas in the case of 1,4 linkages the necessary glycol system is located at carbon atoms 2 and 3 of the mannose units. As previously described, the results of the present investigation indicated the presence of 1,4 linkages and it may be postulated that the ability of these carbohydrates to form the gels with borax is due to the free hydroxyl groups possessing the *cis* configuration on carbon atoms 2 and 3 of the mannose units. This is of some interest since mannose is the only naturally occurring hexose sugar known wherein the *cis* configuration is found for the hydroxyl groups at the C_2 and C_3 positions.

Acknowledgment.—The authors wish to thank Dr. Lee I. Smith for helpful advice and constructive criticism during the course of this investigation and the preparation of this manuscript.

Experimental

Purification of the Mannogalactans.—The finely-powdered mannogalactans[15] from carob, honey locust and guar seeds were purified in the following manner.

The carbohydrates were dissolved (dispersed) in water at 70–80° to yield 0.3–0.4% solutions (cloudy). After standing overnight, the solutions were centrifuged and then filtered and the polysaccharides were precipitated from the clear, water-white filtrates by addition of an excess of methanol. The precipitated products were washed with fresh methanol, collected by filtration and dried *in vacuo*. The average moisture and ash contents of these purified mannogalactans were 1.5 and 0.3%, respectively. The mannogalactans purified in this manner were used for both the buffered and the unbuffered oxidations.

Solutions of the Oxidant.—The buffered periodate solutions (0.05 *M*, *p*H 4.2–4.3) were prepared from trisodium paraperiodate[16] ($Na_3H_2IO_6$) and acetic acid essentially as described by Purves and co-workers.[17] The unbuffered solutions (0.5 *M*) of the oxidant were prepared from periodic acid.

Buffered Oxidations at 16–17° and 31°.—All buffered oxidations were carried out on accurately weighed samples (0.4–0.5 g., dry basis) of the carbohydrates. The amount of buffered periodate solution (300–350 cc.) employed gave a molar ratio of periodate to carbohydrate of 5.6–6.2 to 1. The amounts of periodate consumed in the oxidation of the reserve carbohydrates from honey locust and guar seeds are shown graphically in Fig. 1. After forty-eight hours, the reserve carbohydrate from locust bean had consumed 0.97 mole of periodate per hexose unit. The titrations of the aliquot solutions of periodate were carried out at various time intervals according to the method of Fleury and Lange[18] as modified by Grangaard, Gladding and Purves.[5] The results obtained from buffered oxidations at 31° are given in Table II.

Unbuffered Oxidations at 16–17° and 31°.—The unbuffered oxidations were carried out on samples (0.5 g., dry basis) of the carbohydrate (honey locust) using 60 cc. of 0.5 *M* periodic acid solution. The average molar ratio of periodate to carbohydrate was approximately 10.5 to 1.

When large amounts (5.4 g.) of the carbohydrates were oxidized in unbuffered media (16–17°), 167 cc. of 0.5 *M* periodic acid solution was used giving a molar ratio of periodate to carbohydrate of approximately 2.5 to 1. After forty-eight hours, the moles of periodate consumed per hexose unit for the mannogalactans from carob, honey locust and guar seeds were 1.06, 1.04 and 1.11, respectively.

Hydrolysis of Polymeric Dialdehydes (cleavage products).—The cleavage products were always washed repeatedly with water and alcohol in order to remove the last traces of periodate and then dried *in vacuo*. The cleavage products from both the buffered (16–17°) and the unbuffered (cleavage products from 5.4 g. runs, 16–17° were used) oxidations were refluxed with 2% sulfuric acid until

(11) A. L. Williams, *Analyst*, **53**, 411 (1928).

(12) R. Hart, *Ind. Eng. Chem., Anal. Ed.*, **2**, 329 (1930).

(13) J. Böeseken and H. Corwert, *Rec. trav. chim.*, **40**, 354 (1921).

(14) S. H. Bauer and J. Y. Beach, THIS JOURNAL, **63**, 1394 (1941).

(15) Obtained from Dr. P. E. Ramstad.

(16) Obtained from the G. Frederick Smith Chemical Co.

(17) D. H. Grangaard, J. H. Michell and C. B. Purves, THIS JOURNAL, **61**, 1290 (1939).

(18) P. Fleury and J. Lange, *J. pharm. chim.*, [8] **17**, 107, 196 (1933).

the rotation of the solution appeared to be constant. One specific example is given (hydrolysis of the polymeric dialdehyde from the buffered oxidation of the polysaccharide from the carob bean): One gram of the polymeric dialdehyde was refluxed with 100 cc. of 2% aqueous sulfuric acid. After eight hours, the reaction mixture had darkened and a small amount of insoluble material was noted. A little norite was added and the mixture was filtered. Refluxing was then continued (total time, fifteen hours) during which the mixture again darkened. It was finally treated with norite and filtered.

Isolation and identification of glyoxal (as pure phenylosazone): The above reaction mixture was neutralized with barium carbonate and the barium sulfate was removed by filtration. A 50-cc. aliquot of the filtrate (corresponding to 0.5 g. of cleavage product) was warmed with sodium acetate (1.0 g.), acetic acid (2 cc.) and phenylhydrazine (2 cc.) for fifteen minutes. After standing at room temperature for three hours, the crude phenylhydrazone was collected by filtration and dried *in vacuo*. The product weighed 0.53 g. and melted at 131–134° with decomposition. The crude product, when recrystallized from benzene, was crystalline; it weighed 0.22 g. (30%) and melted at 162–164°. After crystallization from ethanol, the pure glyoxal phenylosazone weighed 0.16 g. (22%) and melted at 168–170° alone or when mixed with an authentic sample. The yields obtained from the other cleavage products are given in detail in Table III.

Summary

1. The periodate oxidation of the reserve carbohydrates from carob, honey locust and guar seeds has been studied. Each polysaccharide consumed one mole of oxidant per hexose unit and the cleavage products yielded glyoxal on hydrolysis in every case. These results are interpreted as being indicative of the presence of 1,4-linkages. In the case of polymeric carbohydrates from carob and honey locust beans these results are at variance from previously published results.

2. The effects of the temperature and the *p*H on the periodate oxidations of these polysaccharides have been discussed.

3. The sensitivity of these mannogalactans to borax has been explained on the basis of the *cis* glycol group found at the C_2 and C_3 positions of the mannose units.

4. The possibility of branching has not been eliminated and no conclusion in regard to the configuration at the C_1 position has been drawn.

RECEIVED MAY 8, 1947

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

Pantothenic Acid Studies. I. Growth Effect of Pantoic Acid Analogs[1]

BY VERNON H. CHELDELIN AND CHESTER A. SCHINK

Analogs and derivatives of pantothenic acid have been prepared in a number of laboratories. The earlier ones resulted from studies on the structure of the vitamin,[1a] and from efforts to produce compounds with similar biological activity.[2-7] Following the preparation of pantoyltaurine,[8-10] however, most syntheses have aimed at the development of inhibitors related to pantothenic acid. *In vitro* growth tests with microörganisms have revealed a number of compounds which competitively inhibit the growth-promoting action of pantothenic acid over a wide range of concentrations.

In general, the most successful inhibitors have been those in which the pantoic acid[10a] moiety of the molecule is coupled to a suitable amino acid,[8,10,11,12] amino ketone,[13] amino alcohol[14] or amine.[15] Alterations in the pantoic acid moiety, on the other hand, have with but one exception[16] given rise to inactive or very slightly stimulatory substances, when tested on organisms requiring the preformed vitamin.

It was felt that an organism such as *Acetobacter*, which utilizes pantoic acid as readily as the intact vitamin,[17] might be inhibited by compounds resembling this acid, as well as by their condensation products with β-alanine. We have found two such compounds to effectively inhibit the coupling of pantoic acid to β-alanine. Other analogs have been prepared which possess considerable growth-promoting activity. The details of these studies and some of their implications are presented below.

(1) The data in this paper are taken from the dissertation presented by one of the authors (C. A. S.) for the Ph.D. degree, Oregon State College, 1947. Presented before the Northwest Regional Meeting of the American Chemical Society, Pullman-Moscow, May 2, 1947. Published with the approval of the Monographs Publication Committee, Oregon State College, Research Paper No. 111, School of Science, Department of Chemistry. This work was supported by the Nutrition Foundation, Inc., Research Corporation, Inc., and by the General Research Council, Oregon State System of Higher Education, Corvallis, Oregon.

(1a) H. K. Mitchell, H. H. Weinstock, E. E. Snell, S. R. Stanbery and R. J. Williams, THIS JOURNAL, **62**, 1776 (1940).

(2) Y. Subbarow and L. Rane, *ibid.*, **61**, 1616 (1939).

(3) H. K. Mitchell, E. E. Snell and R. J. Williams, *ibid.*, **62**, 1791 (1940).

(4) H. H. Weinstock, *J. Biol. Chem.*, **135**, 343 (1940).

(5) T. Reichstein, *Helv. Chim. Acta*, **23**, 650 (1940).

(6) J. W. Barnett and F. A. Robinson, *Biochem. J.*, **36**, 357 (1942).

(7) A. H. Nease, dissertation, University of Texas, 1943.

(8) E. E. Snell, *J. Biol. Chem.*, **139**, 975 (1941); **141**, 121 (1941).

(9) R. Kuhn, T. Wieland and E. F. Müller, *Ber.*, **74**, 1605 (1941).

(10) J. W. Barnett and F. A. Robinson, *Biochem. J.*, **36**, 364 (1942).

(10a) The nomenclature developed by Barnett and Robinson (ref. 6) is used in the present communication. Pantoic acid refers to levo-α,γ-dihydroxy-β,β-dimethylbutyric acid. It is used in solution as the sodium salt, obtained by alkaline hydrolysis of pantolactone (see ref. 17).

(11) N. Nielsen, V. Hartelius and G. Johansen, *Naturwissenschaften*, **32**, 294 (1944).

(12) W. Shive and E. E. Snell, *J. Biol. Chem.*, **160**, 287 (1945).

(13) D. W. Woolley and M. L. Collyer, *ibid.*, **159**, 263 (1945).

(14) E. E. Snell and W. Shive, *ibid.*, **158**, 551 (1945).

(15) W. Shive and E. E. Snell, *ibid.*, **160**, 287 (1945).

(16) W. Drell and M. S. Dunn, THIS JOURNAL, **68**, 1868 (1946).

(17) H. P. Sarett and V. H. Cheldelin, *J. Biol. Chem.*, **159**, 311 (1945).