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Reactions of Carbohydrates with Nitrogenous Substances. I. Mutarotations of Some Glycosylamines

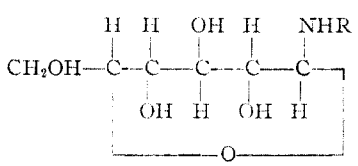
BY WARD PIGMAN,[1] E. A. CLEVELAND, D. H. COUCH AND J. H. CLEVELAND

Glycosylamines may undergo rapid hydrolysis or isomerization in aqueous solutions. Hydrolysis may only be partial at equilibrium. For several members of this class the reactions at 30° were followed by mutarotation measurements and determinations of the amount of free amine present. Dilute acetic acid (3 *N*) always caused hydrolysis. The effects of dilute hydrochloric acid and of sodium hydroxide were variable and dependent upon the nature of the alkyl or aryl radical. Generally dilute hydrochloric acid (0.5 *N*) was a less effective hydrolyzing agent than dilute acetic acid (3 *N*). In glacial acetic acid, an unknown and apparently different type of reaction took place with the final formation of deeply colored solutions. A number of new compounds were prepared.

Previous studies of the mutarotation of glycosylamines have shown that the reactions involved are isomerizations of some type accompanied in aqueous solution by hydrolysis.[2-4] The present paper is a further study of these reactions for several representative types of glycosylamines, particularly as they are affected by the acidity of the solutions. The preparation of a number of new compounds is described. A subsequent paper will present additional observations on glucosylaniline in non-aqueous solutions.

Results

The mutarotations of the glucosylamines shown in the accompanying formulas (written arbitrarily in the ring forms with β-configuration) was followed at 30° generally in the solvents: water, 0.01 *N* sodium hydroxide, 0.5 *N* hydrochloric acid, 3 *N* acetic acid and glacial acetic acid.



R = H, glucosylamine
R = *n*-C_4H_9, glucosyl-*n*-butylamine
R = *n*-C_6H_{13}, glucosyl-*n*-hexylamine
R = C_6H_5, glucosylaniline

An amorphous maltosyl-*n*-dodecylamine and an impure barium salt of glucosylglycine were also studied. The results are given in Figs. 1 to 4, and the rotational data are summarized in Table II.

At some critical points, the dissociation of the glycosylamines into glucose and amine was measured by determinations of Van Slyke amino-nitrogen. The glucosylalkylamines were found to be stable under the conditions of the determination. Free aniline was determined by extraction of a neutral alkaline solution with ether, conversion to the hydrochloride and weighing. These results are shown in Table I.

Glucosylamine (Fig. 1 and Table I).—In agreement with earlier workers[5] glucosylamine (glucose ammonia) was unaffected by water and by 0.01 *N* sodium hydroxide. Hydrochloric acid (0.5 *N*) caused a slight increase in rotation (reaction A).[6] Dilute acetic acid, as indicated by the value of the initial rotation, apparently brought about reaction A rapidly prior to the first reading, and the observed reaction which followed the first-order equation was a complete hydrolysis. The rotation of the equilibrium solution was close to that calculated for the equivalent amount of equilibrated D-glucose and the reaction followed the first-order equation. In glacial acetic acid, levorotatory substances were formed, but a reaction constant could not be calculated because the solution darkened before equilibrium was reached; this reaction is termed reaction B.[6]

TABLE I

EQUILIBRIUM DATA FOR SOLUTIONS OF GLUCOSYLAMINES[a] 5% SOLUTIONS AT 30°

Compound	3 *N* HOAc	Hydrolysis at equilibrium, % 0.5 *N* HCl	H_2O	0.01 *N* NaOH
Glucosylamine (glucose ammonia)	100	100	0	..
Glucosyl-*n*-butylamine[a]	100	13[c]	55	62
Glucosyl-*n*-hexylamine	100	4[c]	73	..
Glucosyl-*n*-octylamine	96	9[c]	..	..
Glucosyl-*n*-decylamine	100	22[c]	..	..
Glucosyl-*n*-dodecylamine	100	100	..	..
Glucosylaniline	80	90[d]	44	0
Maltosyl-*n*-dodecylamine	*ca.* 100	0	0	..
Ba salt of glucosylglycine[b]	100	...	75	..

[a] For 10% solutions. [b] For 2.5% solutions. [c] Forty-eight hours values; probably not equilibrium values. [d] Complete hydrolysis within the experimental error. [e] Except for glucosylaniline, the hydrolysis was determined by the Van Slyke amino-N method. Free aniline was determined by extraction of alkaline solution with ether, conversion to hydrochloride and weighing as hydrochloride. For the water and 0.01 *N* sodium hydroxide solutions, the same equilibria were reached when equimolar solutions of the amine and glucose were allowed to react under the same conditions.

Glucosyl-*n*-butylamine (Fig. 2 and Table I).—This compound underwent a partial hydrolysis in water and 0.01 *N* sodium hydroxide. Dilute acetic acid produced essentially a complete hydrolysis, whereas 0.5 *N* hydrochloric acid produced extensive hydrolysis only at 100°. These reactions followed the first-order equation. The difference between the initial rotations of the alkaline and acid solutions may indicate that a prior reaction (reaction A) has occurred. Glacial acetic acid again caused a marked increase of levorotation (reaction B) which followed the first-order equation.

Glucosyl-*n*-hexylamine (Tables I and II).—Slow hydrolysis occurred at 30° in 0.5 *N* hydrochloric acid. Using as the equilibrium value, that ($[\alpha]^{30}D$ +29°) found by heating the solution at 100° for one hour, good first-order reaction constants were obtained. The reaction is then a

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(2) J. C. Irvine and R. Gilmour, *J. Chem. Soc.*, **93**, 1429 (1908); **95**, 1545 (1909).

(3) J. W. Baker, *ibid.*, 1205 (1929); 1583 (1928).

(4) K. Hanaoka, *J. Biochem. (Japan)*, **28**, 109 (1938); **31**, 95 (1940).

(5) E. Mitts and R. M. Hixon, THIS JOURNAL, **66**, 483 (1944).

(6) The reactions other than hydrolysis (hydrolytic reaction) and that occurring in glacial acetic acid (reaction B) will be called reaction A. For the various glycosylamines in aqueous solution, reaction A follows the first-order equation and presumably is a ring or α,β-conversion (see below).

TABLE II

SUMMARY OF OPTICAL ROTATIONS AND MUTAROTATIONS OF GLUCOSYLAMINES (T 30°; 5% SOLUTIONS)

Compound	Solvent	Approx. pH	Initial $[\alpha]_D$	Final $[\alpha]_D$	$[\alpha]^{30}D$ at t minutes after dissolution	Probable mutarotation reaction[6]
Glucosylamine (glucose ammonia)	H_2O	7.0	20.2	20.2	20.2	None
	0.01 N NaOH	12	18.8	18.8	18.8	None
	0.5 N HCl	0.6	17.4	25.1	$25.1 - 7.7 \times 10^{-0.038t}$	Reaction A
	3 N HOAc	3.0	24.2	53.5	$53.3 - 29.3 \times 10^{-0.0185t}$	Hydrolysis (prior reacn. A)
	Glacial acetic	..	20.0	>-30	.....	Reaction B
Glucosyl-n-butylamine	H_2O	7.0	−24.2	7.7	$7.7 - 31.9 \times 10^{-0.0059t}$	Hydrolysis
	0.01 N NaOH	12	18.9	6.4	$6.4 - 25.3 \times 10^{-0.0074t}$	Hydrolysis
	0.5 N HCl	0.6	− 9.5	31.2	$31.2 - 40.7 \times 10^{-0.000014t}$	Slow hydrolysis (prior reaction A)
	3 N HOAc	3.0	− 9.3	35.1	$35.1 - 44.4 \times 10^{-0.0120t}$	Hydrolysis (prior reacn. A)
	Glacial acetic	..	− 2.9	−51.5	$-51.5 + 48.6 \times 10^{-0.0095t}$	Reaction B
Glucosyl-n-hexylamine	0.5 N HCl	0.6	−10.1	29.5	$29.5 - 39.6 \times 10^{-0.000016t}$	Hydrolysis
Glucosyl-aniline	H_2O	7.0	14.8	−14.3 (Complex)	$-14.3 - 65.3 \times 10^{-0.000163t} + 94.4 \times 10^{-0.0218t}$	Reaction A + hydrolysis
	0.01 N NaOH	12	37.2	−85.6	$-85.6 + 122.8 \times 10^{-0.0091t}$	Reaction A
	0.5 N HCl	0.6	36.9	36.9	36.9	Prior hydrolysis
	3 N HOAc	3.0	27.7	27.7	27.7	Prior hydrolysis
Maltosyl-n-hexylamine	H_2O	7.0	92.0	>97.4	.. ..	Hydrolysis
	0.5 N HCl	0.6	93.4	93.4	93.4	None
Maltosyl-n-dodecylamine	H_2O	7.0	67	67	67	None
	0.5 N HCl	0.6	73	73	73	(Prior reaction A)
	3 N HOAc	3.0	72	86	$86 - 14 \times 10^{-0.062t}$	Hydrolysis (prior reacn. A)
	Glacial acetic	..	68	65	.. ..	Slow reaction B
Ba salt of glucosylglycine	H_2O	8.1	− 6.6	18.2	$18.2 - 24.8 \times 10^{-0.0008t}$	Hydrolysis
	3 N HOAc	3.3	24.8	24.8	24.8	Hydrolysis

slow essentially complete hydrolysis. Water caused a partial hydrolysis, and dilute acetic acid caused complete hydrolysis. Glucosyl-n-decylamine (Table I) behaved similarly as far as studied.

Glucosylaniline (Fig. 3 and Table I).—In alkaline solution (0.01 N sodium hydroxide), hydrolysis of glucosylaniline was completely prevented, but a reaction (type A) occurred which followed the first-order equation. In water, a complex reaction occurred which appeared to be the same as that in dilute alkali accompanied by partial hydrolysis. At equilibrium, only 44% hydrolysis had occurred, whereas at 400 minutes, when the first reaction (reaction A) was completed, only 9% hydrolysis had taken place. Good reaction constants could be calculated[7] for the solutions in water from the equation for two simultaneous or consecutive reactions (see Table II). In acid solution, a high degree of hydrolysis occurred before the first measurements were made. Because of the inaccuracy of the method used for the estimation of free aniline, it is not certain whether complete hydrolysis occurred. In 3 N acetic acid, the hydrolysis seemed only to reach about 80% at equilibrium. Darkening occurred so rapidly in glacial acetic acid that no measurements could be made.

Maltosyl-n-dodecylamine (Fig. 4).—This compound showed no change of rotation or hydrolysis in water or 0.5 N hydrochloric acid. The somewhat higher initial value of the acid solution may indicate a prior reaction (reaction A). In weak acid (3 N acetic), rapid complete hydrolysis following the first-order equation occurred. A slow decrease in rotation took place in glacial acetic acid.

Maltosyl-n-hexylamine (Table II).—The rotation remained constant in 0.5 N hydrochloric acid with little or no hydrolysis. In water, the rotation increased toward that for maltose, but equilibrium observations could not be made because of darkening of the solution.

Glucosylglycine (Impure Barium Salt) (Table II).—A high degree of rapid hydrolysis was brought about by dilute acetic acid. In water, a slow partial hydrolysis (75%)

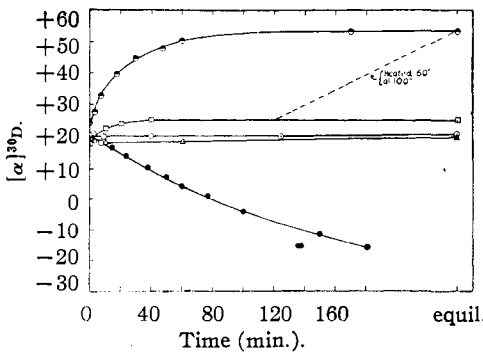

Fig. 1.—Mutarotation of glucosylamine (glucose ammonia) in 5% solutions at 30°: ⊙, water; ▲, 0.01 N sodium hydroxide; ◑, 3 N acetic acid; ●, glacial acetic acid.

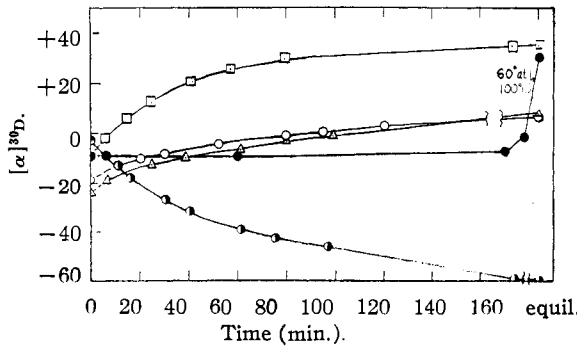

Fig. 2.—Mutarotation of glucosyl-n-butylamine in 5% solutions at 30°: ▲, water; ⊙, 0.01 N sodium hydroxide; ●, 0.5 N hydrochloric acid; ⊡, 3 N acetic acid; ◑, glacial acetic acid.

(7) See H. S. Isbell and W. W. Pigman, *J. Research Natl. Bur. Standards*, **18**, 141 (1937).

TABLE III

PROPERTIES, ANALYSIS AND PREPARATION OF GLYCOSYLAMINES

Compound	M.p., °C.	Preparatory method	Carbon, % Found	Carbon, % Theory	Hydrogen, % Found	Hydrogen, % Theory	Nitrogen, %[c] Found	Nitrogen, %[c] Theory
Glucosylamine	128–129	[d]	40.4	40.3	7.0	7.3	6.7	7.8
Glucosyl-*n*-butylamine	88–90	2	..	..	..	..	6.0	6.0
Glucosyl-*n*-hexylamine	93–95	2	54.6	54.8	9.1	9.5	4.9	5.3
Glucosyl-*n*-octylamine[a]	102	2	..	..	..	..	4.6	4.8
Glucosyl-*n*-decylamine[a]	103–104	2	60.3	60.2	10.1	10.4	4.3	4.4
Glucosyl-*n*-dodecylamine[a]	105.5	2	62.7	62.2	10.1	10.7	3.9	4.0
Glucosyl-*n*-octadecylamine	103.5	2	..	..	..	..	3.3	3.2
Glucosylaniline	140	1	56.1	56.5	6.9	6.7	5.3	5.5
Glucosyl-4-aminobiphenyl·H_2O[a]	118–120 (dec.)	1	62.5	61.9	6.5	6.6	4.1	4.0
Glucosyl-4-amino-azobenzene·H_2O[a]	134–135 (dec.)	1	57.7	57.3	6.2	6.1	11.1	11.1
Maltosyl-*n*-hexylamine·H_2O[a]	.....	2 and pptn. into acetone	48.9	48.8	7.6	8.3	2.4	3.2
Maltosyl-*n*-dodecylamine·H_2O[a,b]	48–84	2 and pptn. into acetone	..	..	..	..	2.3	2.7
Maltosyl-*n*-octadecylamine[a,b]	80–115	2 and pptn. into acetone	..	..	..	..	2.8	2.3
Lactosyl-*n*-dodecylamine[a]	117–119 (dec.)	2	..	..	..	..	3.0	2.75
Lactosyl-4-aminobiphenyl·$2H_2O$[a]	144–148 (dec.)	1	53.3	54.4	6.5	6.7	2.35	2.65

[a] New compounds. [b] Probably amorphous. [c] Analyses by T. S. Ma. The nitrogen determinations were by the Dumas method, because Kjeldahl determinations gave low values. [d] Prepared initially by evaporation of a solution of glucose in liquid ammonia.

occurred which followed the first-order equation. Wolfrom, Schuetz and Cavalieri[8] had previously shown that glucosylglycine ethyl ester is rapidly hydrolyzed in aqueous solution.

Discussion

As shown by the present results and those of previous workers, the glycosylamines are extremely labile, and the stability and type of reaction are markedly dependent upon the nature of the aglycon group and the acidity of the solution. There is evidence for three types[9] of reactions: Hydrolysis, reaction A (presumably isomerization) and reaction B which occurs in glacial acetic acid.

One remarkable generalization which holds for all of the compounds studied is that dilute acetic acid is generally an effective hydrolyzing agent (Table I), whereas dilute hydrochloric acid (0.5 *N*) of much higher hydrogen-ion concentration frequently is ineffective at 30°. These results agree generally with those obtained by Isbell and Frush[10]

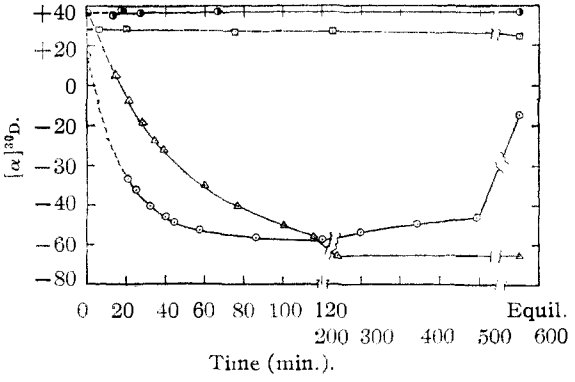

Fig. 3.—Mutarotations of 5% solutions of glucosylaniline at 30°: ⊙, water; △, 0.01 *N* sodium hydroxide; ◑, 0.5 *N* hydrochloric acid; ⊡, 3 *N* acetic acid.

(8) M. L. Wolfrom, R. D. Schuetz and L. F. Cavalieri, THIS JOURNAL, **71**, 3518 (1949).

(9) In a subsequent publication now in preparation, it will be shown that the amount of water present is also an important influencing factor.

(10) H. S. Isbell and H. L. Frush, THIS JOURNAL, **72**, 1043 (1950).

for arabinosylamine; the curve for the rate of hydrolysis for this compound as a function of *p*H was an inverted catenary, with a maximum at *p*H 5 and virtually no hydrolysis occurred at *p*H 1.5 and 9.

The effect of 0.5 *N* hydrochloric acid was extremely dependent upon the nature of the aglycon amine. With some compounds no hydrolysis occurred at 30° (glucosylamine, maltosylhexylamine and maltosyldodecylamine). For glucosylaniline the hydrolysis was over before the first measurements were made. Glucosylbutyl- and hexylamines were hydrolyzed at measurable rates. For representatives of the series of glucosyl-*n*-alkylamines from glucosyl-*n*-dodecylamine, hydrolysis was complete at both ends of the series but was only partial for intermediate members (Table I) after 48 hours at 30°. It is unlikely that these represent equilibrium values.

Dilute alkali (0.01 *N* sodium hydroxide) prevented the dissociation of glucosylaniline, but catalyzed the hydrolysis of glucosyl-*n*-butylamine more than 0.5 *N* hydrochloric acid. Except for glucosylamine and maltosyl-*n*-dodecylamine, which were stable, distilled water generally produced

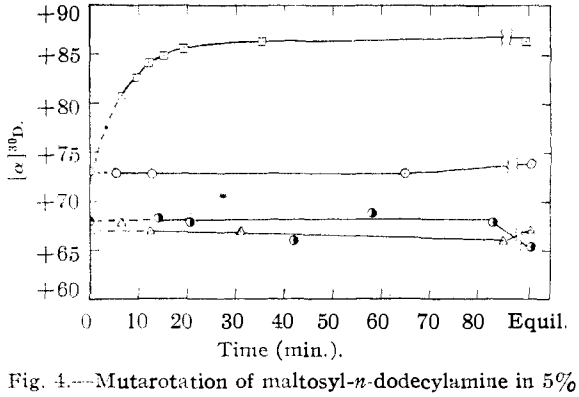

Fig. 4.—Mutarotation of maltosyl-*n*-dodecylamine in 5% solutions at 30°: △, water; ⊙, 0.5 *N* hydrochloric acid; ⊡, 3 *N* acetic acid; ◑, glacial acetic acid.

partial hydrolysis. Sometimes, as with glucosylaniline particularly, a simultaneous or prior reaction (reaction A) took place.

Under some conditions a mutarotation took place without hydrolysis, or the prior occurrence of such a reaction was inferred from the initial rotations. This has been called reaction A and presumably arises from reversible isomerization reactions involving ring or α,β-changes.

All of the glucosylamines when dissolved in glacial acetic acid developed a brown color, and when the rotations could be observed, they became more levorotatory. The nature of this reaction (reaction B) is also unknown, but it may be of importance in the development of colored bodies which are formed readily from glucosylamines. Possibly a reaction like the Amadori-type rearrangement is involved.

Mitts and Hixon[5] have pointed out that with the exception of glucosylamine itself, the hydrolysis of glycosylamines seems to parallel the dissociation constant of the aglycon amine. This relation seems to hold generally for the glucosyl derivatives of simple alkyl and aryl amines and agrees with the known stability of N-acetylglucosylamine and nucleosides. However, not only is glucosylamine an exception, but also glucosylglycine which dissociates readily. Before such a generalization can be made a distinction must be made between the rate and the extent of hydrolysis, and in view of the work of Isbell and Frush,[10] the *p*H at which comparisons are made must be carefully considered. The stability of maltosyldodecylamine also is not consistent with the strong basicity of the parent amine. Since the product is highly surface active, possibly its lack of hydrolysis may arise from its presumed orientation and high concentration in the surface layer. The maltosylhexylamine dissociated readily in water.[11]

Experimental

Preparation of Alkyl Glucosylamines.—Method 1 (based on Weygand[12]). A mixture of 18 g. (0.1 mole) anhydrous glucose, 20.3 g. (0.12 mole) 4-aminobiphenyl and 5.4 g. (0.3 mole) water was heated in a bath of boiling water for 15 minutes. To the clear solution was added 25 ml. of methanol, and the solution was allowed to crystallize in the refrigerator. The crystals were filtered and washed with ether; yield 17.9 g. (66%).

Method 2 (Based on Sorokin).[13]—To a solution of 36 g. (0.5 mole) of *n*-butylamine in 50 ml. of methanol was added 90 g. (0.5 mole) of anhydrous glucose. The stirred solution was heated at 60–65° for 15 to 20 minutes, diluted with 300 ml. of hot ethanol and allowed to cool to room temperature. Crystallization occurred; yield (of two crops) 120 g. (94%). Methanol was found preferable to ethanol as used by Sorokin and others. Higher yields and less discoloration resulted.

The melting points and analyses of the products used are given in Table III.

(11) Several crude maltosylamines and their reduction products have been described previously by J. H. Werntz, U. S. Patent 2,181,929, December 5, 1939.

(12) F. Weygand, *Ber.*, **72**, 1663 (1939).

(13) W. Sorokin, *ibid.*, **20**, 783 (1887).

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A Synthetic Compound with Folinic Acid Activity

BY EDWIN H. FLYNN, THOMAS J. BOND, THOMAS J. BARDOS AND WILLIAM SHIVE

The preparation and properties of a synthetic compound having biological activities similar to folinic acid, a new B-vitamin related to folic acid, are described. The synthetic factor, folinic acid-SF, is prepared by reduction of formylfolic acid or by reduction of folic acid in the presence of compounds capable of donating a single carbon unit.

In attempts to develop assays for the anti-pernicious anemia principle(s) in refined liver extracts, numerous testing procedures for unknown principles were developed as a result of their particular effects on the toxicities of inhibitory analogs of folic acid and *p*-aminobenzoic acid for certain organisms.[1]

One group of related factors were detected as a result of their enhanced ability, when compared with folic acid, to prevent the toxicity of *x*-methylfolic acid for either *Lactobacillus casei* or *Streptococcus faecalis* R.[2] This group of factors has been termed the folinic acid group, and one of the factors closely related to folic acid has been termed folinic acid.

Purified liver extracts have been reported to contain a factor or factors necessary for the growth of *Leuconostoc citrovorum* 8081 under specified conditions.[3] This growth promoting effect of purified liver extracts has been reported to be the result of a synergistic effect of a combination of thymidine with factors which appear to be identical with the folinic acid group[4]; however, thymidine[5] or folinic acid[4] alone promote growth of this organism at concentrations considerably higher than those required for the two factors combined.

A recent communication has described the preparation from folic acid of a reaction mixture which has the biological activities of folinic acid derived from liver.[6] It is the purpose of this paper to report in detail the preparation in crystalline form and the properties of a synthetic compound, folinic

(1) W. Shive, *Trans. N. Y. Acad. Sci.*, **52**, 1212 (1950); presented before the New York Acad. Science, Feb., 1949.

(2) T. J. Bond, T. J. Bardos, M. Sibley and W. Shive, THIS JOURNAL, 71, 3852 (1949).

(3) H. E. Sauberlich and C. A. Baumann, *J. Biol. Chem.*, **176**, 165 (1948).

(4) T. J. Bardos, T. J. Bond, J. Humphreys and W. Shive, THIS JOURNAL, **71**, 3852 (1949).

(5) E. E. Snell, E. Kitay and W. S. McNutt, *J. Biol. Chem.*, **175**, 478 (1948).

(6) W. Shive, T. J. Bardos, T. J. Bond and L. L. Rogers, THIS JOURNAL, **72**, 2817 (1950).