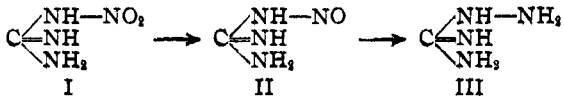
either 0.4 g. of platinum oxide or 5 g. of Raney nickel catalyst. The volume of solvent medium was 120 ml. The reductions were carried out at 25, 75 and 125°. The aminoguanidine obtained was determined by titration.[3]

Figure 1 and Table I summarize the main results obtained. It will be observed that, except for platinum oxide catalyst in 15% aqueous acetic acid solution and Raney nickel catalyst in *n*-propyl alcohol, the yields of aminoguanidine are higher in starting from pure nitrosoguanidine than from nitroguanidine. This is especially evident for the platinum catalyst in neutral aqueous media.

Discussion

The data on the conversion of nitrosoguanidine to aminoguanidine indicate that the greatest loss in the reduction of nitroguanidine to aminoguanidine in neutral or basic media occurs in step I to II



I II III

Since in acid media the conversion is directly I to III without the formation of II, this factor is not operative and it will be observed that the yields of aminoguanidine, starting from nitrosoguanidine in acid media, are very much lower over the whole temperature range than when the starting material is nitroguanidine. This is due to the instability of nitrosoguanidine in acid solution.[5] From an examination of the last columns of Table I, it will be of interest to observe that nitrosoguanidine is much more resistant to reduction by catalytic hydrogenation than nitroguanidine, as measured by the times of reduction. The times as noted are for three molar proportions of hydrogen for nitroguanidine and two molar proportions of hydrogen for nitrosoguanidine to complete reduction to aminoguanidine. This probably accounts for the ability to isolate nitrosoguanidine as an intermediate product of reduction of nitroguanidine in neutral or basic media.[1a,5] As in the reduction of nitroguanidine,[1c] the yields of aminoguanidine for a nickel catalyst are conditioned by the type of solvent used. In absolute methyl alcohol, at room temperature, practically a quantitative conversion to aminoguanidine is obtained, while at 75°, the solvents may be placed in the order, ethyl, methyl and propyl alcohols, water and dioxane.

(5) Sabetta, Himmelfarb and Smith, THIS JOURNAL, **57**, 2478 (1935).

Summary

The reduction of nitrosoguanidine to aminoguanidine has been studied by the method of catalytic hydrogenation and the isolation of aminoguanidine described. The effect of temperature and solvent media on the yields of aminoguanidine have been determined and a comparison made with the conversion of nitroguanidine to aminoguanidine.

BROOKLYN, NEW YORK RECEIVED JULY 10, 1937

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY]

A Quantitative Study of the Influence of Certain Factors upon the Activity of the Amylase of Aspergillus Oryzae[1]

BY M. L. CALDWELL AND S. E. DOEBBELING

Recent work with the amylase of *Aspergillus Oryzae* made evident the need for more exact information concerning the influence upon its action of the hydrogen ion activities and electrolyte concentrations of its substrates, information which would establish the conditions necessary for the full activity of this enzyme at different stages in its purification and which would insure the attainment and consistent control of these conditions in comparative measurements of its activities. Such information is essential to further studies of the purification and properties of this enzyme and to comparisons of its characteristics with those of other amylases which have been investigated more intensively.[2] Results of work carried out to meet this need are briefly reported here.

(1) We are greatly indebted to The Takamine Ferment Company, New York, N. Y., for a grant in aid of this investigation.

Experimental

A number of amylase preparations of widely different degrees of concentration and purification were used. These included commercial products[3] and others, which

(2) (a) Sherman, Caldwell and Adams, THIS JOURNAL, **50**, 2529, 2535, 2538 (1928); (b) *J. Biol. Chem.*, **88**, 295 (1930); (c) Sherman, Caldwell and Doebbeling, *ibid.*, **104**, 501 (1934); (d) Caldwell and Doebbeling, *ibid.*, **110**, 739 (1935).

(3) These products were kindly furnished by the Takamine Laboratory, Inc., Clifton, New Jersey.

had been subjected to more or less extensive purification in the Laboratory.[4] The activities of these products expressed as powers[5] ranged from 34 to 880. Each product was studied both for its amyloclastic and for its saccharogenic activity. In each case the amylase acted upon 1 or 2% soluble potato starch for thirty minutes at 40°. The amyloclastic action was determined by two independent methods: by a modification[6] of the Wohlgemuth[7] procedure, which gives the weight of enzyme preparation necessary to hydrolyze a given weight of starch to products which give a clear red and no blue color with iodine in potassium iodide, and by the direct quantitative determination of residual starch.[8] For the saccharogenic action, the reducing sugar formed from the starch was determined either by the modified gravimetric Fehling's method[9] or iodimetrically,[10] and calculated to maltose.

The total electrolyte concentrations of the substrates were varied by the use of different concentrations of sodium chloride. This salt was chosen because previous work with a number of neutral salts[11] had shown that any favorable influence upon the activity of this amylase of additional electrolyte beyond that needed adequately to buffer its substrate is due to the total electrolyte concentration of the substrate rather than to any specific ion effect such as has been observed with pancreatic amylase.[2a] Each concentration of sodium chloride in the substrate was studied at a series of different hydrogen ion activities adjusted by the use of sodium acetate–acetic acid mixtures present in different proportions but in a constant total concentration of 0.01 *M* acetate.[9]

Conditions for the Amyloclastic Activity of the Amylase.—It was found that sodium chloride markedly increases the amyloclastic activity of this amylase whether this is measured by the modified Wohlgemuth[7] or by the residual starch precipitation[8] method. In either case, this activity of the enzyme was higher and more consistent in the presence of sodium chloride, the most favorable influence being reached under the conditions of these experiments at a concentration of this salt of 0.05 *M* and at *p*H 5.0. This is shown by the strictly comparable data summarized in Table I which were obtained with highly purified products but which are also typical of the results given by the other less active products[3] studied.

Conditions for the Saccharogenic Activity of the Amylase.—When the hydrogen ion activities of its substrate are suitably adjusted by the presence of 0.01 *M* acetate, sodium chloride has no appreciable influence upon the saccharogenic activity of this amylase until the relatively high concentration of 0.20 *M* is reached when a slight inhibitory influence becomes evident. This is shown by the data given in Table II which again were obtained with a highly purified preparation but which also are typical of the results with the other enzyme products studied.

(4) (a) Chester, Dissertation, Columbia University, 1933; (b) unpublished data.

(5) Sherman, Kendall and Clark, THIS JOURNAL, **32**, 1073 (1910).

(6) Sherman and Thomas, *ibid.*, **37**, 623 (1915).

(7) Wohlgemuth, *Biochem. Z.*, **9**, 1 (1908).

(8) Caldwell and Hildebrand, *J. Biol. Chem.*, **111**, 411 (1935).

(9) Caldwell and Tyler, THIS JOURNAL, **53**, 2316 (1931).

(10) Caldwell, Doebbeling and Manian, *Ind. Eng. Chem., Anal. Ed.*, **8**, 181 (1936).

(11) Coppersmith, Dissertation, Columbia University, 1929.

TABLE I

DEPENDENCE OF THE AMYLOCLASTIC ACTIVITY OF THE AMYLASE OF *Aspergillus Oryzae* UPON THE INTERRELATIONSHIP BETWEEN HYDROGEN ION ACTIVITY AND ELECTROLYTE CONCENTRATION OF ITS SUBSTRATE

*p*H of substrate	Molar concentration of sodium chloride in substrate: None	0.01	0.02	0.05	0.10	0.20
A. As measured[a] by Wohlgemuth procedure. Activity expressed as Wohlgemuth units[b]						
4.0	2777	3571	3906	3571	2777	2273
4.5	2777	4630	4630	4808	4808	3571
4.7	2500	3906	4630	4808	4808	4167
5.0	2500	3371	5000	5000	5000	4630
5.3	2500	2777	3571	4167	4167	4167
5.5	2500	2500	3571	3906	4167	3906
6.0	2273	2500	3125	3571	3571	3125
B. As measured[a] by residual starch method. Activity expressed as starch in mg. hydrolyzed[c] by 0.056 mg. enzyme preparation						
4.0	143			126	119	
4.5	142			145	142	
4.7	141			143	137	139
5.0	138	144		145	144	
5.3	137	142		139	140	

[a] The purified amlyase preparations with powers[5] of 660 in A and 315 in B reacted for thirty minutes at 40° with 1% soluble potato starch containing 0.01 *M* acetate. [b] Wohlgemuth units represent weight of starch hydrolyzed (50 mg.) divided by the weight of enzyme preparation (mg.) required to bring this starch to products which give a clear red and no blue color with iodine in potassium iodide. [c] To products no longer precipitated by 55% alcohol.[8]

TABLE II

DEPENDENCE OF THE SACCHAROGENIC ACTIVITY OF THE AMYLASE OF *Aspergillus Oryzae* UPON THE INTERRELATIONSHIP BETWEEN THE HYDROGEN ION ACTIVITY AND THE ELECTROLYTE CONCENTRATION OF ITS SUBSTRATE

Hydrogen ion activity of substrate, *p*H	Molar concentration of sodium chloride in substrate: None	0.01	0.02	0.05	0.10	0.20
	Maltose formed by 0.08 mg. enzyme preparation,[a] mg.					
4.0	112.0	111.2	109.3	101.8	89.5	71.2
4.5	119.3	124.1	131.3	135.7	133.4	127.5
4.7	132.7	136.4	140.9	143.7	142.2	138.3
5.0	145.0	146.0	149.1	149.0	147.3	142.4
5.3	148.6	148.7	148.7	146.6	143.8	137.5
5.5	150.5	149.6	146.0	143.3	131.5	127.9
6.0	141.9	139.1	133.2	123.5	104.7	91.9

[a] The purified amylase preparation with a power[5] of 660 reacted for thirty minutes at 40° with 1% soluble potato starch containing 0.01 *M* acetate.

Discussion

From all of the data it is concluded that the optimal amyloclastic action of the amylase of *Aspergillus Oryzae*, in measurements of thirty minutes at 40° with 1% soluble potato starch made 0.01 *M* in acetate, is obtained in the presence of 0.05 *M* sodium chloride and at *p*H 5.0. Similarly, the optimal saccharogenic action of this amylase, in measurements of thirty minutes

at 40° with 1 or 2% soluble potato starch, made 0.01 *M* in acetate, is obtained at *p*H 5.0 in the presence of 0.02, 0.05 or 0.10 *M* sodium chloride, or at *p*H 5.3 to 5.5 when no sodium chloride is added. These conditions hold for amylase preparations of widely different degrees of concentration and purification, including crude commercial and highly purified products.

The significance of the data summarized here is evident when it is remembered that each step in the purification of enzyme material and every study of its properties, to be of real value, must be accompanied by quantitative activity measurements which in turn depend upon the chemical environment of the enzyme.

Whether the slight differences in the conditions which favor the two kinds of activity indicate that more than one amylase is present can only be decided by future work.

Summary

The conditions which favor the action of the amylase of *Aspergillus Oryzae*, both saccharogenic and amyloclastic, have been established and are reported briefly.

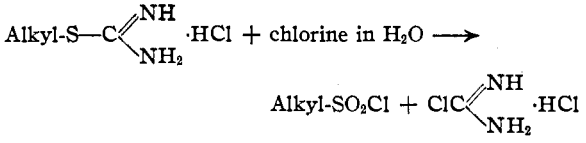
NEW YORK, N. Y. RECEIVED JULY 6, 1937

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, YALE UNIVERSITY]

The Preparation of Alkyl Sulfonyl Chlorides from Isothioureas. II

BY JAMES M. SPRAGUE[1] AND TREAT B. JOHNSON

A study of the action of chlorine gas on salts of isothioureas in aqueous solution has opened up a new and practical method of preparing alkyl sulfonyl chlorides. A number of applications of this method of synthesis were described in our first paper,[2] and the complete change may be expressed tentatively according to the equation



The question of the true mechanism of this interesting reaction will be discussed in a future paper from this Laboratory.[3]

Our method of synthesis has now been applied successfully with several other alkyl isothioureas, and the corresponding sulfonyl chlorides prepared without difficulty. Three of the sulfonyl chlorides previously reported have been prepared in much larger quantities and the technique described for practical work. Also the new applications of the chlorination reaction have necessitated the synthesis of several new representatives of the isothiourea type, thereby increasing our knowledge of the chemistry of this class of sulfur compounds. It is of interest to note here that in the chlorination of *s*-butyl and also cyclohexyl isothioureas, there was a partial elimination of the sulfur as sulfate, while in the case of *t*-butyl isothiourea, the sulfur was eliminated completely as sulfate and no sulfonyl chloride was obtained. Several of these isothiourea combinations promise to prove of future physiological interest and their pharmacological study will receive immediate attention.

(1) Sterling Professorship of Chemistry Research Assistant, 1936–37.

(2) Paper I, Johnson and Sprague, THIS JOURNAL, **58**, 1348 (1936); see also *Science*, **83**, 528 (1936).

(3) In our previous paper[2] an error occurs, which we desire to correct at this time. In Table II on page 1350 the data recorded for the *n-butyl* radical are the results of experimentation with *iso-butyl*, and the data for *n-butyl* (recorded below) were omitted entirely.

R	Hours	Yield, %	B. p., °C.	Mm.	$n^{25}D$
n-C_4H_9	24	68	81–82	10	1.4524
	48	76			
	72	82			
	120	80			

Experimental Part

If not otherwise described the S-alkyl isothiourea hydrohalides were prepared by the general procedure previously outlined.[2] The sulfonyl chlorides, recorded in Table I,

TABLE I

FORMATION OF SULFONYL CHLORIDES

R—(SO_2Cl)	Yield, %	Procedure	B. p. or m. p., °C.	Mm.
$C_{12}H_{25}$	75–85	A	M. 42–43	
$C_{16}H_{33}$	70–80	A	M. 52–53	
s-C_4H_9	50	A	B. 86–88	18
	56	B		
s-C_8H_{17}	65	A	B. 110–112	4
C_6H_{11}	40	A	B. 122–123	14
	43–55			
p-$NO_2C_6H_4CH_2$	90	A	M. 91–92	
C_2H_5	66	A	B. 71–72	20
n-C_4H_9	75–83	B	B. 94–96	18
$C_6H_5CH_2$	81	A	M. 91–92	