This equation is applicable over the experimental range with an error of about 1–2%. The table presented in the paper on the determination of alpha-amylase was compiled with the aid of this equation.

It is evident that the equation should be applicable to other enzyme preparations such as taka-diastase, pancreatin, etc., so long as the rate curves of these preparations were the same as those of malt amylase. This has not been demonstrated as yet but it is probable that when each preparation is properly treated with sodium chloride as a desorption or activating agent the rate curves would follow closely those of malt amylase. If the rate curves are totally different it is only necessary that we change the empirical expression to fit the preparation if great accuracy is desired.

The enzyme-unit method is generally applicable in enzyme studies. It has been applied to the study of invertase with excellent results which will soon be published. Work is now in progress on units for β-amylase and for proteolytic enzymes such as papain.

The authors wish to express their appreciation to Dr. C. N. Frey and Mr. Q. Landis for valuable suggestions in carrying out this investigation.

Summary

Experimental evidence has been presented which permits the definition and use of a rational amylase unit termed the liquefon. Its use in the determination of alpha-amylase has been described.

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Nitroguanylhydrazones of Some Common Aldehydes and Ketones[1]

BY W. F. WHITMORE, A. J. REVUKAS AND G. B. L. SMITH

Phillips and Williams[2] have reported that nitroaminoguanidine reacts with certain aldehydes and ketones to form crystalline substances. These investigators suggested that the reaction was probably that characteristic of carbonyl groups and that nitroguanylhydrazones were formed. This postulation is supported by the work of Thiele,[3] who found that aminoguanidine formed a guanylhydrazone with benzaldehyde. Woods[4] confirmed the observations of Phillips and Williams and studied in some detail the crystallographic and optical properties of certain of the nitroguanylhydrazones.[5] This paper describes the methods of preparation, analyses and some physical properties of a series of nitroguanylhydrazones of some common aldehydes and ketones. It is hoped that these and future data will establish the usefulness of nitroaminoguanidine as a reagent for the identification of aldehydes and ketones.

Experimental Part

Materials.—Nitroaminoguanidine ($H_2NNHC(=NH)NHNO_2$) was prepared by the method described by Phillips and Williams.[2] The aldehydes and ketones were purchased from Schering–Kahlbaum and were the "pure" grade.

General Procedure.—One to five drops of the pure aldehyde or ketone (if liquid), or approximately 0.05 g. (if solid) is dissolved in one ml. of water or if not water-soluble in one ml. of acetone-free methyl alcohol. To this solution is added 5 ml. of an aqueous solution of nitroaminoguanidine saturated at 70° (approximately 0.15 g. of nitroaminoguanidine) together with 0.25 ml. of glacial acetic acid. If precipitation does not take place within five minutes the solution is cooled in an ice-bath. The crystalline product is separated by filtration and recrystallized. The recrystallization medium is either hot water, hot 50% methyl alcohol, or hot absolute methyl alcohol, depending upon the solubility of the respective nitroguanylhydrazones. In general, the water-soluble aldehydes and ketones yield nitroguanylhydrazones which may be readily recrystallized from water, and those insoluble in water must be recrystallized from methyl alcohol. Precipitation occurs when the concentration of the aldehyde or

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[1] An abstract of part of the thesis submitted to the Faculty of the Polytechnic Institute of Brooklyn in June, 1934, by Mr. Revukas in partial fulfilment of the requirements for the degree of Master of Science in Chemistry.

[2] Phillips and Williams, THIS JOURNAL, **50**, 2465 (1928).

[3] Thiele, *Ann.*, **270**, 35 (1892).

[4] Woods, Thesis, M.S. in Chemistry, Polytechnic Institute of Brooklyn, 1932.

[5] These studies are being extended and will be reported in a future communication.

ketone is to the extent of 1 part in 1000 parts of the final reaction medium. The analyses, melting points and other data are listed in the accompanying table.

TABLE I

Aldehyde or ketone	Recryst. from	Corr. m. p., °C.	Carbon, %			Hydrogen, %			Nitrogen, %		
			Calcd.	Found		Calcd.	Found		Calcd.	Found	
Anisaldehyde	Abs. MeOH	200	45.54	46.2	45.5	4.68	4.76	4.74			
Benzaldehyde	Abs. MeOH	188	46.35						33.82	34.5	33.8
Cinnamaldehyde	Abs. MeOH	186	51.47	51.8	51.9	4.76	5.03	4.99	30.04	29.8	30.9
Citral	Abs. MeOH	135–136.5	52.14	52.4	52.3	7.56	7.61	7.61			
Formaldehyde	Distd. H_2O	169	18.31	18.3	18.2	3.84	3.80	3.88	53.43	54.7	54.2
Piperonal	Abs. MeOH	220	43.01	43.2		3.61	3.83				
Vanillin	50% MeOH	186–187	42.67	42.7		4.38	4.47				
Salicylaldehyde	Abs. MeOH	213	43.03	43.2	43.1	4.07	4.13	4.12			
Acetone	Distd. H_2O	164–165	30.17	30.0	30.1	5.70	5.90	5.78	44.02	43.9	42.5
Acetophenone	Abs. MeOH	161–162	48.84	48.9	48.9	5.02	5.23	5.09			
Acetoacetic ester	50% MeOH	130–131	36.34	37.6	36.8	5.67	6.35	5.99			
Cyclohexanone	Abs. MeOH	161.5									
Me Et ketone	Distd. H_2O	136.5	34.59	35.3	34.7	6.41	5.81	6.58			
Me Pr ketone	Distd. H_2O	109–110	38.47	38.8	38.7	7.00	7.14	7.11			
Me Bu ketone	Distd. H_2O	109–110	41.55	42.0	41.8	7.98	7.56	7.98			
Pyruvic acid	Abs. MeOH	181.5	25.25	25.9	25.4	3.71	3.81	3.76			

Discussion of Results

The analyses of the several compounds show that the reaction between nitroaminoguanidine and an aldehyde or a ketone is a condensation involving the elimination of water from the carbonyl group and the hydrogen of the hydrazine group. This interpretation is further substantiated by the fact that when the substances are heated with a 20% solution of hydrochloric acid for half an hour the original aldehyde or ketone is formed. Attempts to form nitroguanylhydrazones of sugars have thus far been unsuccessful. The low solubility of the nitroguanylhydrazones of formaldehyde and acetone suggest the possibility that nitroaminoguanidine might be used as a reagent for the gravimetric determination of these substances.

All the nitroguanylhydrazones investigated are definitely crystalline substances and relatively very stable. The hydrazone of cyclohexanone is explosive at higher temperatures and it was not possible to get an analysis but the compound, however, is beautifully crystalline. Of especial interest is the crystalline character of the nitroguanylhydrazones of acetone and formaldehyde as well as the extreme sensitiveness of the reagent toward the carbonyl compounds. On long standing (several months) and exposure to light the hydrazones slowly undergo decomposition with discoloration.

From these results it appears that nitroaminoguanidine should be a useful reagent for the identification of many aldehydes and ketones. The nitroguanylhydrazones are readily prepared and precipitation takes place in most cases almost instantaneously or within five minutes.

Summary

The reaction between nitroaminoguanidine and aldehydes and ketones yields the corresponding nitroguanyl hydrazones. The substances are sparingly soluble crystalline compounds with well defined melting points. The aldehyde or ketone may be recovered by hydrolysis of the nitroguanylhydrazone in an aqueous 20% solution of hydrochloric acid.

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