four carbon atoms on either the alcohol or aldehyde side of the acetal. The aldehydes which have a double bond in the 2-3 position, such as benzaldehyde, cinnamic aldehyde and furfural, have a very low affinity in the acetal reaction.

Methanol shows the slowest rate of reaction of all the alcohols. Next to it in order of reactivity is *n*-butanol. The secondary alcohols and especially the tertiary alcohols show a very much higher rate of reaction. Butyraldehyde reacts more slowly than does acetaldehyde or any other aldehyde studied. Furfural, benzaldehyde and cinnamic aldehyde apparently react hundreds of times more rapidly then do the lower aliphatic aldehydes. Heptaldehyde is *apparently* like these aldehydes both in rate of reaction and in affinity values.

Michael's speculation that there is no necessary relationship between the extent of a reaction and its velocity has been completely substantiated.

The results are in harmony with the conceptions that the extent of a reaction is determined by the relative stability of the electronic systems that are represented on the right and left hand sides of the equation, while the rate of a reaction is in part dependent upon the ease or extent to which the electronic system of a given compound must be dislocated by the catalyst before reaction may ensue. There appears to be no correlation between these two characteristics of a compound or a reaction.

MADISON, WISCONSIN

[CONTRIBUTION FROM THE BUREAU OF CHEMISTRY, UNITED STATES DEPARTMENT OF AGRICULTURE]

A METHOD FOR THE ESTIMATION OF HYDROGEN SULFIDE IN PROTEINACEOUS FOOD PRODUCTS[1]

BY L. H. ALMY

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Hydrogen sulfide is one of the end products resulting from the action of many varieties of bacteria on organic material containing protein. Though the presence in protein of sulfur-containing compounds other than cystine has been hinted at, it is generally recognized that this amino acid is the chief source of hydrogen sulfide formed during the putrefaction of flesh products.

A large number of bacteria produce hydrogen sulfide from protein containing the cystine linkage, and it is probable, therefore, that among the organisms causing decomposition of any flesh product will be found one or more of these hydrogen sulfide formers. Indeed, Rettger[2] states that "Hydrogen sulfide is, no doubt, one of the first substances which are split

[1] This paper was presented before the Division of Biological Chemistry at the Washington Meeting of the American Chemical Society, April, 1924.

[2] Rettger, *J. Biol. Chem.*, **2**, 71 (1906–7).

off from the protein molecule during putrefaction." It is evident that a reliable and convenient method for the estimation of small amounts of this gas in biological materials would be of value in connection with studies on the rate of decomposition of food products. It was with this purpose in mind that the present study was inaugurated.

Fischer[3] called attention to the possibility of using *p*-amino-dimethylaniline as a reagent for the detection of hydrogen sulfide in exceedingly small quantities. The reaction between this compound and hydrogen sulfide in the presence of hydrochloric acid and an oxidizing agent, results in the formation of methylene blue. This is generally known as Lauth's reaction. The method was applied practically by Lindsay[4] for the estimation of sulfur in pig iron. It was also studied by Mecklenburg and Rosenkränzer,[5] who clearly defined the conditions necessary for the best results, and one of them[6] used the method in an investigation concerning the speed of solution of zinc blend and galena in dil. sulfuric acid. In carrying out the method Mecklenburg and Rosenkränzer treated about 490 cc. of the test solution with 10 cc. of concd. hydrochloric acid solution, followed by 10 mg. of *p*-amino-dimethylaniline sulfate and 1 cc. of 0.1 *M* ferric chloride solution. After several hours the colored solution was compared in a colorimeter with standards prepared in a similar manner by treatment of aqueous solutions of definite hydrogen sulfide concentration.

Description of the Proposed Method

Following the method of Mecklenburg and Rosenkränzer, several sets of standards were prepared and compared one with the other. A Schreiner colorimeter[7] was employed. It was found that when the conditions of preparation were identical, any difference in the calculated and observed value for the concentration of the hydrogen sulfide was well within the experimental error of matching the colors. It was concluded, therefore, that it would be possible to determine hydrogen sulfide in food products in exceedingly small amounts if some method of separating it from the solid matter and interfering dissolved material could be devised.

Attempts to determine hydrogen sulfide in the filtrate after precipitation of the protein material from an aqueous suspension with the usual reagents were unsuccessful. Removal of the gas by aëration seemed to be feasible and a study of the possibilities in this direction led to the development of a method that gave satisfactory results. It was found that the sulfide radical could be completely removed from aqueous solutions of sodium

[3] Fischer, *Ber.*, **16**, 2234 (1883).

[4] Lindsay, *School of Mines Quarterly*, **23**, 24 (1901–2).

[5] Mecklenburg and Rosenkränzer, *Z. anorg. Chem.*, **86**, 143 (1914).

[6] Rosenkränzer, *ibid.*, **87**, 319 (1914).

[7] Schreiner, THIS JOURNAL, **27**, 1192 (1905).

sulfide on treatment with an acid and a current of carbon dioxide. For the success of this procedure it was necessary to find a suitable solution for absorbing the hydrogen sulfide driven out by the carbon dioxide. Zinc acetate solution commended itself for the reasons that it absorbs the hydrogen sulfide quantitatively, while the carbon dioxide is not sufficiently acidic to decompose the zinc sulfide formed, and does not affect the color reaction. The method as finally adopted is as follows.

Reagents.—1. A 1:1 solution of hydrochloric acid.

2. *p*-Amino-dimethylaniline hydrochloride. On the day the tests are to be made the diamine salt is dissolved in 1:1 hydrochloric acid in the proportion of 0.04 g. of the salt to 100 cc. of acid.

3. Ferric chloride solution (approximately 0.02 *M*). This may be conveniently made up as required by dilution, with distilled water, of an 0.1 *M* ferric chloride solution, which is prepared as in the Mecklenburg and Rosenkränzer method, that is, 27 g. of ferric chloride hexahydrate is dissolved in 500 cc. of concd. hydrochloric acid and the solution is diluted to a total volume of 1000 cc.

4. Two per cent. and 0.6% zinc acetate solutions. A quantity of each solution sufficient for the standards and for several determinations may be conveniently prepared as required by dilution of a stock solution (about 20%) made up by treating 135 g. of glacial acetic acid with a slight excess of an aqueous suspension of zinc oxide and bringing to 1000 cc. with water. After the dilution the solutions are filtered to remove undissolved zinc oxide and any basic zinc acetate which may have separated.

5. Carbon dioxide in a pressure cylinder.

6. Diphenyl ether.

Preparation of Standards.—Into each of 15 to 20 volumetric flasks of 500cc. capacity is placed 75 cc. of 2% zinc acetate solution. From 315 to 375 cc. of cool, recently boiled, distilled water (the exact amount being dependent upon the amount of hydrogen sulfide solution to be added subsequently) is added to the contents of each flask. The requisite amount of hydrogen sulfide solution B (see below) is then introduced, promptly followed, in order, by 25 cc. of the diamine reagent and 5 cc. of the 0.02 *M* ferric chloride solution. The volume of the standard hydrogen sulfide solution in each flask before addition of the diamine reagent should be about 450 cc. The temperature of the solutions in the flasks at the time of adding these reagents should be uniform, preferably not varying more than 0.5° from the mean in any flask. To be strictly comparable, the temperature of these solutions should be the same as that of the test solutions, at the time the hydrogen sulfide reagents are added to either; otherwise, the specific temperature at this stage is immaterial. For the addition of the larger quantities of sulfide solution pipets are satisfactory, but in the case of the smaller portions it is advisable to use a buret supplied with an elongated exit tube, which permits the tip to extend below the surface of the liquid in the flask. The solution is agitated after the addition of each of the three reagents by a gentle horizontal motion of the flask. After about two hours the solutions are diluted to the mark with water. Except in the case of

the first two or three of the weaker standards the content of hydrogen sulfide of no solution should be less than 70% of that in the one immediately above it in the series.

The hydrogen sulfide solution (B) employed in making the standards is prepared by dilution of a stronger solution (A) whose concentration is determined iodimetrically. To make Solution A, the hydrogen sulfide evolved from ferrous sulfide and hydrochloric acid is washed with water and passed slowly for one or two minutes into about 300 cc. of cool, boiled, distilled water. An aliquot portion of the resulting solution is added to an excess of a known amount of 0.01 *N* iodine solution and the mixture titrated with 0.01 *N* sodium thiosulfate solution. An amount of Solution A corresponding to about 30 cc. of 0.01 *N* solution is added to approximately 450 cc. of dil. zinc acetate solution (about 15 cc. of 2% zinc acetate solution plus about 435 cc. of water) in a 500cc. volumetric flask and the solution diluted to the mark with water. This Solution B, each cubic centimeter of which contains about 10 micromilligrams of hydrogen sulfide, is then used *immediately* for the preparation of the standards. A convenient set of standards may be obtained by using 1, 2, 3, 4, 5, 7, 10, 12.5, 15, 20, 25, 30, 40, 50 and 60cc. portions of Solution B.

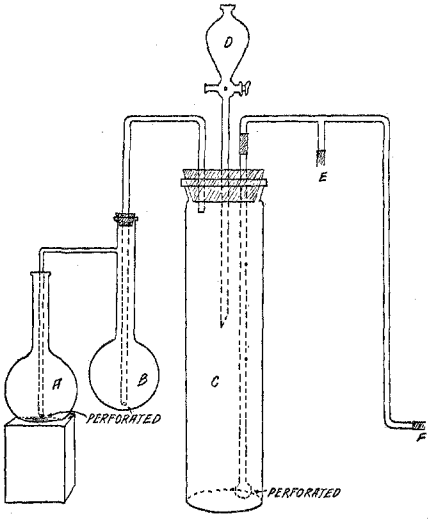

Fig. 1.—Apparatus used in the estimation of hydrogen sulfide in food products. *A*, 100 cc. volumetric flask. *B*, Round-bottom distilling flask of 80–100 cc. capacity. *C*, Aerating cylinder approximately 5 cm. in diameter and 35 cm. high. *D*, Dropping funnel of about 60cc. bulb capacity. *E*, Connection to manometer. *F*, Connection to carbon dioxide cylinder.

Procedure.—Twenty-five g. of the finely ground sample is added to 50 cc. of water in the tall cylinder (C), shown in Fig. 1. The 3-hole stopper carrying the dropping funnel is fitted to the cylinder and 50 cc. of 1:1 hydrochloric acid is placed in the funnel. The 100cc. volumetric flask (A) receives 20 cc. of 0.6% zinc acetate solution and the small distilling flask (B) receives 30 cc. of the same solution, after which they are connected as shown. The stopcock in the funnel is turned to allow the acid to run into the mixture, the flow being stopped in time to prevent the last few drops from leaving the bulb. Carbon dioxide is then passed through the train at a pressure of about 400 mm. of water, the aeration being continued at this pressure for 15 minutes. The carbon dioxide is then shut off, the

two receiving vessels are disconnected and the solution in the distilling flask is washed into the volumetric flask. The addition of the original 50 cc. of solution to the washings should bring the volume of the liquid to about 90 cc. This is then treated with 5 cc. of diamine reagent, followed by 1 cc. of the 0.02 *M* ferric chloride solution, the solution being gently agitated after each addition. After two hours the volume is brought to 100 cc. The solution is then compared in a colorimeter with the standard whose depth of color most closely approximates that of the test solution. The results may be expressed as parts per billion, micromilligrams per 100 g., or similarly convenient units.

When the zinc acetate solution after the aeration is markedly turbid, due to precipitated zinc sulfide, it probably contains a concentration of sulfide greater than that represented by the highest standard and must, therefore, be diluted before the test reagents are added. For this purpose the liquid is diluted to the mark with or without the previous addition of a small amount of dil. hydrochloric acid solution (to dissolve zinc sulfide in case it is in the form of a flocculent precipitate), an appropriate aliquot portion removed to another 100cc. volumetric flask containing 15 cc. of 2% zinc acetate solution and the resulting mixture diluted and treated as above described.

Foaming may be minimized or entirely prevented by the use of a few drops of diphenyl ether. If this treatment fails to control the foaming, as might be true in the case of some albuminous materials such as egg products, the addition of about 2 cc. of a 40% solution of sodium tungstate may be found to be efficacious.

As the flow of gas through the valves of some carbon dioxide cylinders is a trifle uneven, the use of a manometer filled with a solution heavier than water is recommended. The author employed a zinc chloride solution, whose specific gravity was 2. A reading with such a solution is one-half that when water is used.

The possibility of rubber stoppers and connections affecting the results is slight, as the method is carried out at room temperature and the apparatus is so designed that a minimum area of rubber is exposed to the current of gas. However, as a matter of precaution all rubber fittings should be thoroughly cleansed before use. Although many trials were made, in no instance has a measurable blank been obtained. These tests also showed that it is not necessary to wash the incoming carbon dioxide for the removal of hydrogen sulfide.

The solution of *p*-amino-dimethylaniline in 1:1 hydrochloric acid has been found to be just as effective after standing 24 hours as it is at the time of preparation. There is no advantage in using this reagent in the dry form, nor in adding it and the hydrochloric acid separately to the test solution, procedures which merely tend to complicate the technique.

Experimental Part

The standard solutions, if kept in a cool and dark place, will remain unaltered for four or five weeks and perhaps longer. At room temperature (about 25°), however, a change in the color of the more dilute solutions to a greenish tint is apparent after about three weeks, the change becoming more pronounced as time goes on. In the initial stages this alteration does not appear to affect the reliability of the standards representing about 140 mmg. of hydrogen sulfide per liter and above, as the change, if at all detectable, seems to be limited to a slight variation in the shade rather than in the total color.

The only difference in the treatment of the standards and of the test solutions, as outlined in the method, is in respect to the aeration with carbon dioxide. It was found that carbon dioxide treatment of zinc acetate solutions containing zinc sulfide does not affect the color value of the solutions upon subsequent addition of the color reagents, except in the case of those solutions representing less than 70 mmg. of hydrogen sulfide per liter. In the case of these dilute solutions the color value was sometimes affected and at other times not, the change, if any, resulting in a loss of not more than 8 or 10% in the color values. As these dilute solutions are the ones which are relatively unimportant on account of the difficulty encountered in making accurate readings with them in the colorimeter and as the efficacy of more concentrated solutions is not affected, the treatment of the standards with carbon dioxide at any stage in their preparation is considered unnecessary.

Using a 25g. sample, the hydrogen sulfide may, therefore, be estimated quantitatively when present in the sample in the proportion of about 28 mmg. per 100 g. and higher. When containing smaller amounts of hydrogen sulfide, the method gives approximately quantitative results down to about 8 mmg. per 100 g.

It was found by repeated trials that methyl and ethyl sulfides and ethyl mercaptan do not interfere in the test. Identical results were obtained when the method was run on samples with or without the addition of these substances. When the hydrogen sulfide reagents are added directly to pure solutions of them, however, a pink color is produced which may later change to a yellowish or slightly greenish shade. The lack of interference of these related sulfur compounds in the test is obviously due to the fact that they are not absorbed by the zinc acetate solution.

The zinc acetate solution absorbs the hydrogen sulfide quantitatively at the rate of aeration specified, all of the gas under ordinary conditions being retained in the first flask, the second flask serving to catch any liquid carried over to it mechanically. Once the gas has reacted with the zinc acetate to give zinc sulfide, further aeration within reasonable limits does not alter the sulfide content of the receiving solution as determined colori-

metrically. This point was settled by aerating, for 15 minutes, zinc acetate solutions containing definite amounts of zinc sulfide and then comparing the aerated sulfide solutions with zinc acetate solutions to which the same amounts of zinc sulfide had been added after aeration.

The following experiment illustrates the accuracy of the method in transferring sulfide sulfur from one medium to another. A 25g. sample of canned salmon[8] was analyzed and found to contain 10 mmg. of hydrogen sulfide. At the time of making this test a second was made with another sample of the same salmon, to which had been added 30 mmg. of hydrogen sulfide. The amount of hydrogen sulfide found in the second test was 39 mmg. instead of 40, indicating practically 100% recovery.

Kind and Strength of Acid.—In the preliminary work phosphoric acid was employed to release the hydrogen sulfide and hold back basic substances. This acid is recommended by Fellers, Shostrom and Clark.[9] For reasons which will be apparent later, it was found necessary to substitute hydrochloric acid for the phosphoric. In order to spare detail, many of the early results with the latter acid will be omitted and the description confined to a brief comparison of the performance of the two acids as subsequently determined.

As it was the intention to develop a method with as broad an application as possible, examination was made of canned, as well as raw products. Canned salmon was used as an example of the former, and raw fish, beef, and pork as types of the latter. On varying the strength of acid added to the aqueous suspension of 25 g. of canned salmon from 4 to 19% in the case of hydrochloric acid and from 10 to 41% in the case of sirupy phosphoric acid, it was found (Table I) that the 34.5% phosphoric acid liberated approximately as much hydrogen sulfide from the salmon as the

TABLE I

VARIATION IN THE STRENGTH OF ACID

Canned salmon; 50 cc. of acid added to mixture of sample with 50 cc. of water

Sample 1. Aeration period, 20 minutes				
Concn. of acid (HCl), %.....	4.0	10.0	15.5	19.0
H_2S, mmg. per 100 g.....	128	188	232	276
Sample 2. Aeration period, 15 minutes				
Concn. of acid (HCl), %.....	10.0	19.0	27.5	35.5
H_2S, mmg. per 100 g.....	252	372	388	383
Sample 3. Aeration period, 20 minutes				
Concn. of acid (H_3PO_4), %.....	10.0	22.5	34.5	41.0
H_2S, mmg. per 100 g.....	160	200	216	228

[8] Until further study has been made, it is not possible to attribute any special significance to the various proportions of hydrogen sulfide found in the canned salmon examined in the course of this investigation.

[9] Fellers, Shostrom and Clark, *J. Bacteriol.*, **9**, 238 (1924).

41%, which suggests that the 34.5% acid under these conditions gives about the maximum effective acidity possible with phosphoric acid. Apparently, there would be no gain in using more concentrated solutions nor would such a procedure appear practical. Increasing amounts of hydrogen sulfide were obtained with hydrochloric acid, by increasing the concentration to as high as 19% with no indication that a maximum had been reached. The tests with more concentrated solutions of hydrochloric acid, however, show that very little, if any, more hydrogen sulfide is obtained with percentages of acid greater than 19.

A large number of determinations were made in which the efficacy of 19% hydrochloric acid solution was compared with that of 34.5% phosphoric acid solution in liberating hydrogen sulfide from portions of the same samples of raw and canned flesh. The two acids gave about the same yields of hydrogen sulfide from raw flesh, but in the case of canned flesh (salmon) the hydrochloric acid resulted in a much higher yield. The reason for this is unknown.

For general purposes, therefore, 19% hydrochloric acid (1:1) is to be preferred to 34.5% phosphoric acid.

Time of Aeration.—When the proposed method was applied to raw and canned salmon, employing aeration periods of increasing duration, it was found (Table II) that no measurable amount of hydrogen sulfide came over after the first 15 minutes. Approximately 200 determinations of 15 minutes' duration each can be made with a cylinder containing initially 23 kg. (50 pounds) of carbon dioxide.

TABLE II

TIME OF AERATION OF SAMPLES OF SALMON

19 PER CENT. HYDROCHLORIC ACID

	Decomposing, raw		Canned, No. 4				Canned, No. 5			
Aeration, min.	15	30	3	7	15	120	3	7	15	120
H_2S, mmg. per 100 g.	72	68	268	304	312	312	264	316	328	328

Examples of the Use of the Method.—To test the method, determinations were made on raw fish, beef and pork at intervals during holding at known temperatures. The beef and pork were from animals which had been slaughtered three hours prior to the analysis, the beef being from the neck and the pork from the belly wall. The flesh of the different fish at the time of the first analysis, with the exception of the kingfish, was in prime condition from the standpoint of edibility. Information in regard to the time the fish had been held in ice between capture and analysis was obtained from the wholesale dealer and is presumed to be approximately correct. The experiments (Table III) showed that the proportion of hydrogen sulfide increased with the time the products were held and was high when putrefaction was indicated by the odor.

TABLE III

HYDROGEN SULFIDE IN FRESH AND DECOMPOSING FLESH PRODUCTS (RAW)

Product		Length of experimental holding period	Temperature at which held	Hydrogen sulfide	
Kind	History	Hours	°C.	Mmg. per 100 g.	Remarks
Beef	Strictly fresh lean meat	0	.....	48	....
		24	about 25	52	No odor of putrefaction
		48	about 25	716	Vile, putrid odor
Pork	Strictly fresh—fatty tissue removed	0	.....	52	....
		24	about 25	200	Rancid odor
		48	about 25	656	Vile, putrid odor
Weakfish (*Cynoscion regalis*)	Caught in Potomac River one day before test	0	.....	0	....
		24	about 25	0	....
		48	about 25	41	Slight putrid odor
Butterfish (*Poronotus triacanthus*)	Caught near Old Point Comfort, Va., one day before test	0	.....	5	....
		24	about 25	42	Slight odor of decomposition
		48	about 25	378	Vile, putrid odor
Spotted Sea Trout (*Cynoscion nebulosus*)	Caught near Beaufort, N. C., about one day before test	0	.....	0	....
		18	31	trace	An "off" odor, not putrid
		24	31	64	A distinctly sour odor
		42	31	2200	Very offensive odor
Kingfish (*Menticirrhus americanus*)	Caught near Beaufort, N. C., two days before test	24	31	48	Obviously spoiled, but odor not vile

Applications

Chemical criteria of food spoilage have been sought for years and much has already been accomplished. We have methods for the estimation of such decomposition products as ammonia, amines, amino acids, volatile acids, indole, etc. The type of end-products resulting from the action of putrefactive organisms depends upon the character of the flora concerned. It is logical to assume that the chemical detection of spoilage, in the case of most products, would require the use of more than one method. It is quite possible, therefore, that the proposed method for the estimation of hydrogen sulfide may be of supplemental value in determining the sanitary quality of certain foods.

The method is applicable to the examination of sewage and may also be used in the analysis of mineral waters. This latter application has recently been suggested by Heath and Lee[10] who state that "In order to eliminate errors due to the action of nitrites, nitrates and alkali salts, we suggest that natural waters be tested for hydrogen sulfide by the colorimetric method of W. Mecklenburg and F. Rosenkränzer in which methylene blue is formed." These authors, however, report no results in which this method is used.

[10] Heath and Lee, THIS JOURNAL, **45**, 1643 (1923).

Further work is planned involving the adaptation of the method to the study of the formation of hydrogen sulfide by bacteria in various prepared culture media. It should be possible to secure some interesting quantitative data on this subject.

Summary

A method for the estimation of hydrogen sulfide in food products which comprises the following features has been developed.

1. Removal of sulfide sulfur from the material by a current of carbon dioxide acting on an aqueous acidified suspension of the product.

2. Absorption of the evolved hydrogen sulfide in dilute zinc acetate solution.

3. Evaluation of the sulfide content of the absorbing solution, by a comparison with standards of the "methylene blue" color produced by interaction of the hydrogen sulfide with *p*-amino-dimethylaniline, hydrochloric acid and ferric chloride, according to the well-known Lauth's reaction.

Analyses of beef, pork and fish showed that hydrogen sulfide was formed progressively during the putrefaction of these products.

WASHINGTON, D. C.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, YALE UNIVERSITY]

THE UTILIZATION OF PARA-TOLUIDINE IN THE SYNTHESIS OF AROMATIC ESTERS OF THE NOVOCAINE TYPE[1]

BY MAGNUS A. SODERMAN AND TREAT B. JOHNSON

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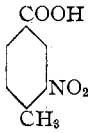
This paper is a report of an investigation in the field of anesthetics, and contains data contributing to our knowledge of the relationship between chemical constitution and pharmacological action. The research was developed around the aromatic base *p*-toluidine, and by application of new syntheses and improvement in the technique of known reactions several new combinations have been prepared which promise to be of immediate physiological and pharmacological interest. The derivatives of major interest are some new esters of aromatic acids prepared from diethyl-amino-ethyl alcohol, $(C_2H_5)_2N.CH_2CH_2.OH$.

The two aromatic acids selected as the basis of our work were 2-nitro-4-methylbenzoic acid I and nitroterephthalic acid II.



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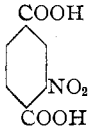

II

[1] Constructed from part of a dissertation presented by Magnus A. Soderman to the Faculty of the Graduate School of Yale University, June, 1922, in candidacy for the degree of Doctor of Philosophy.