minutes in acetic anhydride. The acetylated product was obtained by precipitating with water. It dried to a semi-crystalline, reddish powder, insoluble in alkalies.

Condensation of 9,10-Diphenic-ketone-4-carboxylic Acid with Resorcinol. Formation of 2′,7′-Dihydroxy-*spiro*(9-fluorene-9′-xanthene)4-carboxylic Acid

Fifteen g. of the ketonic acid, 16 g. of resorcinol and 25 g. of fused zinc chloride[9] were heated 5 hours at 115–120°. The melt was boiled with hydrochloric acid, dissolved in dil. caustic soda solution and precipitated with hydrochloric acid. The product so obtained was not so sticky as that from the anhydride. It was dissolved in boiling alcohol and reprecipitated with water. The resinous solid was dried at 105° and powdered. Its alkaline solution was brownish-yellow and had a green fluorescence; 22.17% of silver was found in the silver salt. A sample was acetylated at 135° for 2 hours, as in the case of the condensation product from the diphenic anhydride. The product was soluble in alkali.

Summary

Diphenic anhydride has been condensed with resorcinol to form a compound similar in structure and properties to fluorescein. The condensation product was brominated to give a compound analagous to eosin.

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THE DETERMINATION OF SULFUR IN ORGANIC COMPOUNDS[1]

By Walter F. Hoffman and Ross Aiken Gortner

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The quantitative determination of sulfur in organic compounds is based on the oxidation of the sulfur to sulfate, under such conditions that the remainder of the molecule is completely decomposed. The sulfate is then precipitated as barium sulfate. In carrying out this oxidation two methods are, in general, used by the organic chemist, the Carius and the peroxide fusion methods. Besides these there is a third method which is occasionally employed, consisting in burning the compound in a stream of oxygen.

The Carius method quantitatively oxidizes the sulfur in all but a very few compounds. It is, however, a difficult determination and requires the technique of a trained chemist to yield satisfactory results. The peroxide fusion method is disagreeable, demands extreme care and is not quantitative for any volatile substance or for a compound that sublimes unless the oxidation is carried out in a calorimetric bomb as described by v. Konek.[2]

The biochemists have developed a number of methods for the determination of total sulfur in body fluids, especially urine. These are likewise based on the oxidation of the sulfur to the sulfate. One of the most

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[9] Ref. 1. Ref. 2, p. 288.

[1] Published with the approval of the Director as Paper No. 363, Journal Series of the Minnesota Agricultural Experiment Station.

[2] v. Konek, *Z. angew. Chem.*, **16**, 518 (1903).

widely used is that of Benedict[3] as modified by Denis.[4] It occurred to us that this method might be advantageously applied to the determination of sulfur in organic compounds, inasmuch as it requires neither special equipment nor exceptional skill.

The procedure which we have adopted is as follows. 0.1000–0.2000 g. of the sample, depending on the sulfur content, is placed in an 11cm. porcelain evaporating dish and dissolved in an appropriate solvent, such as alcohol, acetic acid or nitric acid, etc.

It is absolutely necessary to have the compound in solution so that it is in a finely divided state and intimately mixed with the copper nitrate when ignited. The solvent used has no effect on the results of the determination provided it contains no sulfur or other compounds that would be weighed as barium sulfate.

Ten cc. of the Benedict-Denis reagent,[5] which is sufficient for a sample as large as 0.5000 g., is added to the contents of the evaporating dish. This mixture is then evaporated on a water-bath to approximate dryness.[6] The contents of the dish are *carefully* ignited, at first over a small flame and finally to dull redness for a few minutes, thus destroying the last traces of organic matter and converting all of the copper nitrate to copper oxide. After the dish cools, the contents are dissolved, with the aid of heat, in dil. hydrochloric acid and the sulfate is precipitated and weighed in the usual manner as barium sulfate.

Inasmuch as the reagents always contain traces of sulfur or sulfates it is necessary to make a "blank" determination on the reagent and to subtract the sulfate so obtained from the total weight of barium sulfate. With the "analytical" grade of chemicals we usually obtain a blank of 2.5 to 3.5 mg. of barium sulfate for 10 cc. of the reagent, but have occasionally obtained chemicals which gave only 1.0 mg. as a blank. It is undesirable to have a blank of more than 4.0 mg. for 10 cc. of the reagent, especially when the determination is to be made on a small amount of a substance of low sulfur content. We have obtained satisfactory results when the samples gave as low as 0.0400–0.0500 g. of barium sulfate, but it is more satisfactory to use enough of the sample to give 0.1000–0.2000 g. The difference between duplicate determinations is very small and should not exceed 2 mg. of barium sulfate.

To ascertain whether this method is adapted to most types of sulfur-containing compounds, the sulfur contents of a number of organic compounds were determined. It is evident from the nature of the procedure

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[3] Benedict, *J. Biol. Chem.*, **6**, 363 (1909).

[4] Denis, *ibid.*, **8**, 401 (1910).

[5] This reagent is prepared by dissolving 25 g. of crystalline copper nitrate, 25 g. of sodium chloride and 10 g. of ammonium nitrate in enough water to make 100 cc.

[6] We have found that when the dishes are removed from the water-bath before they are completely dry and the ignition carried out in a short time, very little, if any, spattering occurs.

that volatile compounds or compounds which sublime would probably not be completely oxidized.

The following compounds presented no difficulty and the theoretical sulfur content was obtained in the first trial: *l*-cystine dissolved in dil. hydrochloric acid; benzoyl-*l*-cystine dissolved in dil. alcohol; keratin[7] (human hair) dissolved in conc. hydrochloric acid; and benzyl-*i*-cystein; *l*-cysteic acid; thio-urea; *d*-camphor-sulfonic acid; sulfanilic acid; sodium phenolsulfonate; sodium benzylsulfonate; thiobarbituric acid; "thiocol" 2-methoxy-3-hydroxy-potassium benzene sulfonate; and saccharin dissolved in water.

In the case of *β*-naphthalene-sulfonamide, it was necessary to heat the material with 10–15 cc. of conc. nitric acid until it was completely dissolved. Diphenyl-thio-urea, although soluble in alcohol, presented certain difficulties. When the alcohol is evaporated, the diphenyl-thio-urea formed a gummy mass on the sides of the dish and much agitation was required to secure a good mixture of the compound with the oxidizing reagent on evaporation. Out of four determinations only two satisfactory results were obtained. Tribromo-thiophenol presented a similar difficulty.

Phenolsulfonephthalein is very insoluble in water or alcohol and had to be dissolved in sodium hydroxide solution. Only when an excess of sodium hydroxide was added were we able to obtain the theoretical value. The presence of a moderate excess of alkali apparently has no effect on the accuracy of the method. Thiophthalic anhydride gave low results due to the volatile nature of the compound. Both alcohol and sodium hydroxide were used as solvent, but in each case some of the compound was lost during evaporation and ignition, crystals being observed on a watch glass covering the evaporating dish. The highest value obtained was 14.24% (calc., 19.56%).

Sulfonal and trional were the only compounds from which no sulfate was obtained. This we believe to be due to their physical properties rather than to the type of sulfur linkage. Both are lost by volatilization before the nitrates are decomposed by heat.

The values for total sulfur in proteins, as determined by this method, agree very well with those obtained by peroxide fusion. The solvent for proteins is boiling hydrochloric acid, and aliquot portions were taken for the sulfur determination. Satisfactory results are obtained by using proteins containing no more than 0.75% of sulfur but it is necessary to employ about 1 g. of protein and 20 cc. of the oxidizing reagent.

Summary

The Benedict-Denis method for determining sulfur in urine may be used to determine sulfur in almost all kinds of organic compounds. The failure

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[7] Theoretical sulfur obtained by peroxide fusion.

of this method to oxidize the sulfur quantitatively in all organic compounds is apparently not due to any particular type of sulfur linkage but rather to the physical properties of the compound. It will not give accurate results if the compound is volatile or sublimes. We believe that for all other organic compounds, it will serve as a simple, rapid and accurate method for determining sulfur.

SAINT PAUL, MINNESOTA

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF DACCA]

RING CLOSURE OF HYDRAZODITHIO- AND -MONOTHIO-DICARBONAMIDES WITH ACETIC ANHYDRIDE

BY PRAPHULLA CHANDRA GUHA

Received January 8, 1923

Ring closure of hydrazodithio-dicarbonamide was first effected by Freund and Wischewiansky[1] by carbonyl chloride in toluene solution, and they assigned a triazole formula to the resulting compound. Later, Freund and his co-workers effected ring closure of the same hydrazo compound with hydrochloric acid and obtained two compounds, to both of which they assigned triazole formulas, one being dithio-urazole and the other iminothio-urazole. The constitution of the former has been established by the present author to be 5-amino-2-thiol-1,3,4-thiodiazole.[2] Arndt and Milde[3] found fault with the constitution of Freund and Imgart's iminothio-urazole and they have been successful in synthesizing the *real* iminothio-urazole by the action of very strong potassium hydroxide solution on the above hydrazo compound. Emil Fromm with E. Kayser, K. Briegleb and E. Fohrenbach[4] arrived at the conclusion that Freund and Imgart's iminothio-urazole is 2,5-diamino-1,3,4-thiodiazole.

With the object of studying the behavior of various organic acids and their anhydrides as ring-closing reagents towards hydrazodithio-dicarbonamide and its derivatives, the present investigation was undertaken. The action of acetic anhydride has been found to be very interesting in the sense that it not only brings about a closure of the ring when 2,5-di-imino-2,3,4,5-tetrahydro-1,3,4-thiodiazole is formed with the splitting up of 1 molecule of hydrogen sulfide, but this thiodiazole compound is also acetylated thus,

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[1] Freund and Wischewiansky, *Ber.*, **26**, 2877 (1893).

[2] Guha, THIS JOURNAL, **44**, 1502 (1922).

[3] Arndt and Milde, *Ber.*, **54**, 2089 (1921).

[4] Fromm, Kayser, Briegleb and Fohrenbach, *Ann.*, **426**, 313 (1922).