of sodium hypochlorite, prepared as described above. The reaction mixture was shaken occasionally during a period of two weeks, and was finally heated for about an hour on a steam-bath. After cooling, the precipitate was filtered and recrystallized from alcohol. It separated in long needles which proved to be identical with those prepared by the other method. The yield was 2 g. or 70% of the theoretical. A mixture of these crystals with a sample of α,α,α-trichloro-2,4,6-trimethyl-3,5-dinitro-acetophenone (prepared by the first method) showed no lowering of the melting point.

Summary

1. The cleavage of the carbon chain of carbonyl compounds under the influence of alkali is ascribed to the coördination of the carbonyl carbon atom with hydroxyl. It is assumed that the polarity of the bond between the carbonyl carbon atom and the α-carbon atom is thus enhanced. In case the substituents on the α-carbon atom are sufficiently "negative" dissociation occurs.

2. It is pointed out that, according to this theory, certain trihalomethyl ketones whose carbonyl groups do not undergo addition reactions should be stable to alkali.

3. Several such trihalomethyl ketones have been prepared and found to be unaffected by alkali.

4. These results strongly support the assumption that chain cleavage of this type is dependent on an *addition* reaction of the carbonyl group. That this is the addition of hydroxyl, as postulated by the theory here developed, seems probable.

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THE ESTIMATION OF SULFUR IN ORGANIC COMPOUNDS[1]

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In 1923 Hoffman and Gortner[2] drew attention to the applicability to organic compounds of the method for estimating sulfur in biological material devised by Benedict[3] and modified by Denis.[4] This procedure which, in the hands of the biological chemist, has practically superseded the methods of Carius and of alkaline fusion, tends to give rise to mild explosions under conditions which cannot always be controlled. In the method described below this disadvantage is almost entirely eliminated by observing suitable precautions.

The process consists in the oxidation of organic matter by means of

[1] This work was aided by the Research Grant from the Chemical Foundation to the Department of Biological Chemistry.

[2] Hoffman and Gortner, THIS JOURNAL, **45**, 1033 (1923).

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

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

nitric acid in the presence of potassium nitrate, followed by evaporation of the nitric acid and fusion of the residue, whereby organic sulfonates are converted into sulfate. The reaction is carried out in an open vessel capable of withstanding the action of the fused nitrate. The latter is decomposed by warming with hydrochloric acid and after again evaporating to dryness the residue is dissolved and treated with barium chloride.

The method, which has proved well adapted to micro-analysis, is applicable to all types of substance except volatile sulfones or compounds (such as alkyl sulfides) capable of yielding them. This limitation applies also to the Benedict–Denis method, and it may be noted that while sulfonal is reported by Hoffman and Gortner[2] as yielding no sulfate at all by the latter process, we have been able to recover 60–70% of the sulfur in sulfonal as barium sulfate. The process here described thus appears to be somewhat more vigorous.

Experimental

The reaction vessel consists of a test-tube of pyrex glass[5] or, better, clear silica. The sample is introduced, followed by an amount of pure potassium nitrate or chlorate (preferably as a 5–10% aqueous solution) equivalent to at least 30 molecular proportions for every atomic proportion of sulfur anticipated. Concentrated (65–70%) nitric acid (1 cc. or more per decigram of sample) is added, and a small conical flask (or conical glass bulb) of suitable dimensions (Fig. 1) is suspended in the mouth of the reaction tube to serve as a reflux condenser. The tube is held in a clamp lined with asbestos paper at an angle of 45°, and the mixture is heated very gently over a micro-burner until the evolution of oxides of nitrogen practically ceases.

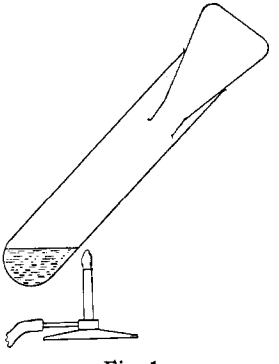

Fig. 1.

The burner is now shifted from a position directly under the liquid to one under the tube at a point just above the level of the liquid, and the flame is increased until it nearly reaches the wall of the vessel. The glass at this point is thus heated above the boiling temperature of the acid; a deposit of salts forms there, and in its superheated condition gradually undergoes a vigorous decomposition, while being continually subjected to the action of fresh nitric acid returning from the reflux condenser. The heating and the height of the tube above the flame are so regulated that a small proportion of the nitric acid vapor escapes without condensation. Oxalic acid and ammonium nitrate, if present, decompose and volatilize at this stage of the process. As the level of liquid in the tube falls, the tube is from time to time raised by sliding it through the jaws of the clamp. This part of the process, which occupies from one to three hours, requires a moment's attention every twenty minutes or so. During this time the vigorous secondary oxidation takes place almost completely, but by small degrees. Care must be taken not to evaporate so rapidly as to displace all of the brown oxides of nitrogen from the tube, for this may result in the removal of the bulk of the acid before oxidation is complete. In such a case the residue may deflagrate with mechanical loss of sulfate.

[5] If a wide, heavy-walled pyrex tube be employed, it should be annealed in a glass blower's flame before use.

When all the liquid has been evaporated, the lower part of the tube is heated with an ordinary Bunsen flame, kept constantly in motion, until the residual potassium nitrate first fuses, then effervesces and finally becomes quiescent. This part of the process, which requires less than a minute, is thus carried out in an atmosphere of nitric acid vapor. The melt is allowed to cool, is treated with a small quantity (0.5–1.0 cc.) of nitric acid, and is again heated, in order to complete the oxidation of any particles which may have escaped decomposition.

After the second fusion, the cold mass is treated with 0.5–2.0 cc. of concentrated hydrochloric acid and gently heated until no more red fumes are evolved. The reflux condenser is then removed, and heating continued very gently until the residue is completely dry. The last traces of acid adhering to the walls are volatilized by warming the whole tube gently with a large flame.

The salts are then dissolved in a suitable quantity of 0.2% hydrochloric acid, the solution is filtered or centrifuged if necessary, and the sulfate precipitated in the usual way with barium chloride. In the case of micro-determinations, this precipitation may very satisfactorily be conducted in a centrifuge-tube, the barium sulfate receiving its first wash by centrifuging and decantation.

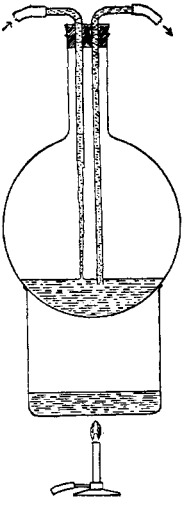

Fig. 2.

When the percentage of sulfur in the substance is extremely low (as for example in cereals), so that a relatively large sample has to be taken, the preliminary oxidation may advantageously be carried out in a pyrex beaker capable of containing about six times the volume of the reaction mixture. A round-bottomed flask containing cold running water, set in the mouth of the beaker, acts as a reflux condenser (Fig. 2). The mixture is heated over a small flame to gentle boiling for fifteen to twenty hours. At the end of this time very little oxidizable organic matter remains. The mixture is transferred to the reaction tube and the decomposition is completed in the manner above described, the nitric acid rinsings being added in the final stages.

This modification of the procedure is exemplified by the estimations of total sulfur in corn starch: in one case 17.9 g. was decomposed with 50 cc. of nitric acid and 5 cc. of 5% potassium chlorate, while in another, 5.153 g. of the starch was decomposed with 25 cc. of nitric acid and 5 cc. of 5% potassium chlorate solution. In such determinations it is necessary to correct for the small amount of sulfate present in the nitric acid which, in the present instance, yielded 0.51 mg. of barium sulfate per 100 cc.

That no loss of sulfate occurs during the process is evident from a series of determinations in which varying amounts of sodium sulfate were treated as above, employing 10 cc. of nitric acid with a mixture of 0.5 g. of sodium nitrate and 0.5 g. of potassium nitrate.

S taken as Na_2SO_4, mg.....	2.82	3.27	6.74	9.39	9.41	15.63	15.96	23.51
S found as $BaSO_4$, mg.....	2.81	3.30	6.91	9.31	9.53	15.75	16.18	23.61

Samples of a solution of sulfuric acid which gave 0.4465 and 0.4462 g. of barium sulfate by direct precipitation gave 0.4463 and 0.4455 g. of barium sulfate when treated as above.

An attempt was made to employ perchloric acid together with the nitric acid, but low results were obtained. It was found that when sodium sulfate is heated with boiling perchloric acid, an appreciable amount of sulfuric acid is volatilized: 2-cc. quantities of sodium sulfate solution which gave 0.1651 g. of barium sulfate by direct precipitation

were treated with 2-cc. portions of 70% perchloric acid and evaporated to dryness during two hours: barium sulfate recovered, 0.1428, 0.1438, 0.1472 g.; loss, 13.5, 12.9, 10.8%.

The following results were obtained with various organic and biological products.

Compound	Wt. of sample	$BaSO_4$	Sulfur, % Found	Calcd.
o-Benzoic Sulfinide	0.1407 g.	0.1810 g.	17.63	17.51
	0.2155 g.	0.2766 g.	17.59	
Cystine (EK)	0.2997 g.	0.5926 g.	27.12	26.69
	6.000 mg.	11.802 mg.	26.97	
Cystine (recrystallized)	0.1694 g.	0.3314 g.	26.78	
	0.0983 g.	0.1918 g.	26.76	
Diphenylsulfone	0.2033 g.	0.1064 g.	7.18	14.67
S-Ethylcysteine	0.1903 g.	0.2907 g.	20.96	21.46
	0.2088 g.	0.3168 g.	20.83	
	0.1183 g.	0.1793 g.	20.79	
Thiocarbanilide (EK)	0.3176 g.	0.3241 g.	14.01	14.05
	0.2233 g.	0.2270 g.	13.93	
	0.3314 g.	0.3369 g.	13.94	
p-Toluenesulfonamide	0.1014 g.	0.1417 g.	18.54	18.73
	0.0690 g.	0.0933 g.	18.55	
Sulfonal	0.1591 g.	0.2241 g.	19.3	28.07
	2.183 mg.	2.674 mg.	16.8	
Casein ("according to Hammarsten," Sample I)	1.3530 g.	0.0757 g.	0.77	
	1.0810 g.	0.0586 g.	0.74	
	1.7622 g.	0.1000 g.	0.78	
	1.1962 g.	0.0680 g.	0.78	
	1.1297 g.	0.0632 g.	0.77	
Casein ("according to Hammarsten," Sample II)	0.4884 g.	0.0257 g.	0.722	
	0.0747 g.	3.839 mg.	0.705	
	0.0625 g.	3.240 mg.	0.712	
Corn Starch (Duryea's)	17.9 g.	25.028 mg.	0.019	
	5.153 g.	6.750 mg.	0.018	
Egg Albumin (Commercial)	0.9948 g.	0.1151 g.	1.59	
	0.9512 g.	0.1123 g.	1.62	
	0.9963 g.	0.1187 g.	1.63	
	0.8076 g.	0.0942 g.	1.60	
Wool	0.1776 g.	40.168 mg.	3.10	
	0.0489 g.	10.325 mg.	2.90	

Urine.—The total sulfur in three different samples was determined, in 10-cc. portions, employing both the method here described, and that due to Benedict and Denis. In the former, the 10 cc. of urine was heated with 10 cc. of nitric acid and 1 g. of potassium nitrate. The results are reported in milligrams of sulfur:

Sample	A	B	C
Present method	4.3	8.5	6.7
Benedict-Denis	4.3	9.1	6.9

Summary

1. Sulfur in organic combination can be estimated with reasonable accuracy as sulfate after oxidizing by fusion with potassium nitrate in presence of nitric acid.

2. The method fails in the case of volatile sulfones and compounds from which such sulfones are produced by the action of nitric acid.

3. Reproducible results can be obtained with biological material.

4. The process is adaptable to the estimation of sulfur in materials containing as little as 0.02%.

NEW YORK, N. Y.

[CONTRIBUTION FROM THE LABORATORY OF PHYSICAL CHEMISTRY OF THE UNIVERSITY OF UPSALA]

THE MOLECULAR WEIGHT OF LEGUMIN

BY BERTIL SJÖGREN AND THE SVEDBERG

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In recent communications from this Laboratory determinations of the molecular weights and of the *P*H-stability regions of some oil seed globulins, *viz.*, edestin,[1] amandin and excelsin[2] by means of the ultracentrifugal method, have been given. These proteins as well as the three other vegetable proteins, R-Phycocyan, R-Phycoerythrin, C-Phycocyan, so far studied with the ultracentrifuge, were all found to possess a molecular weight of 208,000, which is approximately six times the weight of egg albumin. Their molecules are spherical with a radius of 3.95$\mu\mu$. The stability regions are different for the different proteins but they all show a tendency to dissociate into lower multiples of 34,500 with increasing *P*H. It was thought to be of interest to study also some representative of the proteins of the *Leguminosae* family by means of the ultracentrifuge analysis. Legumin from vetch (*Vicia sativa*) was chosen. According to the investigations of Osborne[3,4] the vetch contains only one globulin, legumin and one albumin, legumelin, while the pea and the horse bean contain legumin, legumelin and one more globulin, the vicilin. Whether the legumins extracted from the various *Leguminosae* seeds are identical is not quite clear. We have not so far made any attempt to answer this question.

Preparation of Material.—Two thousand grams of vetch flour was divided in two equal portions and each of them stirred at room temperature during twenty-four hours with 4000 cc. of 10% sodium chloride solution.[5] Enough toluene to form a layer about 1 cm. thick was added. It served the double purpose of dissolving the fat and preventing bacterial growth. The bulk of the insoluble part was removed by means of a cloth filter and the rest by centrifuging. Most of the fat had been taken up by the toluene.

[1] T. Svedberg and A. T. Stamm, THIS JOURNAL, **51**, 2170 (1929).

[2] T. Svedberg and B. Sjögren, *ibid.*, **52**, 279 (1930).

[3] T. B. Osborne and G. F. Campbell, *ibid.*, **18**, 583 (1896); **20**, 406, 410 (1898).

[4] T. B. Osborne, "Vegetable Proteins," Longmans, Green and Co., London, **1916.**

[5] The grinding of the vetch seed was kindly done for us in an experimental mill at the laboratory of Upsala Ångkvarn.