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USE OF HEAVY METAL SALTS IN THE ANALYSIS OF ORGANIC SULPHUR COMPOUNDS

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SUMMARY

The possibilities of using heavy metal salts for the analysis of sulphur compounds were investigated. A method for regenerating the products of the reaction between sulphur compounds and mercuric chloride is suggested, which permits the quantitative regeneration of sulphides and mercaptans and 50% regeneration of disulphides. The procedure described was used for investigating the volatile products which arise from the Maillard reaction and which have a smell similar to cooked beef.

INTRODUCTION

Reaction gas chromatographic (GC) methods have been widely applied in the analysis of complex mixtures in which the components are compounds of different classes. The condensates of food flavours belong to such complex mixtures of previously unknown composition, comprising several hundreds of compounds. Therefore, great care needs to be exercised in selecting the chemical reactions to be used for concentrating the components and for subsequent regeneration in order to avoid mistakes in the identification of the components. In the latter case, the quantitative estimation of the reactions chosen is of great importance. As our experience has shown, reactions which are considered in the literature to be quantitative should not be used without a preliminary verification, especially if the results were obtained before the advent of the GC methods in chemistry.

In this paper, the importance of the verification of such a reaction is illustrated by flavour analysis for sulphur compounds in a condensate. As the sensitivity threshold of some organic sulphur compounds contained in a flavour condensate is several orders lower than the sensitivity limit of chromatographs, we had to use chemical reactions to isolate these compounds as solid derivatives and then apply such a regeneration method that would be quantitative and yet cause no change in the structure of the initial organic sulphur compounds.

It should be noted that many studies on the synthesis and properties of sulphur compounds have been reported in the literature, in particular in the series on divalent sulphur compounds by REID[1], a monograph by CHALLENGER[2], as well as in a number of original publications. Based on these studies, 20% $Pb(C_2H_3O_2)_2$, 4% $Hg(CN)_2$ and 3% $HgCl_2$ solutions are suggested for concentrating hydrogen sulphide, mercaptans and sulphides plus disulphides, respectively.

It is recommended that the regeneration is carried out with an acid or alkali. Such procedures have found wide application in the analysis of sulphur compounds in food flavours such as potato[3,4], milk[5], cheese[6] and chicken[7,8]. However, we decided to use GC for checking the above reactions and quantitatively estimating their suitability for isolating sulphur compounds. The investigation involved two stages. The first was the evaluation of the ability of heavy metal salts to extract and then regenerate mercaptans, as shown by the case with isopropyl mercaptan. In the second the possibility of isolating and regenerating the mixture of sulphides, mercaptans and disulphides was investigated on the model system *n*-butyl mercaptan–*n*-propyl sulphide–diethyl disulphide. The mixture was prepared so that all possible transformation products of these compounds were detectable on the chromatograms.

EXPERIMENTAL

Preparation of mercaptides from isopropyl mercaptan and heavy metal salts, and regeneration

Solutions of the following heavy metal salts in distilled water were prepared: 20% $Pb(C_2H_3O_2)_2$; 4% $Hg(CN)_2$; 3% $HgCl_2$; 50% $AgNO_3$; and 2% $AgC_2H_3O_2$. Isopropyl mercaptan (5 μl) was injected with a syringe below the liquid layer in a test-tube containing 1 ml of the solution of a salt. The precipitate in the test-tube was then centrifuged and the solution was removed by means of capillary tubes. The test-tube was closed with a ground-glass stopper provided with a tap carrying, at the end, a rubber cap through which 1 ml of chromatographically pure dodecane and 1 ml of concentrated hydrochloric acid (sp. gr. 1.18) were injected with a syringe. The regeneration was carried out based on procedures described elsewhere[2,9,10]. For the GC analysis, 1 μl of sulphur compounds in dodecane was injected with a Hamilton syringe into the chromatograph. The regeneration products are listed in Table I.

TABLE I

REGENERATION OF ISOPROPYL MERCAPTAN FROM HEAVY METAL MERCAPTIDES

Mercaptide-producing reagent	*Reaction time (min)*	*Regeneration products (%)*	
		Isopropyl mercaptan	*Diisopropyl disulphide*
$AgNO_3$	1	40	43
$AgC_2H_3O_2$	2	36	4
$Pb(C_2H_3O_2)_2$	180	44	2
$Hg(CN)_2$	180	36	9

We tried to regenerate mercury mercaptide with thiophenol. When the regeneration was carried out with thiophenol, 100 μl of thiophenol were added to 1 ml of dodecane solution covering the mercaptide precipitate, instead of hydrochloric acid, but the recovery of isopropyl mercaptan was only about 60%.

Precipitates produced from mercuric chloride and the mixture of n-butyl mercaptan, di-n-propyl sulphide and diethyl disulphide

To 4 ml of 3% aqueous mercuric chloride solution were added 50 μl of saturated aqueous sodium acetate solution, 4 μl of diethyl disulphide, 3.5 μl of di-*n*-propyl sulphide and 1.5 µl of *n*-butyl mercaptan. The precipitate was allowed to stand for 3 h, then it was centrifuged and the mercuric chloride solution above the precipitate was decanted with a capillary tube. A 2-ml volume of chromatographically pure dodecane was added to the precipitate and the test-tube was closed with a ground-glass stopper with a plug. Then 1 ml of concentrated hydrochloric acid (sp. gr. 1.18) was injected through the rubber cap on the plug. After vigorously shaking the precipitate, it dissolved at a rate dependent on the reaction temperature (Table II). A 2-µl dodecane sample was passed through the rubber cap and injected into the chromatograph. The chromatographic results are shown in Table II.

TABLE II

REGENERATION OF *n*-BUTYL MERCAPTAN, DI-*n*-PROPYL SULPHIDE AND DIETHYL DISULPHIDE FROM THE PRECIPITATE WITH MERCURIC CHLORIDE

Regeneration conditions	*Regeneration, (%)*			
	n-Butyl mercaptan	*Di-n-propyl sulphide*	*Diethyl disulphide*[a]	*Di-n-butyl disulphide*[b]
HCl; 20°; 40 min	38	92	28	13
HCl; 50°; 10 min	49	91	28	2
HCl; 60°; 8 min	81	92	17	2
HCl; 70°; 4 min	97	95	23	3
HCl; 70°; 4 min; $K_2C_2O_4$[c]	98	97	51	3
C_6H_5SH; 20°; 20 min	52	67	11	8
C_6H_5SH; 100°; 3 min	63	99	11	27

[a] Ethyl mercaptan was detected chromatographically as a decomposition product of diethyl sulphide and was not determined quantitatively at the analysis temperature.

[b] Di-*n*-butyl disulphide is a product of the oxidation of *n*-butyl mercaptan.

[c] In this experiment, aqueous potassium oxalate solution (100 µl) was introduced before adding the concentrated hydrochloric acid.

Precipitate formation from mercuric chloride and volatile products from the Maillard reaction and subsequent regeneration

A saturated solution of 2,4-dinitrophenylhydrazine in 5 *N* hydrochloric acid was added to 1 l of solution prepared by the Maillard reaction and having a smell similar to cooked beef; this resulted in no change in the flavour. Then the volatiles were isolated at room temperature under vacuum and trapped with a 3% solution of mercuric chloride in ethanol. The precipitate formed was centrifuged and dried, and 60–80 mg were obtained. A 25-mg amount of the precipitate was placed in a capillary tube of diameter 3 mm and length 150 mm, 100 µl of dodecane were injected and the capillary tube was frozen, followed by the addition of 100 µl of concentrated hydrochloric acid and 10 µl of 10% aqueous potassium oxalate solution and freezing again. The capillary tube was sealed and warmed to room temperature, and then after vigorous shaking it was heated in a water-bath at 70° for 4 min. The capillary tube was cooled again, opened and hermetically sealed with a silicone-rubber cap. The sample (8 µl) was removed through the cap with a syringe and injected into the chromatograph.

Calibrated solutions. An absolute calibration method was applied for the quantitative determination. The calibration solutions were prepared in chromatographically pure dodecane. The organic sulphur compounds were injected below the dodecane layer with a Hamilton syringe in the same amounts as those taken for the formation of precipitates from the salts of heavy metals.

Chromatographic conditions. A Yanagimoto GCG-5DH chromatograph with a double-flame detector (Tswett) and glass columns (1.5 m × 0.4 cm) packed with 10% of Apiezon M on Chromosorb W, 80–100 mesh was used.

The analysis of the regeneration products from isopropyl mercaptides of heavy metals was carried out with temperature-programming from 40° to 140° at the rate of 6°/min.

The GC analysis and identification of the products regenerated from the precipitate produced by the interaction of flavour condensate from the Maillard reaction and the mercuric chloride salt was carried out by an appropriate method described elsewhere[11]. The index values shown in Table III were used for the identification.

RESULTS AND DISCUSSION

The mercaptides that resulted from the interaction of isopropyl mercaptan with the heavy metal salts are listed in Table I. It can be seen that, in addition to the isopropyl mercaptan regenerated, its oxidation product, diisopropyl disulphide, is always present. When regenerating silver mercaptides, almost equal amounts of mercaptan and disulphide are produced. The presence of Ag^+ ions and nitric acid produced during the regeneration presumably contribute to the formation of disulphide from mercaptide. For this reason, silver nitrate was substituted for silver acetate, which reduced slightly the degree of mercaptan regeneration and decreased sharply the extent of disulphide formation. This can be explained by the formation of mercaptide complexes dissolved in the silver acetate used and by the losses of mercaptan when centrifuging the precipitate before the regeneration. It should be noted that, in the experiments with silver salts, the reaction with mercaptan is quantitative; this was proved by the complete disappearance of the characteristic mercaptan smell when injected below the salt solution layer. However, the regeneration does not proceed quantitatively (Table I).

Some interesting results have been obtained with lead acetate, which is generally used for the separation of hydrogen sulphide if it is assumed that mercaptans are not trapped in such cases. The results obtained have shown that isopropyl mercaptan gives lead mercaptide, although the formation of lead mercaptide does not proceed quantitatively. The regeneration yields about 45% of mercaptan and a small amount of disulphide, which is probably formed during regeneration (Table I).

Therefore, lead acetate should not be used for the separation of hydrogen sulphide from mercaptan mixtures, as inaccurate results might be obtained owing to the formation of lead mercaptides. A complete regeneration of mercaptan also cannot be achieved with mercuric cyanide (Table I), as the formation of soluble complexes of metal mercaptides in excess of hydrochloric acid accounts for the losses of mercaptan. For instance, silver mercaptide precipitate produced by the action of silver nitrate dissolved completely within 50 min. The same phenomenon is observed with mercaptide produced from mercuric chloride in that it can be dissolved by moderate heating at pH 1, resulting in the regeneration of 49% of mercaptans only (Table II). However, the detailed reasons for the non-quantitative regeneration of mercaptans from mercaptides of heavy metals require special investigation.

The conclusion that can be drawn from the results obtained is that the methods for the regeneration of heavy metal mercaptides recommended in the literature cannot be applied to the quantitative analysis of mercaptans in a mixture that has a complex composition. Futher investigations have been carried out with mercuric chloride as it gives complexes with sulphides and disulphides and mercaptides from mercaptans. The selection of the optimum conditions for the regeneration of the precipitates produced from the interaction between mercuric chloride and a mixture of mercaptans, sulphides and disulphides with concentrated hydrochloric acid was particularly studied.

Table II shows the regeneration conditions and compounds resulting from the decomposition of the products of reaction with *n*-butyl mercaptan, di-*n*-propyl sulphide and diethyl disulphide. In the first case, the regeneration conditions recommended in the literature were used: room temperature, hydrochloric acid, 40 min. The precipitate formed had completely dissolved within this period, however, and the regeneration yielded only 38% of mercaptan, 92% of sulphide and 28% of disulphide. These results are of particular interest because it is generally considered[2,9,10] that a disulphide complex with mercuric chloride disintegrates completely during regeneration owing to the rupture of the S–S bond and gives mercaptan and alkylsulphinic acid, which remains in the solution. The fact that the mercuric chloride complex can definitely be regenerated has been proved by us. The regeneration products have been found to include also 3% of dibutyl disulphide formed by the oxidation of butyl mercaptide.

By varying the reaction temperature and time, we succeeded in increasing the extent of regeneration of the initial organic sulphur compounds except for the disulphides. Then the regeneration was carried out in the presence of potassium oxalate as reducing agent, which was convenient to use as gaseous carbon dioxide and potassium chloride were produced from its interaction. In this case, the yields of mercaptan and

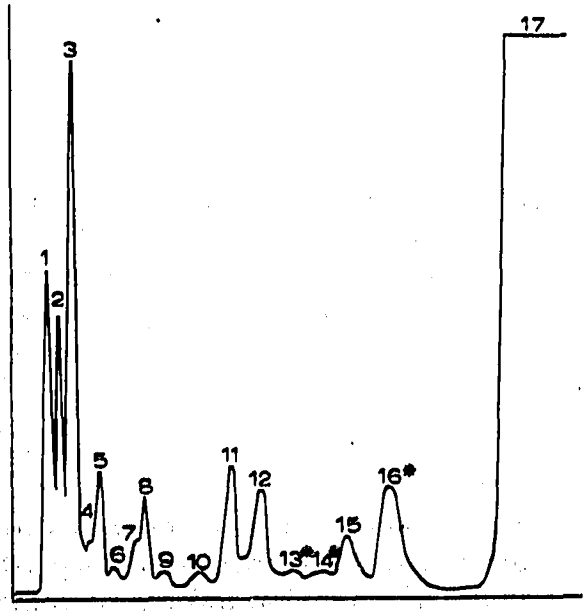

Fig. 1. Chromatogram of sulphur compounds isolated from the flavour condensate from the Maillard reaction on a column of Apiezon-M at 130°. For identification of peaks see Table V.

disulphide were in fact almost quantitative, while the disulphide regeneration amounted to 51% (Table II).

The results obtained were applied to the GC analysis of the cooked beef flavour that formed in the Maillard reaction. This reaction between amino acids and sugars is similar in mechanism to the production of a flavour by the heat treatment of food products. In our case, cysteine was the only sulphur compound contained in the initial mixture of the Maillard reaction. For example, Fig. 1 shows the chromatogram of the sulphur compounds isolated from the flavour condensate on a column of Apiezon-M at 130°. The right-hand peak (17) is due to the dodecane used as the solvent. The chromatogram in Fig. 2 was obtained using a PEG-1000 polar stationary phase at 60°.

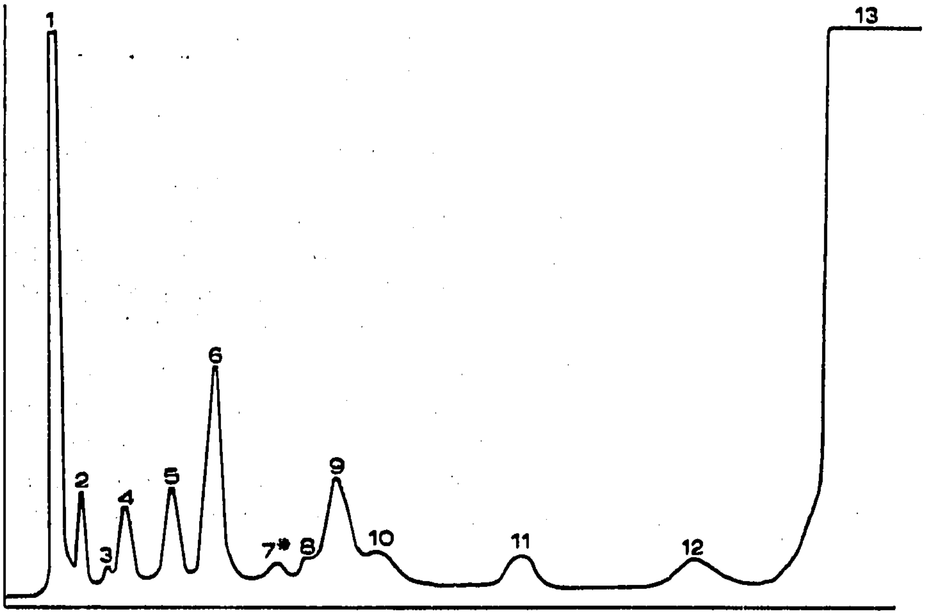

Fig. 2. Chromatogram of sulphur compounds isolated from the flavour condensate from the Maillard reaction using a PEG-1000 polar stationary phase at 60°. For identification of peaks see Table V.

The identification of sulphur compounds in the form of mercaptans, sulphides and disulphides was carried out by a GC method developed in our laboratory[11].

Using the procedure suggested by us[12] for the estimation of the selectivity of the stationary phase based on the difference between the excessive mixing energies calculated from index values by the equation:

$$\Delta\Delta F^E_{2,1} = -2.3\,RT\left[\log\left(\frac{P_2}{P_1}\right)+\frac{b\delta I}{100}\right]$$

(where $\Delta\Delta F^E_{2,1}$ is the difference in the partial molar excessive mixing energies at infinite dilution for the compounds 2 and 1; P_1 and P_2 are the saturated vapour pressures for compounds 1 and 2; δI is the difference in the retention indices of compounds 2 and 1; b is the constant of the equation $\log V_R = a + b_n$ for the n-paraffins used to calculate indices) a column system was chosen that enabled us to identify

TABLE III

EQUATIONS RELATING RETENTION INDICES WITH CARBON NUMBER (n) AND BOILING POINT OF SULPHUR COMPOUNDS

n-Mercaptans	*n-Sulphides*	*n-Disulphides*
$I_{60}^{\mathrm{Ap}} = 100n + 315$	$I_{60}^{\mathrm{Ap}} = 87n + 369$	
$I_{60}^{\mathrm{PEG\text{-}1000}} = 98n + 543$	$I_{60}^{\mathrm{PEG\text{-}1000}} = 80n + 584$	
$I_{130}^{\mathrm{Ap}} = 101n + 324$	$I_{130}^{\mathrm{Ap}} = 98n + 301$	$I_{130}^{\mathrm{Ap}} = 90n + 578$
$I_{130}^{\mathrm{Triton}} = 100n + 519$	$I_{130}^{\mathrm{Triton}} = 96n + 476$	$I_{130}^{\mathrm{Triton}} = 85n + 836$
B.p. (°C) = $(0.26\ I_{60}^{\mathrm{Ap}} - 88)$	B.p. (°C) = $(0.23\ I_{130}^{\mathrm{Ap}} - 64)$	

TABLE IV

RETENTION INDICES OF SOME CYCLIC SULPHUR COMPOUNDS

Compound	I_{60}^{Ap}	$I_{60}^{PEG\text{-}1000}$	I_{130}^{Ap}	$I_{130}^{PEG\text{-}20000}$	I_{130}^{Triton}	$\Delta I_{130}^{PEG\text{-}20000-Ap}$
Thiophane	807	1097	834	1168	1080	334
Thiophene	672	1017	702	1054	961	352
Thiophenol			1002	1525	1411	523
3,5-Dimethyl-1,2,4-trithiolan			1158	1535	1449	377
"*b*"	0.390	0.322	0.266	0.210	0.224	

TABLE V

SULPHUR COMPOUNDS IN VOLATILES FROM THE MAILLARD REACTION

Peak No. in		*Compound*	*Peak No. in*		*Compound*
Fig. 1	*Fig. 2*		*Fig. 1*	*Fig. 2*	
—	—	Hydrogen sulphide	3	5	Diethyl sulphide
1	1	Methyl mercaptan	7	11	Di-*n*-propyl sulphide
1	2	Ethyl mercaptan	5	6	Diisopropyl sulphide
2	4	*n*-Propyl mercaptan	15	—	Di-*n*-butyl sulphide[a]
2	3	Isopropyl mercaptan	8	11	Di-*tert.*-butyl sulphide
4	6	*n*-Butyl mercaptan	11	—	Propyl butyl sulphide[a]
2	3	*tert.*-Butyl mercaptan	5	11	Dimethyl disulphide
6	10	*n*-Amyl mercaptan	10	—	Diethyl disulphide
6	8	*tert.*-Hexyl mercaptan[a]	12	—	Diisopropyl disulphide[a]
9	12	*n*-Hexyl mercaptan	3	9	Thiophene
12	—	*n*-Heptyl mercaptan[a]			

[a] Tentatively identified compounds.

mercaptans, symmetrical sulphides and disulphides in terms of ΔI values by means of the equation system shown in Table III.

The equations in Table III can be used for determining the carbon number in a compound and the boiling-point in terms of the index values available.

The GC behaviour of heterocyclic compounds cannot be described by these equations. Their identification was carried out by using the maximum values of ΔI and the index values listed in Table IV.

The identification results for sulphur compounds in cooked beef flavour arising from the Maillard reaction are summarized in Table V. Some compounds were tentatively identified from the calculated index values. The results obtained make it possible to judge critically the opinion in the literature that the presence of thioaldehydes and thioketones is essential for cooked beef flavour[13-16]. In our experiment, the formation of thioaldehydes and thioketones was not possible owing to the experimental conditions used. However, our samples exhibited a distinctive cooked beef flavour. The specific nature of the cooked beef flavour is the subject of research being conducted in our laboratory.

We feel that a systematic study of chemical reactions is essential for use in GC analysis. We believe that it is necessary to obtain the complete GC characterization of such reactions, as well as a knowledge of their possibilities and limitations.

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END OF THE SYMPOSIUM CONTRIBUTIONS