Pyrolytic Sulfurization Gas Chromatography. XII. The Simultaneous Determination of the Atomic Ratio between C, H, O, and N in an Organic Compound at the Centimilligram Level

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The atomic ratio between C, H, O, and N in an ordinary organic compound and a metal organic chelate compound could be simultaneously and satisfactorily determined by the use of 30—130 μg of a sample which cannot be accurately weighed by a microbalance or an ultramicrobalance. This was done by adopting the following modifications: 1) The amount of S was reduced to 0.5 mg from the 5 mg used in the previous studies; 2) the stainless steel column in the gas chromatograph was replaced by a Teflon column; 3) the poly(vinyl chloride) and silicone rubber tubes were replaced by stainless steel and copper tubes; and 4) a correction procedure was established for the analysis of a small amount of a sample.

With the objective of establishing a new elemental analysis method by which several elements can be simultaneously determined, pyrolytic sulfurization gas chromatography(PSGC) was originated and has been investigated fundamentally by the present authors. It has been successfully applied to the simultaneous determination of the atomic ratio between C, H, O, and N in an ordinary organic compound,[1,2] a metal organic chelate compound,[3] a polymer,[4] and an organic halogen compound.[5,6]

In conventional organic elemental analysis, the atomic ratio between C, H, O, and N is determined by combining the content of C, H, and N obtained by a CHN analyzer with the content of O obtained by an oxygen analyzer. To obtain the exact atomic ratio between C, H, O, and N, a sample of more than either 1 mg or 100 μg must be weighed by a microbalance(sensitivity 1 μg) or an ultramicrobalance(sensitivity 0.1 μg) respectively. On the other hand, since one of the most remarkable characteristics of PSGC is that the atomic ratio between C, H, O, and N can be simultaneously determined without weighing the sample, the amount of sample necessary for an analysis by PSGC can be decreased to a minimum so long as the products can be detected by gas chromatography. The present study has been carried out for the purpose of decreasing the amount of sample necessary for an analysis by PSGC.

When about 50 μg of a sample, which is one tenth of the amount of a sample in the ordinary PSGC method, was analyzed by the conventional PSGC method, the carbon content was found to be less than the theoretical value. This was attributed to the partial dissolution of carbon disulfide(CS_2) into residual sulfur. Therefore, the amount of S was reduced to 0.5 mg from the 5 mg used in the conventional PSGC method. Thus, the atomic ratio between C, H, O, and N could be simultaneously determined by the use of 30—130 μg of a sample and 0.5 mg of S. In addition to ordinary compounds, metal organic chelate compounds, which are difficult to analyze even by mass spectrometry, could be analyzed with satisfactory results.

Experimental

Reagents. All the reagents used as the analytical samples were of a reagent grade for elemental analysis except for cyanoguanidine, which was of a reagent grade for the melting-point standard.

Apparatus. The displacement apparatus,[1] which had been used for the preparation of the ampule containing a sample and S, was modified as follows: As the charging tube of He, a stainless steel tube (1 mm i.d., 1.5 mm o.d.) was used instead of a poly(vinyl chloride) tube, and as the exhaust tube of air, a copper tube (4 mm i.d., 5 mm o.d.) was used instead of a silicone rubber tube. To improve the precision and accuracy of the gas-chromatographic analysis, Teflon columns were used instead of the stainless steel columns used in the previous studies.

The other equipment used in the experiment was the same as that previously reported.

Procedure. A definite volume of a powdered sample was placed in a reaction tube by the use of the sampler[1]-I, which was composed of a glass capillary tube and a plunger (13%Rh–Pt alloy, 0.2 mm diam.) used to push the sample into the reaction tube. About 0.5 mg of S was added to it by the use of the sampler-II, which was also composed of a glass capillary tube and a plunger (Ni–Cr alloy, 0.4 mm diam.). According to this procedure, S could be taken in the range from about 0.45 mg to 0.55 mg. The reaction tube containing the sample and S thus obtained was treated by the same procedure as in the previous study.[7]

Results and Discussion

Amounts of Sample and S. In order to minimize the amount of sample necessary for the PSGC analysis, the effect of the amount of the sample on the analytical values was examined by the use of alanine as a standard sample in the following manner. A known amount of a sample was made to react with 5 mg of S in an ampule, and the reaction products were analyzed by the ordinary PSGC method. The peak areas of the products were then plotted against the weight of the sample (Fig. 1). Figure 1 shows that the calibration curves of all products pass through the point of origin of the coordinate axis except for that of CS_2. Since the peak-area ratio of each product to hydrogen sulfide(H_2S) is required to be a definite value in PSGC, these ratios were also calculated

Table 1. Effect of column material on the fluctuation of the peak-area ratios[a]

Column material	Sulfur (mg)	Sample (μg)	C.V.(%)[b]				
			N_2	CO_2	H_2S	COS	CS_2
Stainless steel	5	500	0.897	2.73	0.614	0.736	0.924
Stainless steel	0.5	50	3.39	10.2	0.793	3.29	3.16
Teflon	5	500	0.451	0.942	0.230	0.484	1.06
Teflon	0.5	50	2.51	4.27	0.714	1.24	1.02

a) The ratios of the peak area of each product to the sum of the peak area of each product. b) Average of 10 runs for alanine.

and plotted against the weight of the sample (Fig. 2). As can be seen from Fig. 2, the peak-area ratio of CS_2 to H_2S decreases with a decrease in the weight of the sample. These results could be explained by the fact that CS_2 as a reaction product partly remained in the residual sulfur because of its dissolution. Therefore, it was necessary to reduce the amount of S with a decrease in the amount of the sample. Almost entirely satisfactory results for 30—130 μg of the sample were obtained by the use of about 0.5 mg of S, as will be shown later.

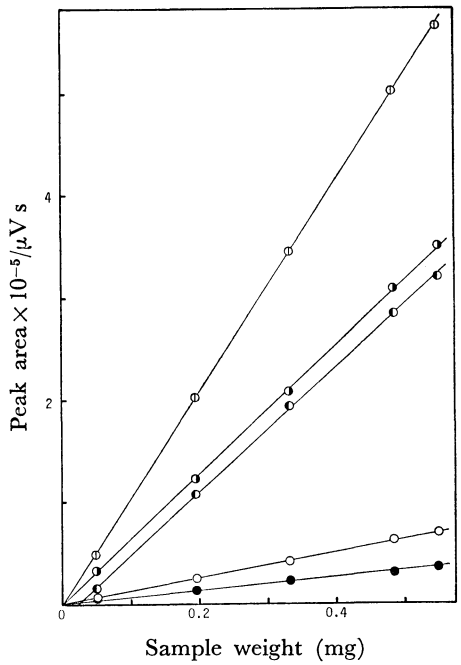

Fig. 1. Relationship between sample weight and peak area of the product.
○: N_2, ●: CO_2, ⓘ: H_2S, ◑: COS, ◐: CS_2.

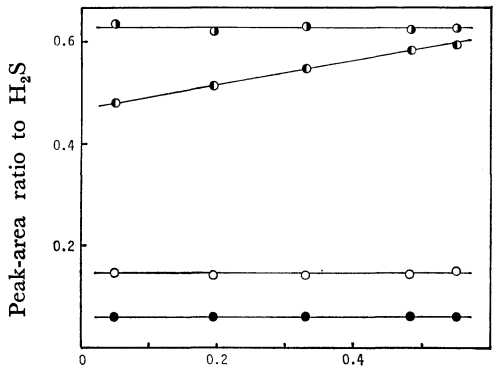

Fig. 2. Relationship between sample weight and peak-area ratio of the product to H_2S (5 mg of S was used).
○: N_2/H_2S, ●: CO_2/H_2S, ◑: COS/H_2S, ◐: CS_2/H_2S.

Improvement of the Apparatus. The analytical value obtained under the experimental conditions of the conventional PSGC method gave an error increasing with a decrease in the amount of sample. In order to improve the precision and accuracy of the analytical data, 1) the column material in a gas chromatograph and 2) the displacement apparatus were modified in the following way. 1) The stainless steel columns used in the previous studies were replaced by Teflon columns under the same gas-chromatographic conditions. The present column material was compared with the previous one by analyzing *ca.* 0.5 mg and *ca.* 50 μg of alanine samples (Table 1). Judging from the results shown in Table 1, the Teflon column is superior to the stainless steel column. 2) To minimize the fluctuation in the analytical value of nitrogen (N_2)(Table 1) which seemed to be due to the contamination of air passing through the tubes of poly(vinyl chloride) and silicone rubber, the displacement apparatus was modified in the following manner. As the charging tube of He, a stainless steel tube(1 mm i.d., 1.5 mm o.d.) was used instead of a poly(vinyl chloride)tube, and as the exhaust tube of air, a copper tube(4 mm i.d., 5 mm o.d.) was used instead of a silicone rubber tube. The present displacement apparatus was compared with the previous one by analyzing *ca.* 50 μg of alanine (Table 2). Table 2 shows that the present apparatus is superior to the previous one with regard to the fluctuation of the analytical data of N_2. As a result of the above-mentioned improvements, it is suggested that the atomic ratio between C, H, O, and N for centimilligrams of a sample can be simultaneously determined with the same pre-

Table 2. Effect of the displacement apparatus on the fluctuation of the peak-area ratios

	C.V.(%)[a]				
	N_2	CO_2	H_2S	COS	CS_2
Present	0.987	3.39	0.767	0.853	1.29
Previous	2.51	4.27	0.714	1.24	1.02

a) Average of 10 runs for alanine.

cision and accuracy as in the ordinary PSGC method. The following investigations were undertaken by the use of the modified apparatus.

Sampling Amounts. Since it was difficult to weigh centimilligrams of a sample accurately by means of a microbalance, the weight of a sample was calculated approximately by the use of Eq. 1 from the analytical data:

$$W(\mathrm{mg}) = \frac{1}{F}\Sigma M(\mathrm{X})K(\mathrm{X})A(X), \qquad (1)$$

where W is the weight of a sample, X is the reaction product, $M(\mathrm{X})$ is the value obtained by subtracting the amount of S from the formula weight of X, $K(\mathrm{X})$ is the calculation factor of X, $A(\mathrm{X})$ is the peak area of X, and F is the conversion factor obtained by the use of Eq. 2 from the calibration curve of H_2S in Fig. 1:

$$F = \frac{M_t}{3.50w}A(\mathrm{H_2S}), \qquad (2)$$

where w is the weight of alanine, M_t is the molecular weight of alanine, and 3.50 indicates the mole number of H_2S produced from 1 mol of alanine. By the use of Eq. 1, the weight of a sample could be calculated within a relative error of about ±10%. Sulfur in the range from about 0.45 mg to 0.55 mg was conveniently sampled by means of the sampler-II.

Effect of the Sample Amount on the Peak-area Ratio. Centimilligrams of alanine as a standard sample was made to react with *ca.* 0.5 mg of S in an ampule and then analyzed by PSGC. The peak-area ratios of the products to H_2S were plotted against the calculated weight of a sample (Fig. 3). It is apparent from Fig. 3 that the atomic ratio between C, H, O, and N in a sample can be determined by the use of a sample in the range from about 50 μg to 130 μg, for the peak-area ratios of all the products to H_2S indicated definite values.

Correction of Blank Values. As can be seen from Fig. 3, the peak-area ratio of carbonyl sulfide(COS) to H_2S increased with a decrease in the amount of a sample to less than 50 μg, while the peak-area ratio of CS_2 to H_2S decreased with a decrease in the amount of a sample to less than 30 μg. Then, the following investigation was made with regard to 1) the blank value of COS and 2) the blank value of CS_2. 1) In the ordinary PSGC, a small peak of COS was still detected in the analysis of a sample consisting of either C and H or C, H, and N, but it was neglected in the calculation of the atomic ratio because it was negligibly small. In the present study, however, a smaller amount of a sample is used, and the peak area of COS is presumed to be influenced by the peak area of COS as a blank value. Then, a 30—100 μg of cyanoguanidine, which consists of C, H, and N, was analyzed by the present method, and the value of the peak area of COS was examined. The peak area of COS as a blank value was approximately a definite value, regardless of the amount of the sample. Therefore, the peak-area ratio of COS to H_2S was calculated from the value which was obtained by subtracting the value of COS as a blank one from the analytical value of alanine as a standard sample; it was then plotted against the sample amount (Fig. 4). Figure 4 shows that the peak-area ratio of COS to H_2S comes to a definite value when the blank value is corrected. 2) The peak-area ratio of CS_2 to H_2S decreased with a decrease in the amount of the sample to less than 30 μg (Fig. 3). It was presumed that a part of the CS_2 remained in the residual sulfur in the ampule. Therefore, the effect of the amount of residual sulfur on the peak area of CS_2 was investigated by the use of the analytical data in Fig. 3 in the following manner. The amount of S(W_c) consumed by pyrolytic sulfurization reaction was calculated by means of Eq. 3:

$$W_c(\mathrm{mg}) = \frac{M_s}{F}(A(\mathrm{H_2S}) + K(\mathrm{COS})A(\mathrm{COS}) + 2K(\mathrm{CS_2})A(\mathrm{CS_2})), \qquad (3)$$

where M_s is the atomic weight of S, F is the same factor as in Eq. 1, X is the reaction product, $A(\mathrm{X})$ is the peak area of X, and $K(\mathrm{X})$ is the calculation factor of X. Therefore, the amount of residual sulfur (W_r) was found approximately by Eq. 4:

$$W_r(\mathrm{mg}) = 0.5 - W_c. \qquad (4)$$

The relationship between W_r and the deficient amount of CS_2, calculated from the data in Fig. 3, was plotted in Fig. 5. Figure 5 shows that the amount of CS_2 dis-

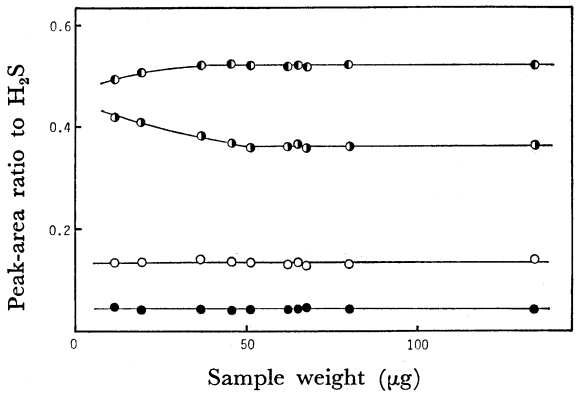

Fig. 3. Relationship between sample weight and peak-area ratio of the product to H_2S.
○: N_2/H_2S, ●: CO_2/H_2S, ◑: COS/H_2S, ◐: CS_2/H_2S.

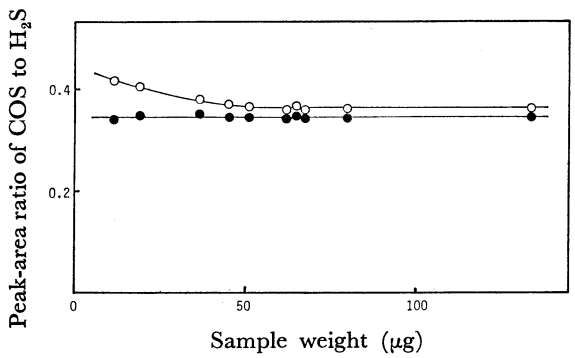

Fig. 4. Relationship between sample weight and peak-area ratio of COS to H_2S.
○: Uncorrected peak-area ratio, ●: corrected peak-area ratio.

Table 3. Corrections for COS and CS_2

Sample	Sample weight (μg)	Correction	C (wt%)			H (wt%)			O (wt%)		
			Calcd	Found	Error	Calcd	Found	Error	Calcd	Found	Error
Cholesterol	62	N[a]	83.87	83.66	−0.21	11.99	11.79	−0.20	4.14	4.55	+0.41
		A[b]	83.87	83.85	−0.02	11.99	11.84	−0.15	4.14	4.31	+0.17
Sucrose	27	N	42.11	41.57	−0.54	6.48	6.23	−0.25	51.41	52.20	+0.79
		B[c]	42.11	42.06	−0.05	6.48	6.31	−0.17	51.41	51.63	+0.22

a) Not corrected, b) corrected for COS, c) corrected for both COS and CS_2.

Table 4. Analytical results of various organic compounds

Sample	Calculated weight (μg)	C (wt%)			H (wt%)			O (wt%)			N (wt%)		
		Calcd	Found	Error	Calcd	Found	Error	Calcd	Found	Error	Calcd	Found	Error
Anthracene	55	94.34	94.37	+0.03	5.66	5.63	−0.03						
Acetanilide	48	71.09	71.30	+0.21	6.71	6.61	−0.10	11.84	11.88	+0.04	10.36	10.21	−0.15
Phenacetin	60	67.02	66.99	−0.03	7.31	7.18	−0.13	17.85	18.06	+0.21	7.82	7.77	−0.05
Nicotinic acid	65	58.54	58.64	+0.10	4.09	3.98	−0.11	25.99	26.19	+0.20	11.38	11.19	−0.19
p-Nitroaniline	53	52.17	52.01	−0.16	4.38	4.30	−0.08	23.17	23.36	+0.19	20.28	20.33	+0.05
Caffeine	61	49.48	49.75	+0.27	5.19	5.00	−0.19	16.48	16.37	−0.11	28.85	28.88	+0.03
Alanine	45	40.44	40.43	−0.01	7.92	7.93	+0.01	35.92	36.03	+0.11	15.72	15.61	−0.11
Triphenylphosphine	70	93.46	93.55	+0.09	6.54	6.45	−0.09						
Phenylmercury(II) acetate	65[a]	70.58	70.39	−0.19	5.92	5.96	+0.04	23.50	23.65	+0.15			
Tris(2,4-pentanedionato)iron(III)	58[a]	60.59	60.78	+0.19	7.12	7.14	+0.02	32.29	32.08	−0.21			
Bis(2,4-pentanedionato) magnesium(II)	67[a]	51.27	51.18	−0.09	7.75	7.84	+0.09	40.98	40.98	0.00			

a) Includes the weight of the metal.

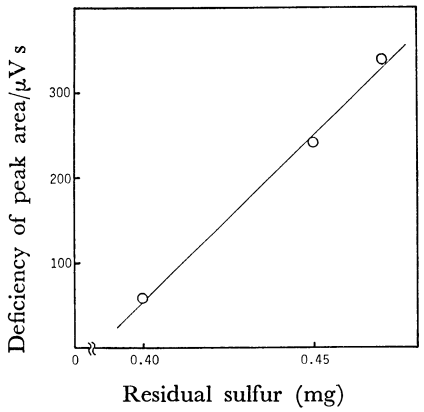

Fig. 5. Relationship between residual sulfur and deficiency of peak area of CS_2.

solved in the residual sulfur is approximately proportional to the amount of the residual sulfur. Therefore, the deficient amount of CS_2 was calculated by the use of Eq. 5, obtained from the calibration curve in Fig. 5:

$$\alpha(\mu V\,s) = 3.8\times10^{3}W_r - 1.5\times10^{3}, \quad (5)$$

where α is the deficient amount of CS_2. Judging from the results shown in Fig. 5, Eq. 5 can be applied when W_r is in the range from 0.39 mg to 0.5 mg. Thus, the atomic ratio between C, H, O, and N should be estimated by calculating α by means of Eqs. 3, 4, and 5 and by correcting the apparent peak area of CS_2. Examples of these corrections are shown in Table 3. As can be seen from Table 3, the atomic ratio between C, H, and O was markedly improved by applying the above-mentioned corrections. Both the oxygen content in cholesterol and the amount of COS produced from 62 μg of cholesterol were small, but a satisfactory result was obtained.

Analysis of Various Organic Compounds. Various organic compounds and metal organic chelate compounds were analyzed by the present procedure; the results are shown in Table 4. Table 4 indicates that the atomic ratio between C, H, O, and N can be simultaneously and satisfactorily determined by the use of centimilligrams of a sample.

The present study is expected to be especially significant for the organic elemental analysis of micro amounts of a sample which cannot be accurately weighed by a microbalance or an ultramicrobalance.

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