The glyoside was chromatographed on 80 g of alumina (activity grade III) with elution by mixtures of ethyl acetate and ethanol of increasing polarity. The convalloside was crystallized from ethanol. Melting poing 198–200°C, $[\alpha]_D^{20}$ 9.6 ± 2° (c 1.0; 80% ethanol). Yield 1.4 g (54%, calculated on the convallatoxin).

SUMMARY

The known natural diglycoside convalloside (strophanthidin 3β-O-[4-O-β-D-glucopyranosyl-α-L-rhamnopyranoside]) has been synthesized from strophanthidin, L-rhamnose, and D-glucose. The synthesis was effected by the Koenigs—Knorr method with the formation of convallatoxin and its 2,3-isopropylidene derivative as intermediates. The yield of convallatoxin was 63% calculated on the strophanthidin, and that of convalloside 54% calculated on the convallotoxin or 34% calculated on the strophanthidin.

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DETERMINATION OF SULFUR IN DRUGS OF NATURAL ORIGIN

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The efficacy of the use of Schoniger's method for the quantitative analysis of sulfur in samples of Allium sativum L. and ichthammol has been shown. The relative error of the determination does not exceed 5%.

As a chemical element, sulfur is of great importance in the structural organization of living matter and the regulation of metabolism. Sulfur participates in various biochemical and physicological processes, especially in redox reactions, the synthesis of protein, the regulation of the permeability of membranes, etc [1]. This element is a component of many natural compounds and is responsible for their biological activity (hormones, enzymes, alkaloids, antibiotics, etc.) [1, 2]. In view of this, various methods for the quantitative determination of this element in natural samples have been proposed, which, as a rule, are based on the preliminary prolonged and laborious mineralization of the material under investigation [3, 4].

The method of combustion in a flask with oxygen which is rapid to perform and free from the above-mentioned deficiency, is widely recommended in the analysis of sulfur-containing inorganic substances but it has not found wide use in the analysis of natural materials, [3, 5]. To a certain extent, this is due to a usually low sulfur content of natural materials, their complex chemical composition, and the resulting possible interference of other chemical elements on the results of determinations. However, the generally recognized advantages of this method [3, 5, 6] have impelled us to investigate the possibility of its use in the analysis of natural sulfur-containing compounds.

As the object of investigation we selected natural drugs: garlic (Allium sativum l.) and ichthammol. This choice was due to the following factors:

1. The most important pharmacologically active compounds of garlic are sulfur-containing compounds (alkyl derivatives of cysteine, alkyl polysulfides, etc.) [7,8];

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TABLE 1. Results of the Determination of Sulfur in Ichthammol

Weight of sample (with allowance for moisture), g	Sulfur found		Metrological characteristics
	mg	%	
Pharmacopeal method			
0,2587	43,72	16,9	$n = 5$
0,4439	67 03	15,10	$\bar{x} = 15,66$
0,4488	71,36	15,90	$S = 0,807$
0,3872	57,42	14,83	$S_{\bar{x}} = 0,361$
0,4334	67,5	15,59	$E_{0,95} = 1,003$
			$A_{rel} = 6,40\%$
Method of combustion in oxygen			
0,0184	3,37	18,29	$n = 5$
0,0130	2,45	18,87	$\bar{x} = 18,55$
0,0122	2,30	18,82	$S = 0,278$
0,0217	3,98	18,32	$S_{\bar{x}} = 0,124$
0,0136	2,51	18,43	$E_{0,95} = 0,345$
			$A_{rel} = 1,8\%$

Ichthammol of batch 1000782, containing 38.84% of moisture, was analyzed.

2. Ichthammol, which is a product of the distillation of bituminous shales and consists of a mixture of ammonium salts of sulfonic acids, also includes sulfur-containing compoounds which are responsible for its medical use [2, 4]);

3. The methods for the quantitative determination of sulfur in these drugs are lengthy and laborious and, in a number of cases, are nonspecific and have a number of other disadvantages [4, 9].

Table 1 gives results on the determination of the total sulfur in ichthammol by the pharmacological method (FS 42-1734-81) [4]* and by the method of combustion in a flask with oxygen [10]. A comparison of these results shows a statistically significant difference between those obtained by the two methods mentioned. In order to determine the reason for this difference, subsequent investigations were performed by the use of "additives." The results obtained (Table 2) permit the conclusion that the pharmacopeal method for determining sulfur in ichthammol leads to systematically low results. This may be due to possible losses of sulfur during the numerous laborious analytical operations used in the pharmacopeal method: filtration, stirring large amounts of the solutions to be analyzed, the separation of the precipitate, repeated transfer of the solutions being investigated, and so on.

At the same time, as the experiments using additives showed (see Table 2), the oxygen flask combustion method enables reliable results to be obtained, which indicates the possibility of its use in the analysis of ichthammol. Subsequently, this method was used in the analysis of medicinal forms of ichthammol (Table 3).

It must be emphasized that the analysis of ichthammol by the oxygen flask combustion method was not only of practical interest but also of interest from the point of view of the possibility of applying this method of elementary analysis to the determination of sulfur in samples of complex composition containing both organic and inorganic sulfur.

In the analysis of sulfur in garlic, allowance must be made for the small amount of this element and the possibility of the interfering influence of nitrogen (in an amount considerably exceeding the amount of sulfur) on the results of the determination. In view

*The method is based on preliminary mineralization followed by the gravimetric determination of sulfur in the form of barium sulfate.

TABLE 2. Determination of Sulfur in Ichthammol and Model Experiments Using "Additives"

Weight of sample (with allowance for moisture), g	Amount of sulfur in the sample, mg*	Sulfur added		Total sulfur in the sample analyzed		Sulfur found		Error
		ml	mg	mg	%	mg	%	
Pharmacopeal method								
0,2684	42,03	0,5	5,00	47,03	17,52	42,62	15,88	—1,64
0,3582	56,09	1,0	10,00	66,09	18,45	60,64	16,93	—1,52
0,3863	60,49	1,5	15,01	75,50	19,54	72,54	18,26	—1,28
0,2863	44,91	2,0	20,01	64,92	22,63	58,48	20,30	—2,24
0,2334	36,55	2,5	25,01	61,56	26,37	48,76	20,89	—5,48
Method of combustion in oxygen								
0,0128	2,37	10	0,10	2,47	19,30	2,44	19,07	—0,23
0,0256	4,75	20	0,20	4,95	19,34	5,04	19,70	+0,36
0,0167	1,98	30	0,30	2,28	21,31	2,28	21,31	0
0,0140	2,60	40	0,40	3,00	21,43	2,96	21,11	—0,32
0,0167	3,10	50	0,50	3,60	21,56	3,60	21,56	0

*In light of the amount of sulfur found by the pharmacopeal method (15.66%) and by the oxygen combustion method (18.55%).

TABLE 3. Quantitative Determination of Sulfur in a 20% Ichtammol Salve by the Proposed Method

Sample, g	Sulfur found		Metrological characteristics
	mg	%	
0,1076	1,94	1,93	$n = 5$
0,0952	1,89	1,99	$\bar{x} = 1,95$
0,0994	1,96	1,97	$S = 0,0320$
0,0968	1,88	1,94	$S_{\bar{x}} = 0,143$
0,1011	1,93	1,91	$E_{0,95} = 0,0397$
			$A_{rel} = 2,03\%$

Salve of batch 211186 was analyzed.

of this, we first investigated standard synthetic organosulfur compounds in the presence of added organonitrogen substances. In particular, in model experiments we anlyzed samples of a sulfur-containing substance — methionine — in the presence of various amounts of an amino acid (glycine) as the source of nitrogen.

The results, which are given in Table 4, indicate the probability of obtaining systematically high results when there are more than 4 mg of nitrogen in the sample being analyzed.

As is known [11], on the combustion of nitrogen-compounds in oxygen, nitrite ions are formed in the absorbing solution which subsequently, during the oxidation of sulfur [10], are obviously oxidized to nitrates under the action of hydrogen peroxide and the heating of the solution. Thus, the high results may be due to the known phenomenon of the coprecipitation of nitrate ions during the precipitation titration of sulfates by barium salts [12].

Further investigation showed that the interfering influence of nitrogen can be eliminated by adding urea to the absorbing solution. Then the well-known decomposition of the nitrite ions to molecular nitrogen [12] takes place. As we see (Table 4), the use of urea enables reliable results to be obtained in the determination of sulfur in the presence of a considerable excess of nitrogen.

TABLE 4. Results of the Quantitative Determination of Sulfur in the Presence of Nitrogen (model experiments)

Sample, g		Nitrogen content in the model sample, mg	Sulfur, %	
methionine	glycine		found	error
		Procedure using [10]		
0,0263	—	2,47	21,47	— 0,02
0,0222	0,0052	3,05	21,99	+ 0,50
0,0286	0,0063	3,86	22,24	+ 0,75
0,0320	0,0083	4,55	22,39	+ 0,90
0,0191	0,0159	4,76	23,04	+ 1,55
0,0299	0,0312	8,63	23,10	+ 1,61
0,0275	0,0593	13,65	23,48	+ 1,99
0,0179	0,0845	17,45	23,87	+ 2,38
		Procedure using urea		
0,0198	0,0621	13,45	21,48	— 0,01
0,0252	0,0610	13,75	22,22	— 0,27
0,0176	0,0664	14,04	21,54	+ 0,05
0,0195	0,0705	14,99	21,50	+ 0,01
0,0157	0,0789	16,19	21,69	+ 0,20

The theoretical sulfur content of methionine is 21.49% and its sulfur content 9.39%; the nitrogen content of glycine is 18.66%

TABLE 5. Quantitative Determination of Sulfur in Garlic by the Proposed Method

Sample, g	Sulfur found		Metrological characteristics
	mg	%	
0,0941	0,5 2	0,597	$n=5$
0,0996	0,572	0,574	$\bar{x}=0,6$ 3
0,0956	0,562	0,5 8	$S=0,0237$
0,0844	0 530	0,628	$S_{\bar{x}}=0.0106$
0,0923	0,578	0,620	$E_{0,95}=0,0295$
			$A_{rel}=4,89\%$

The analysis of sulfur in samples of garlic by combustion in a flask containing oxygen showed a content of this element of about 1.15% at a relative error of more than 10% (by the usual procedure [10]). However, the same procedure with the use of urea enabled more accurate results to be obatined (Table 5). The correctness of the results obtained was confirmed by the results of the model experiments (Table 4).

The methods for analyzing ichthammol and garlic that have been developed can be used in the analysis of various medicinal forms (extemporaneous formulations with ichthammol, garlic extract, "Allakhol" tablets), and other sulfur-containing natural medicinal agents.

On the whole, the investigations performed indicate the possibility of using the method of combustion in a flask with oxygen for the quantitative determination of sulfur in drugs of natural origin.

EXPERIMENTAL

Procedure for Determining Sulfur in Ichthammol by Combustion in Oxygen. About 0.02 g of ichthammol (accurately weighed) was transferred by pipette to a previously weighed piece of dense ash-free filter paper, 15 × 15 mm, folded in the form of a cone (carrier). After the deposition of the sample, it was weighed again and in this way the weight of the sample was determined. Then the carrier with the sample was fixed in the spiral of the combustion

apparatus and analysis was performed in the way usually adopted in the determination of sulfur by the method of combustion in a flask with oxygen [10].

Procedure for Determining Sulfur in Ichthammol Using "Additives." As additive was used a 4.5% aqueous solution of sodium sulfate kh.ch. ["chemically pure"] prepared with analytical accuracy (from an accurately weighed sample in a measuring flask).

In the analysis of ichthammol by the pharmacopeal method, the total sulfur was determined as described in FS 42-1734-81 [4], but to each accurately weighed sample was added an accurate volume (from 0.5 to 5.0 ml) of the standard sodium sulfate solution.

When the oxygen flask combustion method was used, the same standard solution of sodium sulfate was added with the aid of a microsyringe in amounts of 10-50 μl to the carrier with the weighed sample of ichthammol fixed in the spiral, and then the sulfur was determined by the usual method [10].

Model Experiments to Determine Sulfur in the Presence of Nitrogen by the Method of Combustion in Oxygen. About 0.2 g (accuratey weighed) of a pharmacopeal sample of methionine was folded into a 15 × 15 mm piece of cellophane, and this was placed in the spiral of the combustion apparatus. In a similar way, various amounts of glycine (Reanal substance) were placed in the same spiral. Then the determination of sulfur in a series of samples prepared by the method described above was carried out by combustion in a flask with oxygen by the traditional procedure [10], and determinations were carried out in parrallel similarly but with the addition to the absorbing solution of 0.5 g of urea kh.ch. after the absorption of the combustion products.

Procedure for Determining Sulfur in Garlic by the Method of Combustion in a Flask with Oxygen. About 0.1 g (accurately weighed) of a dried sample of garlic carefully ground in a mortar was folded into a piece of cellophane (20 × 20 mm) and placed in the spiral of the combustion apparatus. Then the sulfur was determined by the usual procedure [10], but after absorption of the combustion products 0.5 g of a powder of urea kh.ch. was added to the solution.

SUMMARY

1. The possibility has been considered of using the method of combustion in a flask with oxygen for the determination of sulfur in medicinal agents of natural origin.

2. On the basis of the results of these investigations, procedures are proposed for determining sulfur in garlic and ichthammol, and also in a number of their medicinal forms. The relative error of the determination does not exceed 5%.

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