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Stability of Thiosulfinates from Garlic (*Allium sativum* L.) Supercritical Extracts in Polar and Nonpolar Solvents

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Abstract—For the first time a systematic investigation of the decomposition process of allicin and other thiosulfinates from garlic supercritical extracts (SCEs) in solvents of different polarity (water, ethanol, acetonitrile, dimethyl sulfoxide, dichloromethane, and hexane) was performed. Qualitative and quantitative analysis of the garlic thiosulfinate decomposition products was carried out. A new HPLC analytical procedure was developed; it allows simultaneous determination of both starting thiosulfinates and sulfur-containing products of their decomposition. It was shown that the decomposition of SCE thiosulfinates can be directed toward the formation of target substances (ajoenes, dithiins, trisulfides, etc.) by changing the solvent polarity. The results obtained and methodical developments can be used in biomedical research of garlic-based drugs, as well as in the processes of their preparation, storage, and analysis.

Keywords: supercritical extraction, garlic, carbon dioxide, thiosulfinates, high performance liquid chromatography (HPLC)

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INTRODUCTION

Garlic (*Allium sativum* L.) always attracts attention of researchers due to a broad spectrum of its biological activity [1]. Meanwhile, despite the large number of publications in this field, such components of garlic as thiosulfinates of general formula $R_1—S(O)—S—R_2$ (where R_1 and R_2 = methyl, allyl, 1-propenyl) are the less studied compounds. The reason for this is the instability of thiosulfinates, which was noted by many authors [2–4].

Stability of one of the most studied garlic thiosulfinates, diallyl thiosulfinate (allicin) in the blood, physiological fluids, and some solvents was examined in [5]. Thus, allicin is quickly decomposed at 37°C and more stable in a protic polar solvent (methanol). About 90% of allicin is retained in an aqueous medium at pH 1.2 and 7.5 for 5 h. In the blood, only trace amounts of allicin are detected at 5 min after incubation. It was shown that exposure of garlic homogenate for 3 h at room temperature leads to complete decomposition of allicin [6]. Pure allicin in an aqueous solution is decomposed by 50% over 4 days [7]. At elevated temperatures (~80°C) allicin is completely decomposed within 25 min [8]. The effect of some food products (mayonnaise, yogurt, honey, various sauces, vegetables, and fruits) on the stability of garlic thiosulfinates was studied [9].

It should be noted that a systematic analysis of thiosulfinate behavior in the solvents depending on their polarity, as well as the data on the decomposition products are missed in the discussed papers. Nevertheless, these issues are very important in the practical application of thiosulfinates, their proper storage, as well as in choosing the methods for their isolation from the garlic matrix and subsequent analysis.

Obviously, extraction with carbon dioxide in the supercritical (SC) state is the optimum method for isolation of labile thiosulfinates from the garlic starting matrix. This was confirmed by studies, the results of which are presented in [10, 11]. We used the method of SC fluid extraction with carbon dioxide for isolation of unchanged thiosulfinates from the garlic homogenate.

It is known that high performance liquid chromatography (HPLC) is the most appropriate method for analysis of the garlic sulfur-containing compounds. HPLC allows conducting qualitative and quantitative analysis of the garlic individual components and the products of their transformations [12, 13], whereas a number of sulfur-containing components of the extract are decomposed in vaporizer during the analysis by gas chromatography [14, 15]. However, there are a number of limitations in the known techniques for HPLC analysis of the plant matrix of garlic. Thus, the analysis of thiosulfinates and the products of their decomposition is carried out in different HPLC systems: the determination of thiosulfinates is conducted by the method of adsorption HPLC, and the transformation products (sulfides, disulfides, etc.) are analyzed by reversed-phase HPLC [16–18]. In addition, gradient eluting is applied for efficient separation of

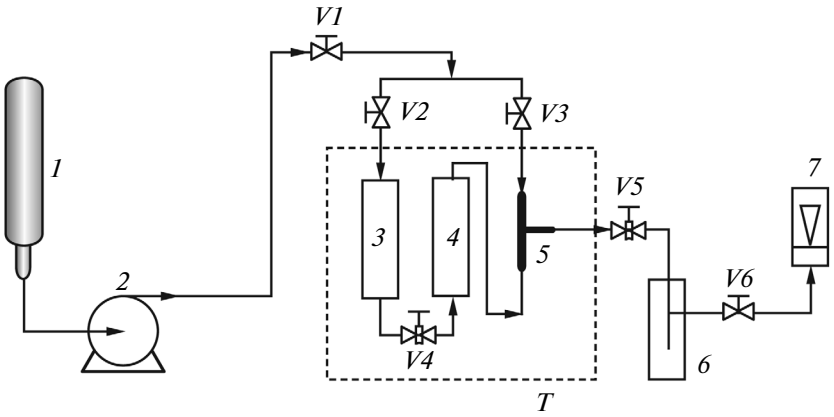

Fig. 1. Scheme of installation for preparation of garlic extract using supercritical CO_2: *1*—CO_2 cylinder, *2*—high-pressure pump with head cooling, *3*—buster cylinder, *4*—high-pressure cell, *5*—tee, *6*—separator, *7*—rotameter; *V1*, *V2*, *V3*—high-pressure valves; *V4*, *V5*—heated valves; *V6*—gas flow regulator; *T*—thermostat.

the components [7]. These analytical methods make it difficult the correct quantification of the target products, so we developed a new HPLC technique that provides simultaneous qualitative and quantitative analysis of both primary thiosulfinates and the products of their decomposition.

The purpose of the work was to study the stability of garlic (*Allium sativum* L.) thiosulfinates, which were obtained by the extraction with supercritical carbon dioxide, in solvents of different polarity.

EXPERIMENTAL

Extraction of the garlic homogenate with SC carbon dioxide was carried out in a SFChem goszmp installation, its schematic diagram is shown in Fig. 1. The installation is made entirely of stainless steel; the sealing material is fluoroplast-4 or graflex. A Knauer pump was used to fill the reactor with liquid carbon dioxide and to create pressure (up to 300 bar); a 100-mL Keystone reactor was used for extraction.

The installation has a bypass line, which is used for presetting the operation mode. Flow rate of CO_2 to the bypass line was set at open valves *V1*, *V3*, *V5*, and *V6* with closed valves *V2* and *V4*. Extraction was carried out as follows: after reaching the required temperature in thermostat *T* and the CO_2 flow rate measured with a calibrated rotameter *7*, valve *V3* was closed with simultaneous opening of valves *V2* and *V4*. Booster cylinder *3* was used to equalize the pressure in the system. If necessary, the pressure was adjusted by valve *V5*. Extract was collected in separator *6*. After completion of the extraction, the CO_2 supply was disconnected by closing valves *V2* and *V4* to preserve CO_2 in the booster cylinder, and the extractor was depressurized to zero through valve *V5*.

Initial raw material (garlic) purchased in a Moscow trading network was crushed to a particle size of 1–4 mm; the obtained garlic homogenate (34 g) was kept in air for 10 min for fermentation [19], then placed in high-pressure cell *4* and thermostated to desired temperature.

Extraction was carried out in the following mode: temperature 40°C, pressure 280 bar, flow rate of SC-CO_2 20 mL/min, extraction time 1 h. After completing the process, 407 mg of extract was obtained. For subsequent experiments, a weighed portion of extract (about 30–32 mg) was dissolved in 5 mL of appropriate solvent. Solvents used in the experiment are listed in Table 1. All organic solvents were of HPLC grade and purchased from Sigma-Aldrich. In the experiments, we used triply distilled water.

Reference materials for qualitative and quantitative analysis of the products of decomposition of garlic supercritical extract (SCE) were obtained by preparative HPLC. The HPLC separation of the garlic extract thiosulfinates was performed according to [13]. Isolation of diallyl thiosulfinate (allicin) was carried out by preparative HPLC, which was described in detail in [21].

Reference materials of the sulfide fraction (sulfides, disulfides, and trisulfides) were obtained from the hexane–isopropanol extract of garlic homogenate as described in [21].

Table 1. The solvents used and their properties

No.	Solvent	Relative polarity* [20]	Note
1	Hexane	0.009	
2	Dichloromethane	0.309	
3	Dimethyl sulfoxide	0.444	
4	Acetonitrile	0.460	
5	Ethanol	0.654	Protic
6	Water	1.000	Protic

* The ratio of the solvent dielectric constant to the dielectric constant of water.

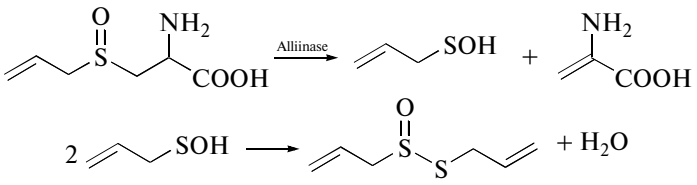

Fig. 2. Scheme of the formation of allicin from alliin.

The structure and identity of the samples obtained was confirmed by the ^{1}H NMR spectroscopic data (a Varian Unity Inova spectrometer, operating frequency 400 MHz) [21]. The content of main components, such as diallyl disulfide, (1-propenyl)-allyl disulfide, diallyl trisulfide, methyl allyl trisulfide, 3-vinyl-4*H*-1,2-dithiin, allicin, in the samples was not less than 98%. All obtained reference samples were stored in a freezer at −18°C.

To analyze the decomposition process of thiosulfinates from supercritical extract, we specially developed a new procedure that allows simultaneous determination of both starting thiosulfinates and the products of their decomposition (sulfides, disulfides, trisulfides, etc.). HPLC analysis was carried out on a Gilson 116 chromatograph equipped with an UV detector (254 nm); mobile phase, acetonitrile–water–isopropanol 40 : 50 : 10; isocratic elution with a rate of 1.5 mL/min. We used a Luna 3u Phenyl-Hexyl column (4.6 × 150 mm, particle size 3 μm). Stability of a thiosulfinate fraction in the eluent was specially studied. It was found that thiosulfinate in the eluent does not undergo any changes at room temperature for 2 h. This ensures the absence of thiosulfinate decomposition in the mobile phase, since the analysis duration is 30 min, including sample preparation. Elution times of the substances were determined by the retention times of external reference compounds.

The data on molar extinction coefficients of thiosulfinates [13] and sulfides [12] were used for quantitative analysis.

RESULTS AND DISCUSSION

The source of all sulfur-containing substances that make up the natural raw material from garlic is an amino acid, S-allyl-cysteine sulfoxide (alliin), which is contained in the vacuoles of plant cells. The enzyme alliinase is contained in the cytosol and quickly converts alliin into diallyl thiosulfinate (allicin) (see Fig. 2) when the plant cells are destroyed (when crushing the garlic cloves).

In addition, since alliinase is a nonspecific enzyme, some amounts of thiosulfinates of general formula R_1—S(O)—S—R_2 (where R_1 and R_2 = methyl, allyl, 1-propenyl), are formed from S-methyl-cystein sulfoxide and S-(1-propenyl)-cystein sulfoxide [22].

Since in HPLC analysis of the decomposition of garlic SCE thiosulfinate we used a polar eluent (water–acetonitrile–isopropanol 50 : 40 : 10), the samples containing polar solvents (water, ethanol, acetonitrile, dimethyl sulfoxide) were injected directly in a chromatograph. The samples containing nonpolar solvents (dichloromethane, hexane) were dissolved in ethanol at a ratio of 1 : 10 before analysis. The thiosulfinate decomposition was studied at room temperature (20 ± 2°C); the samples were taken every day except in the experiment with hexane, when samples were taken every hour.

Decomposition of Thiosulfinates from Garlic Supercritical Extract in Water

The aqueous solution of garlic supercritical extract was kept at room temperature. A sample of 20-μL volume was taken and injected into the liquid chromatograph. The data on changing concentration of the major garlic thiosulfinate, i.e., allicin, total concentration of minor thiosulfinates (i.e., thiosulfinates that are present in smaller concentration as compared with allicin), as well as the products of their decomposition are given in Table 2.

HPLC chromatograms of decomposition of garlic SCE thiosulfinates in water are shown in Fig. 3.

Thus, in the aqueous solution of garlic supercritical extract, allicin and other thiosulfinates are decomposed approximately by 72% within 15 days. It is interesting to note that sulfides are not virtually formed during decomposition. The formation of (1-propenyl)-allyl disulfide and diallyl trisulfide only in insignificant amounts was detected; these compounds were also completely decomposed within 15 days.

Decomposition of Thiosulfinates from Garlic Supercritical Extract in Ethanol

A solution of garlic SCE in ethanol was studied in a similar way. The experimental data are presented in Table 3.

HPLC chromatograms of decomposition of garlic SCE thiosulfinates in ethanol are shown in Fig. 4.

In solutions containing ethanol, the formation of *E*- and *Z*-ajoenes begins already on the first day; moreover, a large amount of diallyl trisulfide and significant amounts of 1-propenyl derivatives are formed. The whole range of the currently best known sulfide components of garlic is formed in ethanol for 15 days; their concentration is growing constantly during the

Table 2. Decomposition of garlic SCE thiosulfinates in water

Analytes	Time-dependent changes in concentration of the solution components, mg/mL					
	beginning of experiment*	4 days	5 days	6 days	8 days	15 days
Allicin	0.32	0.26	0.24	0.19	0.17	0.09
Sum of minor thiosulfinates	0.32	0.23	0.20	0.17	0.14	0.07
2-Vinyl-4*H*-1,3-dithiin	0	0	0	0	0	0
3-Vinyl-4*H*-1,2-dithiin	0	0	0	0	0	0
Diallyl disulfide	0	0	0	0	0	0
Methyl allyl trisulfide	0	0	0	0	0	0
(1-Propenyl)-allyl disulfide	0	0.009	0.010	0.007	0.006	0
Diallyl trisulfide	0	0.009	0.010	0.010	0.009	0

* Hereinafter, in the column "Beginning of experiment," the data of quantitative analysis obtained immediately after preparation of the analyzed solution are given.

Table 3. Decomposition of garlic SCE thiosulfinates in ethanol

Analytes	Time-dependent changes in concentration of the solution components, mg/mL							
	beginning of experiment	1 day	2 day	5 day	6 day	8 day	12 day	15 day
Allicin	0.50	0.47	0.29	0.18	0.15	0.12	0.071	0.060
Sum of minor thiosulfinates	0.47	0.32	0.31	0.30	0.27	0.25	0.20	0.18
Sum of *E*- and *Z*-ajoenes	0	0.17	0.18	0.21	0.22	0.23	0.19	0.18
2-Vinyl-4*H*-1,3-dithiin	0	0	0.005	0.010	0.011	0.013	0.012	0.012
3-Vinyl-4*H*-1,2-dithiin	0	0	0	0.009	0.010	0.012	0.013	0.013
Diallyl disulfide	0	0	0	0.022	0.023	0.026	0.028	0.030
Methyl allyl trisulfide	0	0	0	0.023	0.027	0.033	0.036	0.045
(1-Propenyl)-allyl disulfide	0	0.015	0.021	0.026	0.027	0.032	0.033	0.034
Diallyl trisulfide	0	0.088	0.12	0.25	0.26	0.32	0.33	0.37
(1-Propenyl)-allyl trisulfide	0	0.028	0.032	0.052	0.061	0.071	0.076	0.089

studied time interval except *E*- and *Z*-ajoenes, for which the concentration decreases after 8 days. It should be noted that if the starting concentration of allicin decreases by an order of magnitude within 15 days, the minor thiosulfinates are decomposed only by 60% during this time interval, which testifies to their relative stability in this solvent.

Decomposition of Thiosulfinates from Garlic Supercritical Extract in Acetonitrile

A solution of garlic SCE in acetonitrile was studied in a similar way. The experimental data are presented in Table 4.

As in the experiment with ethanol, in polar acetonitrile, the minor thiosulfinates are more stable than allicin. However, polysulfides formed within 4 days then begin to slowly decompose, and in 15 days their concentration is reduced by half in 15 days.

Decomposition of Thiosulfinates from Garlic Supercritical Extract in Dimethyl Sulfoxide

The experimental data on decomposition of garlic supercritical extract in dimethyl sulfoxide are presented in Table 5.

As it follows from Table 5, the character of decomposition of SCE thiosulfinates in dimethyl sulfoxide is similar to that in ethanol. Feature of the decomposi-

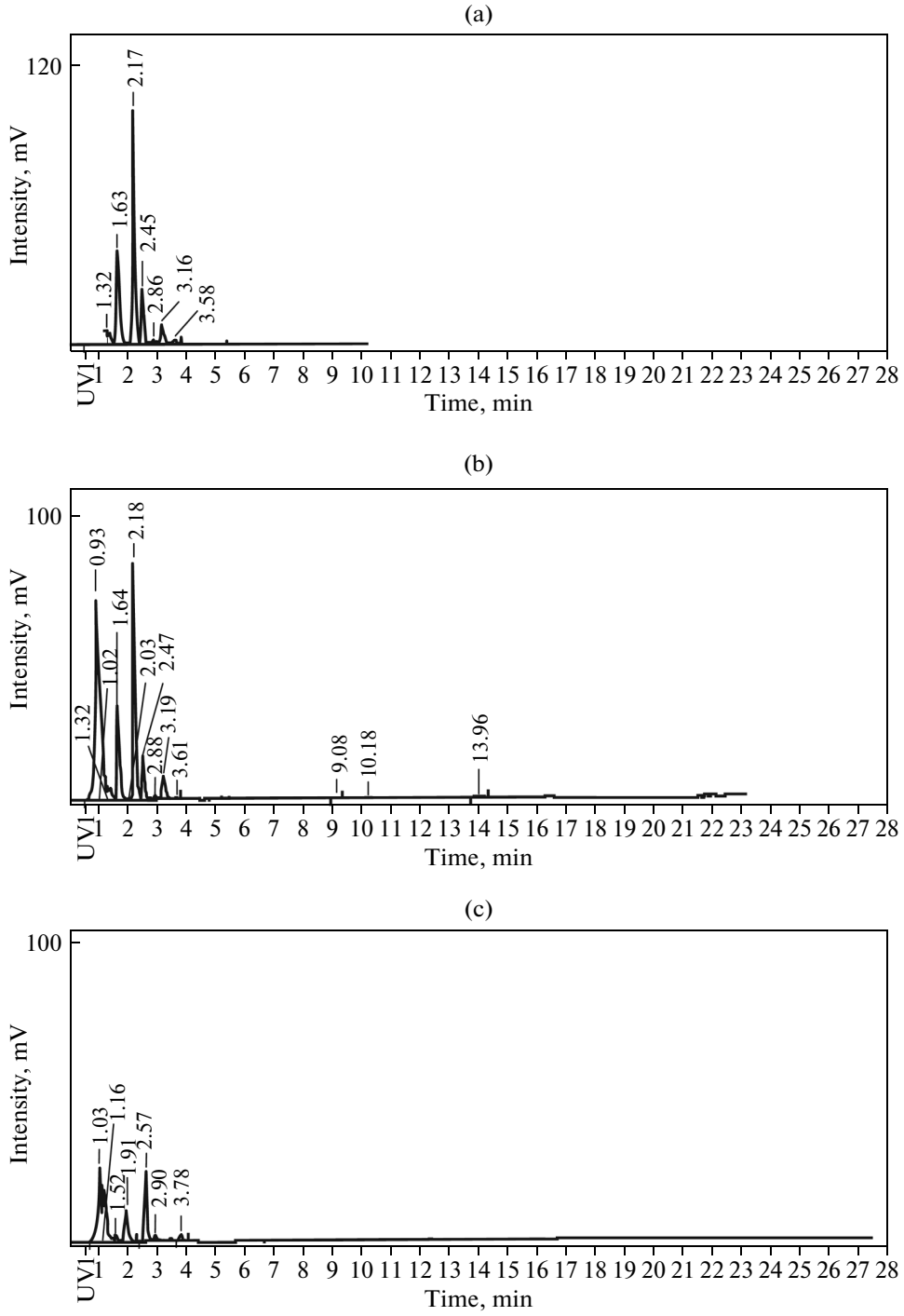

Fig. 3. HPLC chromatograms of thiosulfinate decomposition in water: (a) beginning of experiment, retention times (min): 2.17—allicin; 1.35–3.58—minor thiosulfinates; (b) 4 days, retention times (min): 2.18—allicin; 1.32–3.61—minor thiosulfinates; 10.18—(1-propenyl)allyl disulfide; 13.96—diallyl trisulfide; (c) 15 days, retention times (min): 2.28—allicin; 1.52–4.30—minor thiosulfinates.

tion in dimethyl sulfoxide is the formation of a significant amount of 3-vinyl-4*H*-1,2-dithiin that is not formed in other solvents, as well as the fact that polysulfides formed during 4 days remain almost unchanged up to the end of the experiment.

Decomposition of Thiosulfinates from Garlic Supercritical Extract in Dichloromethane

The experimental data on decomposition of garlic supercritical extract in dichloromethane are given in Table 6.

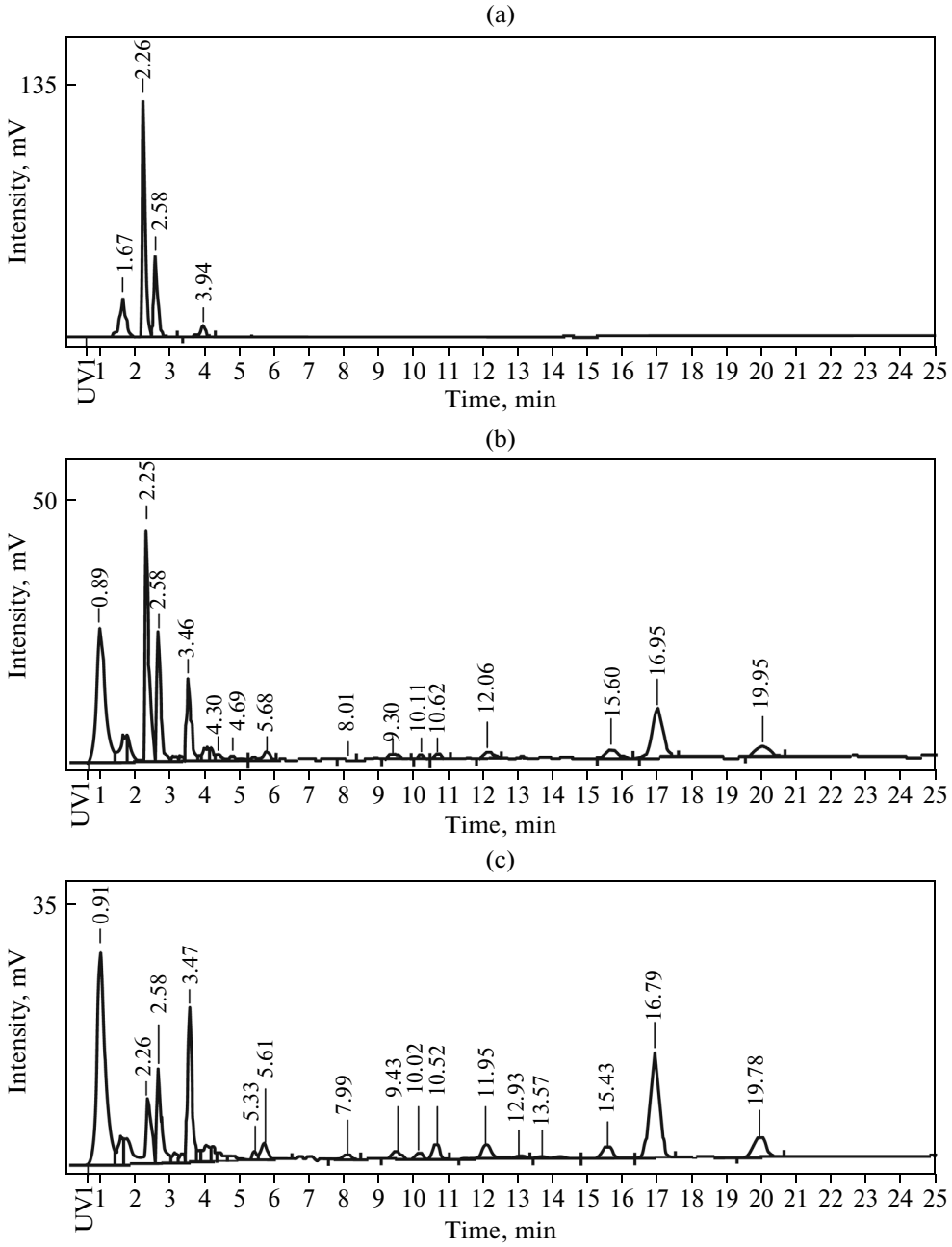

Fig. 4. HPLC chromatograms of thiosulfinate decomposition in ethanol: (a) beginning of experiment, retention times (min): 2.26—allicin; 1.67–3.94—minor thiosulfinates; (b) 5 days, retention times (min): 0.89—*E*/*Z*-isomers of ajoene; 2.25—allicin; 1.59–3.46—minor thiosulfinates; 5.68—2-vinyl-4*H*-1,3-dithiin; 8.01—3-vinyl-4*H*-1,2-dithiin; 10.11—diallyl disulfide; 10.62—methyl allyl trisulfide; 12.06—(1-propenyl)allyl disulfide; 16.95—diallyl trisulfide; 19.95—(1-propenyl)allyl trisulfide; (c) 15 days, retention times (min): 0.91—*E*/*Z*-isomers of ajoene; 2.26—allicin; 1.52–3.47—minor thiosulfinates; 5.61—2-vinyl-4*H*-1,3-dithiin; 7.99—3-vinyl-4*H*-1,2-dithiin; 10.02—diallyl disulfide; 10.52—methyl allyl trisulfide; 11.95—(1-propenyl)allyl disulfide; 16.79—diallyl trisulfide; 19.78—(1-propenyl)allyl trisulfide.

A typical HPLC chromatogram of the products of garlic SCE thiosulfinate decomposition in dichloromethane within 4 days is shown in Fig. 5.

A characteristic feature of SCE thiosulfinate decomposition in dichloromethane is the predominant formation of 2-vinyl-4*H*-1,3-dithiin and 3-vinyl-4*H*-1,2-dithiin. One can also note a higher stability of the minor thiosulfinates compared with allicin: their content remains almost unchanged whereas the allicin concentration is decreased by an order of magnitude.

Decomposition of Thiosulfinates from Garlic Supercritical Extract in Hexane

The experiments were carried out at room temperature; samples were taken every 1–3 h because of the

Table 4. Decomposition of garlic SCE thiosulfinates in acetonitrile

Analytes	Time-dependent changes in concentration of the solution components, mg/mL					
	beginning of experiment	4 days	5 days	6 days	8 days	15 days
Allicin	0.044	0.021	0.013	0.009	0.006	0.004
Sum of minor thiosulfinates	0.050	0.045	0.045	0.038	0.030	0.018
2-Vinyl-4*H*-1,3-dithiin	0	0.016	0.011	0.010	0.009	0.008
3-Vinyl-4*H*-1,2-dithiin	0	0	0	0	0	0
Diallyl disulfide	0	0.010	0.008	0.007	0.006	0.006
Methyl allyl trisulfide	0	0.005	0.004	0.003	0.002	0.002
(1-Propenyl)-allyl disulfide	0	0.010	0.009	0.007	0.007	0.006
Diallyl trisulfide	0	0.014	0.012	0.010	0.010	0.009

Table 5. Decomposition of garlic SCE thiosulfinates in dimethyl sulfoxide

Analytes	Time-dependent changes in concentration of the solution components, mg/mL				
	beginning of experiment	4 days	5 days	8 days	15 days
Allicin	0.050	0.020	0.016	0.014	0.009
Sum of minor thiosulfinates	0.048	0.040	0.015	0.014	0.010
2-Vinyl-4*H*-1,3-dithiin	0	0.012	0.011	0.011	0.009
3-Vinyl-4*H*-1,2-dithiin	0	0.02	0.032	0.034	0.035
Diallyl disulfide	0	0.015	0.015	0.015	0.015
Methyl allyl trisulfide	0	0.005	0.005	0.005	0.006
(1-Propenyl)-allyl disulfide	0	0.022	0.024	0.023	0.025
Diallyl trisulfide	0	0.021	0.025	0.027	0.028

Table 6. Decomposition of garlic SCE thiosulfinates in dichloromethane

Analytes	Time-dependent changes in concentration of the solution components, mg/mL					
	beginning of experiment	4 days	5 days	6 days	8 days	15 days
Allicin	0.25	0.09	0.09	0.06	0.04	0.03
Sum of minor thiosulfinates	0.23	0.20	0.20	0.18	0.18	0.17
2-Vinyl-4*H*-1,3-dithiin	0	0.025	0.028	0.029	0.030	0.031
3-Vinyl-4*H*-1,2-dithiin	0	0.005	0.005	0.005	0.004	0.004
Diallyl disulfide	0	0	0	0	0	0
Methyl allyl trisulfide	0	0	0	0	0	0
(1-Propenyl)-allyl disulfide	0	0	0	0	0	0
Diallyl trisulfide	0	0	0	0	0	0

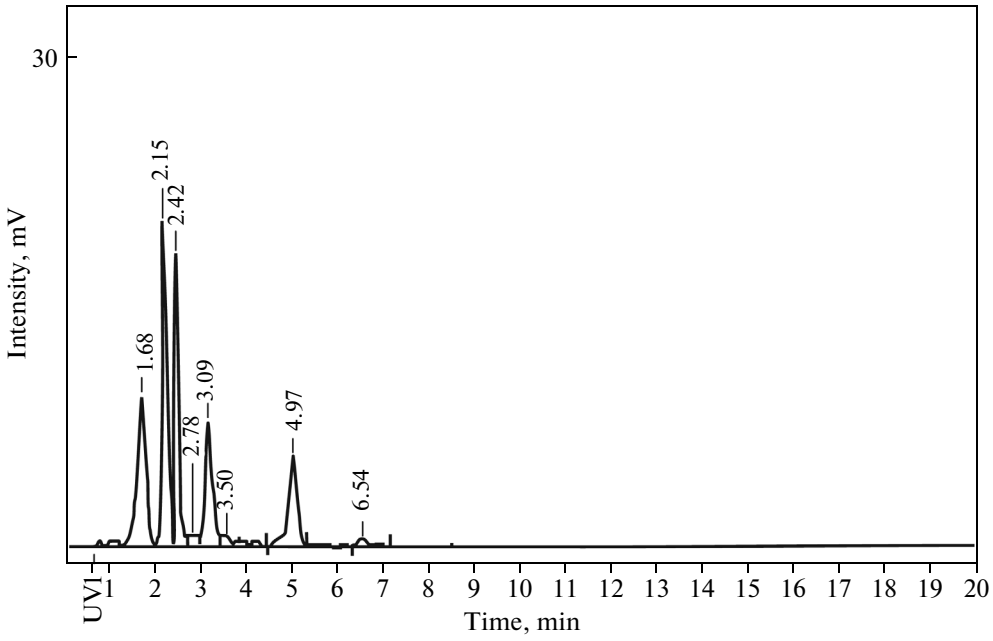

Fig. 5. HPLC chromatogram of thiosulfinate decomposition in dichloromethane in 4 days, retention times (min): 2.15—allicin; 1.62–3.50—minor thiosulfinates; 4.97—2-vinyl-4*H*-1,3-dithiin; 6.54—3-vinyl-4*H*-1,2-dithiin.

high rate of thiosulfinate decomposition in this solvent.

The data on changing concentration of the major garlic thiosulfinate, i.e., allicin, total concentration of minor thiosulfinates, as well as the products of their decomposition are given in Table 7.

HPLC chromatogram of the products of decomposition of garlic SCE thiosulfinates in hexane after 28 h is shown in Fig. 6.

A character of the SCE thiosulfinate decomposition in hexane resembles the process in dichloromethane. The predominate formation of 2-vinyl-4*H*-1,3-dithiin and some amount of 3-vinyl-4*H*-1,2-dithiin is observed. However, the rate of allicin decomposition is significantly higher than in dichloromethane. The allicin concentration is reduced by an order of magnitude in 28 h, which corresponds to the degree of decomposition in dichloromethane for

Table 7. Decomposition of garlic SCE thiosulfinates in hexane

Analytes	Time-dependent changes in concentration of the solution components, mg/mL							
	beginning of experiment	3.5 h	6.5 h	8.5 h	13.5 h	16.5 h	21 h	28 h
Allicin	0.038	0.023	0.016	0.012	0.009	0.008	0.004	0.004
Sum of minor thiosulfinates	0.044	0.044	0.043	0.043	0.042	0.041	0.039	0.039
Sum of *E*- and *Z*-ajoenes	0	0.011	0.013	0.022	0.017	0.019	0.017	0.015
2-Vinyl-4*H*-1,3-dithiin	0	0.004	0.005	0.007	0.008	0.009	0.007	0.005
3-Vinyl-4*H*-1,2-dithiin	0	0	0	0	0	0	0	0
Diallyl disulfide	0	0	0	0	0	0	0	0
Methyl allyl trisulfide	0	0	0	0	0	0	0	0
(1-Propenyl)-allyl disulfide	0	0	0	0	0	0	0	0
(1-Propenyl)-allyl trisulfide	0	0	0	0	0	0	0	0

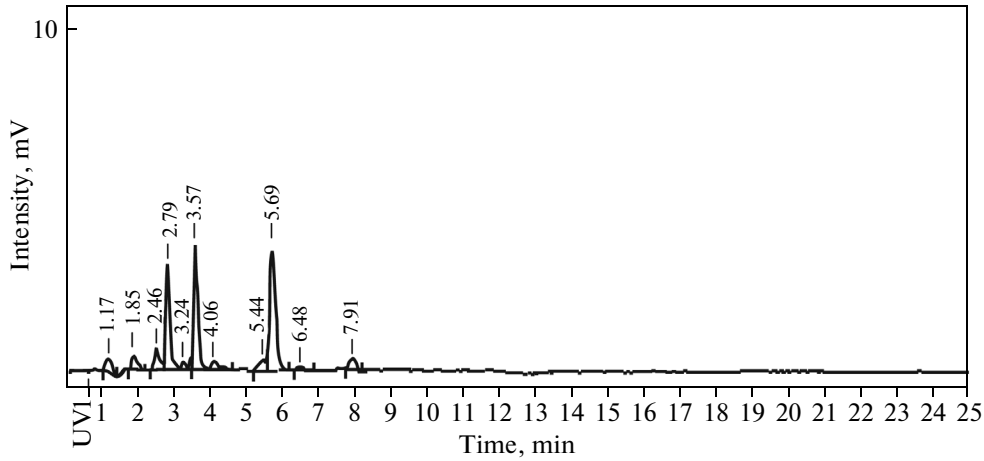

Fig. 6. HPLC chromatogram of thiosulfinate decomposition in hexane in 28 h, retention times (min): 2.46—allicin; 1.85–4.06—minor thiosulfinates; 5.69—2-vinyl-4*H*-1,3-dithiin; 7.91—3-vinyl-4*H*-1,2-dithiin.

15 days. Concentration of minor thiosulfinates remains almost unchanged within the experiment (28 h).

General Regularities of Decomposition of Garlic Supercritical Extracts in Polar and Nonpolar Solvents

The rates of decomposition of allicin and minor thiosulfinates in the most polar solvent, i.e., water are almost the same: 28–30% of the starting thiosulfinates are retained at room temperature within 15 days. After 4–5 days of the experiment, there are traces of polysulfides in the solution, they disappear by day 15. Apparently, the main process that occurs in the aqueous solution is the hydrolysis of sulfur-containing compounds, the hydrolysis products are failed to be identified.

The diagram of decomposition of allicin and minor thiosulfinates are shown in Fig. 7.

In ethanol, allicin and other thiosulfinates are decomposed in different ways. The initial concentration of minor thiosulfinates is reduced by 60–65%, whereas the allicin concentration is reduced almost by an order of magnitude. Allicin is mainly converted to *E*- and *Z*-ajoenes, as it is shown in Fig. 8, which corresponds to the published data [23].

Concentration of ajoenes in an ethanol solution grows during the first 8 days, and then slowly decreases. Allicin is likely to be partially converted to 2-vinyl-4*H*-1,3-dithiin and 3-vinyl-4*H*-1,2-dithiin through the formation of thioacrolein [24] according to the scheme given in Fig. 9.

The minor thiosulfinates are decomposed to form methyl and 1-propenyl analogues, as well as symmetrical polysulfides. All compounds, excluding ajoenes, formed in ethanol solution are well preserved throughout the whole experiment.

In acetonitrile, decomposition of the components of supercritical extract resembles the process in ethanol. A significant difference is the absence of *E*- and *Z*-ajoenes, 3-vinyl-4*H*-1,2-dithiin, and (1-propenyl)allyl trisulfide among the reaction products. All polysulfides formed within 4 days undergo a significant (1.5–2 times) degradation to the 15th day. The decomposition rate of the minor thiosulfinates is about three times less than the rate of allicin, which is almost completely decomposed in 15 days.

The rate of thiosulfinate decomposition in dimethyl sulfoxide is close to that observed in water (up to 20% of the initial thiosulfinates is retained). However, polysulfides formed within 4 days are well preserved to the end of experiment (15 days).

In nonpolar solvents, decomposition of thiosulfinates occurs somewhat differently. In dichloromethane, allicin is almost completely decomposed within 15 days, with about 75% of minor thiosulfinates retained during the same time. Only 3-vinyl-4*H*-1,2-dithiin and 2-vinyl-4*H*-1,3-dithiin were detected in solution among the decomposition products.

In another nonpolar solvent, hexane, the garlic SCE thiosulfinates behave themselves in a similar way and form the same decomposition products, namely 3-vinyl-4*H*-1,2-dithiin and 2-vinyl-4*H*-1,3-dithiin. The minor thiosulfinates are also not subjected to decomposition; however, the allicin decomposition proceeds almost 12 times faster than in dichloromethane.

In general, our investigations show that minor thiosulfinates are more stable in all studied solvents (especially, in nonpolar ones) than allicin. The products of allicin decomposition at room temperature are only 3-vinyl-4*H*-1,2-dithiin and *E*-, *Z*-ajoenes. Di- and trisulfides, such as diallyl disulfide, (1-propenyl)allyl disulfide, methyl allyl trisulfide, diallyl trisulfide, and (1-propenyl)allyl trisulfide are formed in

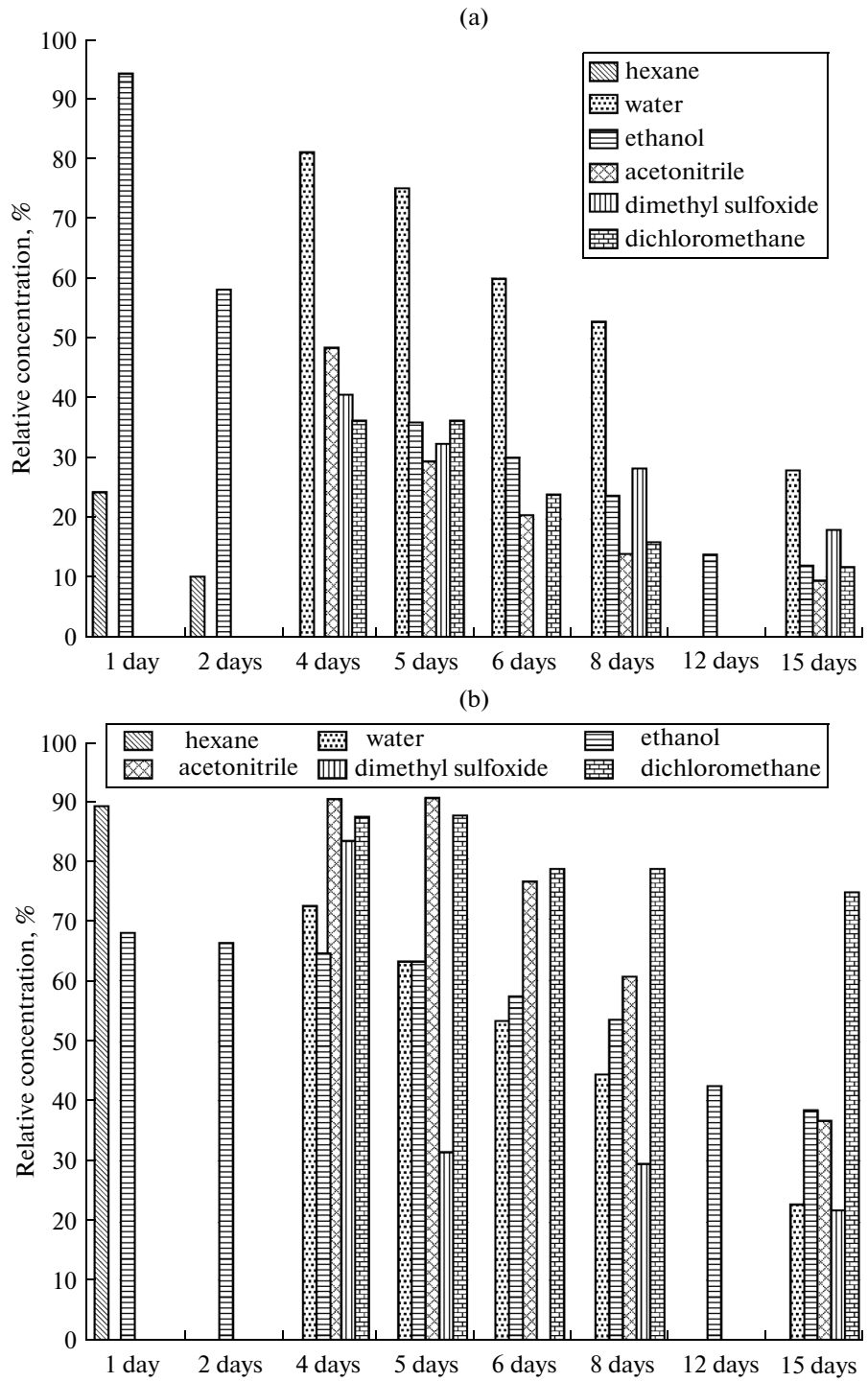

Fig. 7. Diagrams of decomposition of (a) allicin and (b) minor thiosulfinates from garlic supercritical extract in different solvents.

those solvents in which the decomposition of minor thiosulfinates does occur.

CONCLUSIONS

For the first time a systematic study of the decomposition of allicin and minor thiosulfinates from garlic supercritical extract was carried out in six solvents of different polarity.

In all cases, the dynamics of decomposition of the initial thiosulfinates was determined, qualitative and quantitative analysis of the products of their decomposition was carried out.

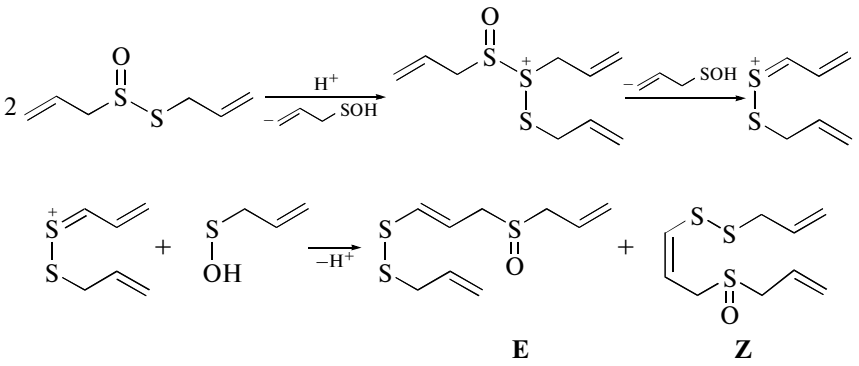

Fig. 8. Scheme of formation of **E**- and **Z**-ajoenes from allicin.

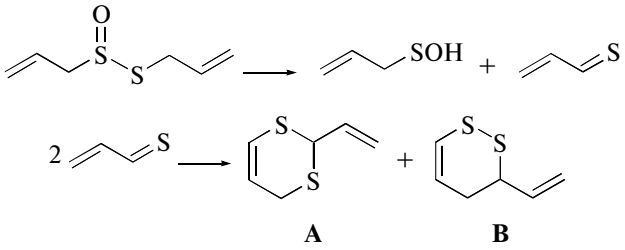

Fig. 9. Scheme of formation of 2-vinyl-4*H*-1,3-dithiin (**A**) and 3-vinyl-4*H*-1,2-dithiin (**B**) from allicin.

A new procedure for HPLC analysis was developed, which allows the simultaneous determination of both the starting thiosulfinates and the polysulfide products of their decomposition.

By changing the solvent polarity, one can direct the process of decomposition of thiosulfinates from supercritical extract toward the formation of the target sulfur-containing substances (ajoenes, dithiins, trisulfides, etc.).

The data obtained and methodical developments can be used in biomedical research of garlic-based drugs, as well as in the processes of their preparation, storage, and analysis.

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