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Allylthiosulfinate: β-cyclodextrin inclusion complex: preparation, characterization and microbiological activity

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An allylthiosulfinate: β-cyclodextrin inclusion complex was synthesized and characterized using X-ray crystallography, IR spectroscopy, thermal analysis and nuclear magnetic resonance. The microbiological activity of the complex was tested on available fungi (*Candida albicans* ATCC 10231, *Aspergillus niger* ATCC 16404) and bacteria (*Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9027). In small concentrations, the complex inhibited the growth of the microorganisms tested. The most susceptible microorganism was *Candida albicans* ATCC 10231, and the least susceptible *Pseudomonas aeruginosa* ATCC 9027.

1. Introduction

Allicin (allylthiosulfinate) is a thioester of sulfenic acid and, pharmacologically, it is the most important constituent of fresh aqueous extract of garlic (Lawson et al. 1991b; Nikolic et al. 2001). Allicin has a wide range of antimicrobial and antifungal effects (Block 1992; Cavallito and Bailey 1944 a; Cavallito et al. 1944 b; Davis et al. 1994). Garlic (*Allium sativum* L.) extract and allylthiosulfinate have a potential anti-tumor effect (Meng and Shyu 1990; Nagabhushan et al. 1992, Weisberger and Pensky 1958), and hypoglycemic activity (Bever and Zahd 1997), and reduce elevated blood pressure (Ernst 1987; Lau et al. 1983; Silagy and Neil 1994) and thrombocyte aggregation in blood of animals and humans (Briggs et al. 2000; Kiesewetter et al. 1991). The main drawback of allylthiosulfinate is its instability, so that, depending on the conditions, it can be transformed to other products that may be pharmacologically active, but are so not necessarily (Lawson et al. 1991a).

Cyclodextrins (CD) contain a hydrophobic cavity as opposed to their hydrophilic molecule periphery. Because of that, CD's are soluble in aqueous media and are capable of encapsulating guest hydrophobic molecules inside their cavities, thus forming CD inclusion complexes (Giastas et al. 2003, Pop et al. 2002). CD inclusion complexes with pharmacologically active substances are important compounds for the pharmaceutical and chemical industries, because they improve the stability, solubility and bioavailability of the guest molecule, i.e. of the inclusion compound (Angelova et al. 1999 a, 1999 b, Cortes et al. 2001).

2. Investigations, results and discussion

2.1. Characterization of the inclusion complex

The spectra of the synthesized allylthiosulfinate, β-cyclodextrin and allylthiosulfinate: β-cyclodextrin molecule inclusion complex are compared in Fig. 1.

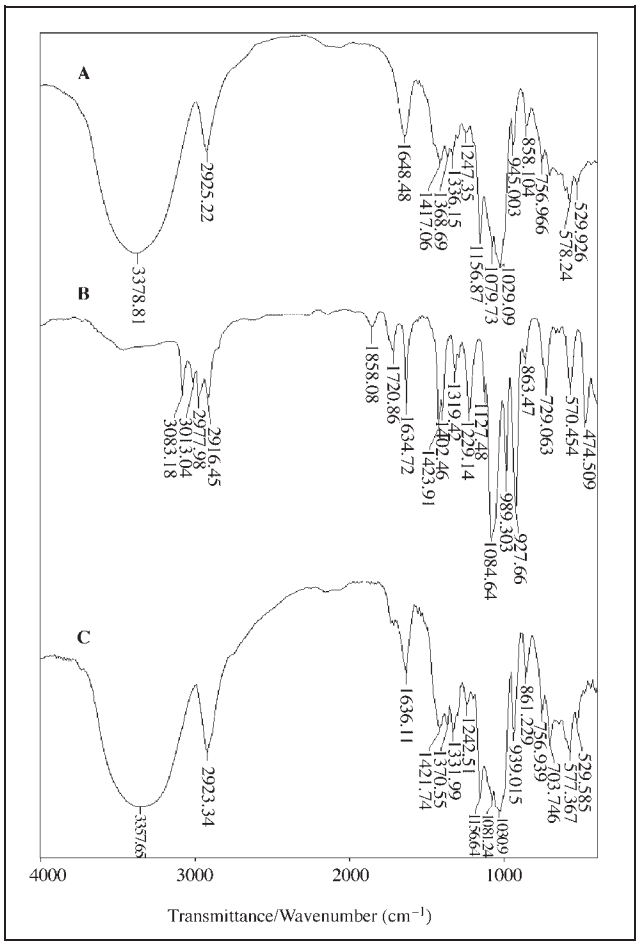


Fig. 1: IR spectra of β-cyclodextrin (A), allylthiosulfinate (B) and allylthiosulfinate : β-cyclodextrin molecular inclusion complex (C)

IR spectral analysis shows differences in some band positions in the complex with respect to the initial compounds. Firstly, the complex band centroid in the spectrum is shifted in the range of valence ν (OH) vibrations by about 20 cm^{-1} towards lower frequencies compared to the position of the similar band in the spectrum of β-cyclodextrin. This indicates that an interaction has occurred between allylthiosulfinate and β-cyclodextrin via hydrogen bonding, and that the complex is not a physical mixture. In the 1200–1000 cm^{-1} range, where in β-cyclodextrin the ν (C–O) and ν (C–O–C) bands could be expected, there are three bands: 1156, 1079 and 1029 cm^{-1}, while in the allylthiosulfinate spectrum there is one band at 1085 cm^{-1} that from its intensity, shape and position may be ascribed to ν (S=O) vibration. As expected, there are two bands in the complex spectrum, at 1156, and 1081 cm^{-1} with weak inflection on the high frequency side, and a band at 1030 cm^{-1}. The 1156 and 1030 cm^{-1} bands have the same frequencies for all compounds, except that the ν (S=O) vibration is shifted towards lower frequencies by 4 cm^{-1}, going from allylthiosulfinate to the complex, which is to be expected if the S=O group is involved in hydrogen bond formation in the complex. In other spectral regions the position, shape and intensity of the bands remained unchanged. Thus, the bands in the 945, 860, and 760 cm^{-1} regions of the complex spectrum characteristic of the C1 conformation of the glucopyranose moiety, have the same intensity, shape and position as those of β-cyclodextrin, indicating that the C1 conformation of the glucopyranose moiety has not been changed during the formation of the complex.

^{1}H NMR and ^{13}C NMR spectra for β-cyclodextrin, allylthiosulfinate and the allylthiosulfinate: β-cyclodextrin inclusion complex are given in Tables 1 and 2, respectively.

^{1}H NMR data clearly indicate the changes in the allylthiosulfinate and β-cyclodextrin spectra after inclusion. When the components, signals are compared with those of the complex, it can be seen that there are changes in the peak shapes and signal chemical shifts, as shown in Table 1. The greatest chemical shift of $\Delta\delta = -0.05$, is recorded with the H_3 proton in β-cyclodextrin, and the conversion of the triplet into a multiplet in the inclusion complex, which is the result of a strong hydrophobic interaction between the 'guest' and 'host' molecules. An approximate Δδ magnitude of −0.021 ppm was recorded for the H_6 and H_5 protons, the signals of these protons in the complex being converted into corresponding multiplets. Also, chemical shifts were observed for all the protons in allylthiosulfinate after formation of the complex, which supports the hydrophobic interaction of the components (Table 2).

^{13}C NMR analysis results show of Δδ chemical shifts 0.31 to 0.033 ppm for carbons in βCD, which also indicates the inclusion of ATS into βCD cavities, in accordance with previous investigations.

Table 1: ^{1}H NMR chemical shifts, δ(ppm) for H protons in β-cyclodextrin (βCD), allylthiosulfinate (ATS) and Δδ for protons in allylthiosulfinate : β-cyclodextrin inclusion complex (ATS : βCD) as compared to βCD and ATS

βCD	ATS	βCD δ	ATS δ	ATS : βCD Δδ	ATS : βCD Δδ
H_1	CH_2=C–	5.114*	5.5*	−0.014*	+0.05*
H_2	C=CH–C	3.694***	5.9***	−0.041***	+0.02***
H_3	C–CH_2–S=O	4.016**	3.84*	−0.05***	−0.053*
H_4	–C=CH_2	3.629**	5.5*	−0.039**	+0.05*
H_5	–C-CH=C	3.894*	5.9***	−0.02***	+0.02***
H_6	–S-CH_2-C	3.925	3.84*	−0.021***	−0.053*

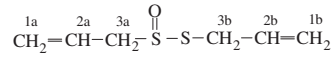
*doublet, **triplet, ***multiplet

Table 2: ^{13}C NMR chemical shifts, δ(ppm) for carbons in βCD, ATS and Δδ for carbons in ATS: βCD inclusion complex as compared to βCD

Carbon		βCD δ	ATS δ	ATS : βCD Δδ
βCD	ATS			
1	1a	104,56	118,556	+0,31
2	2a	74,77	133,366	+0,033
3	3a	75,77	53,603	+0,31
4	1b	83,82	118,366	+0,14
5	2b	74,49	123,967	+0,23
6	3b	62,97	33,178	− 0,18

The diffractograms are shown in Fig. 2 and correspond to the JCPDS-chart data for β-cyclodextrin (32–1626; 27; 28). The samples obtained are a white powder with a low degree of crystallinity.

Comparative analysis of the diffractograms in Fig. 2 shows a change of the position and intensity of certain peaks (indicated in the diffractograms), demonstrating that the inclusion of allicin into the β-cyclodextrin cavities has occurred without provoking changes in the structure of the host. The fact that no amorphous zones appear in the diffractograms



of the complex may indicate the possible inclusion of several allylthiosulfinate molecules in the β-cyclodextrin molecule cavity, depending on the β-cyclodextrin cavity size and the size of the allylthiosulfinate molecule, on one hand, and on the molecule forces interacting between the 'guest' and 'host' molecules, on the other.

Thermogravimetry (TG) curves for pure β-cyclodextrin and allylthiosulfinate and for the allylthiosulfinate: β−cyclodextrin inclusion complex are shown in Fig. 3.

The β-cyclodextrin TG curve shows high stability over a wide temperature interval between 303 and 593 K, above which the thermal decomposition process follows. The

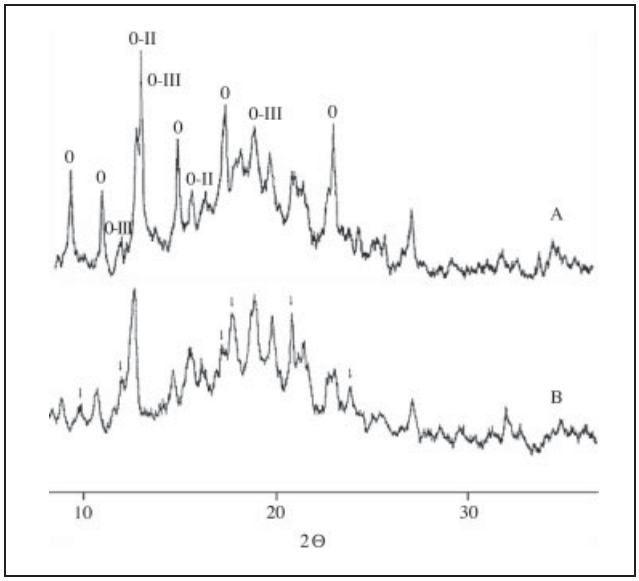


Fig. 2: Diffractograms of pure βCD (A) and ATS : βCD molecular inclusion complex (B)

Table 3: Microbiological activity of allylthiosulfinate and allylthiosulfinate : β-cyclodextrin complex with respect to the microorganisms tested

t, day	Inhibition zones, mm									
	C. albicans		*A. niger*		*E. coli*		*S. aureus*		*P. aeruginosa*	
	ATS	C	ATS	C	ATS	C	ATS	C	ATS	C
0	45	45.5	29	29	29.5	30	30	31	14.9	15
8	35.6	42	23.5	27.5	27.5	29.5	27.5	31	14	14.8
23	26	36	14	22	14	22	20.5	23	12.8	13
40	18.8	24.5	14	18.5	14	18.5	17.6	19	–	–
52	15.4	19	–	15.7	–	15.5	14.1	15.3	–	–
62	–	18.5	–	15.5	–	15.3	–	15	–	–

ATS – allylthiosulfinate, C – complex ATS:βCD

TG curve of allylthiosulfinate shows loss of mass beginning even at room temperature, and in a very narrow temperature interval from 303 to 393 K, the curve displays a steep fall, which shows that allylthiosulfinate is very unstable and volatile. The thermal decomposition regime of the complex shows obvious differences when compared to the pure compounds. The TG curve is more complex and has two transformations. The first transformation comes from decomposition of allylthiosulfinate, which is much slower when it is bonded than for the free compound, and continues from 313 K to 543 K, when complete decomposition is achieved. The second transformation comes from the β-cyclodextrin in the complex, which indicates a gradual loss of mass, and, infact, increased stability of β-cyclodextrin in the complex (the curve is shifted towards higher temperature by 40–60 K). These test results agree with previous results confirming that the complex is not a physical mixture and that some form of molecular interaction of the components in the complex has occurred.

The UV spectra given in Fig. 4 show a great difference between the absorption of allyl thiosulfinate and that of the inclusion complex, while the absorption of pure ß-cyclodextrin was negligible. The concentration of allyl thiosulfinate in the aqueous solution was the same as in the complex solution, and this demonstrates that a complex of allyl thiosulfinate and cyclodextrin had formed, not just a physical mixture of the two compounds.

2.2. Microbiological study

The fungicidal and bactericidal properties of allylthiosulfinate and the inclusion complex were investigated at different time intervals after preparation, as it is well known that allylthiosulfinate is unstable and undergoes degradation and loss of microbiological activity during storage. The results of these tests are given in Table 3.

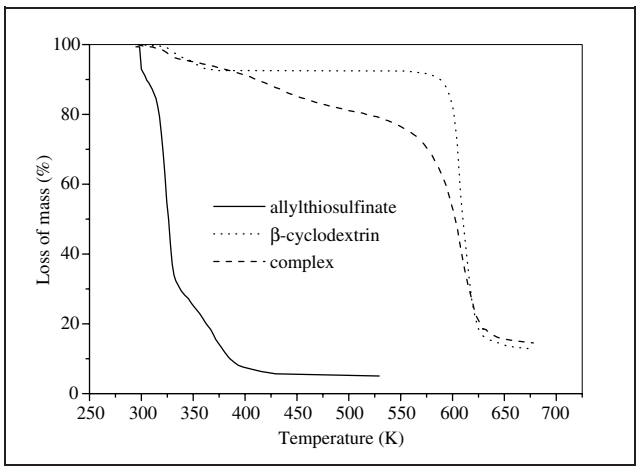


Fig. 3: TG curves of β-cyclodextrin, allylthiosulfinate and ATS : βCD inclusion complex

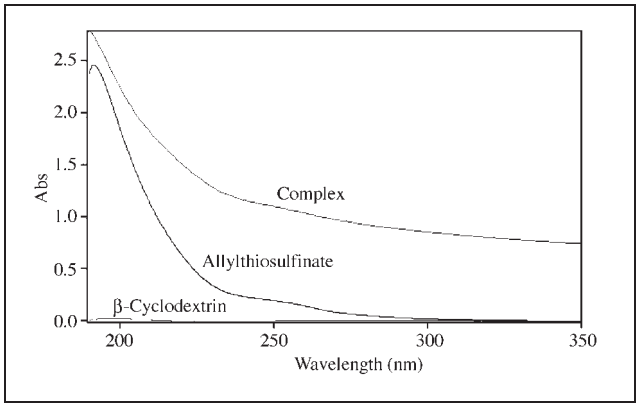


Fig. 4: UV spectra of aqueous solution of ß-cyclodextrin (300 μg/cm^3), allyl thiosulfinate (42.9 μg/cm^3) and allyl thiosulfinate: ß-cyclodextrin inclusion complex (1 : 1) for identical concentrations of allyl thiosulfinate both in the complex and in the aqueous solution of allyl thiosulfinate

The allylthiosulfinate: β-cyclodextrin inclusion complex shows greater stability and microbiological activity against all the microorganisms tested, compared to allylthiosulfinate. Minimal microorganism growth inhibition zones were reached thirty days after the synthesis of allylthiosulfinate, while the complex retained its activity to a significant degree even after sixty days. The most susceptible microorganism was *Candida albicans*, and the least susceptible *Pseudomonas aeruginosa*. These investigations confirm the formation of a new supramolecular compound ATS : βCD, in which allylthiosulfinate stability is maintained and its microbiological activity and shelf life prolonged.

3. Experimental

3.1. Reagents

Allyldisulfide (80%, ϱ 1.008 g/cm^3) was bought from Aldrich Chemical Co. and β-cyclodextrin (98%) was bought from Merck. Remaining reagents used in our work are of analytical quality.

3.2. Synthesis of allylthiosulfinate

Allylthiosulfinate was synthesized from allyldisulfide by the procedure described elsewhere (Nikolic et al. 2004), and was used as such for the preparation of the inclusion complex. The allyl thiosulfinate concentration was determined by the method given in the literature (Nakata et al. 1970).

3.3. Preparation of the inclusion complex

For preparation of the molecular inclusion complex of allicin with β-cyclodextrin the solid complex formation technique was used. Allylthiosulfinate,

synthesized and purified by multiphase ether extraction, was added to a dense mixture of β-cyclodextrin and distilled water, mixed at room temperature until a thick paste was obtained and then dried in a desiccator over a dehydrating agent (concentrated sulfuric acid) at about 10 °C for 24 hours. The quantities of allyl thiosulfinate and ß-cyclodextrin used for the complex preparation were such that the mole ratio in the complex was 1 : 1. The concentration of allyl thiosulfinate in the inclusion complex was determined by extraction with diethyl ether, evaporation of diethyl ether, dissolving the residue in 2-propanol, and proceeding according to the method described by Nakata et al. (1970).

3.4. UV spectroscopy

UV spectra were recorded in water on a Cary 100 Conc. UV VIS spectrophotometer in quartz cells with 1 cm pathlength. UV, λ_{max} (H_2O): 198 and 254 nm.

3.5. FT-IR spectroscopy

IR spectra were recorded on a MB-series Bomem Hartmann & Braun FT-IR spectrophotometer in the wavelength range from 4000 to 400 cm^{-1}, with allylthiosulfinate between KBr plates with a 0.1 μm film thickness, and the complex and β-cyclodextrin in KBr pellets.

3.6. ^{1}H NMR and ^{13}C NMR spectrometry

^{1}H NMR and ^{13}C NMR spectra were recorded in D_2O on a Bruker AC 250 E spectrometer with operating frequencies of 250 and 62.5 MHz, respectively, in 5 mm dia glass cuvettes at room temperature by the impulse method with multiple repetitions of scans to obtain ^{13}C NMR spectra.

3.7. X-ray crystallography

Diffractograms were made on a Philips X'pert powder diffractometer under the following conditions: samples were radiated by a monochromic CuK_α beam and diffraction angles analyzed between 5 and 40°. The voltage, current strength, step and time per step were 45 kV, 40 mA, 0.02 and 5 s/step, respectively.

3.8. Thermogravimetric analysis

Thermogravimetric curves were recorded on a Bomem TG/plus apparatus in the temperatures range from 303–673 K with 10 K/min linear increments. The sample mass was 10 mg.

3.9. Microbiological tests

The microbiological activity of allylthiosulfinate and the complex were observed by the diffusion method with different time intervals. 180 μg of allylthiosulfinate, in complex or free, was applied to the 12.7 mm dia disk. The samples were incubated at 37 °C (bacteria) and 25 °C (fungi) for 24 h. The following microorganisms were used: Bacteria: *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9027, and fungi: *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404. For bacteria B-1 *Bacto antibiotic medium 1 dehydrated* (Difco Laboratories, Detroit, U.S.A.) medium was used, and *Tripton soya-agar* (Torlak Institut za imunologiju i virusologiju, Belgrade) for fungi.

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