water and 7.7 g (55 mmole) of bromoacetic acid were added. The solution was stirred for 15 min at 0°. After that time all the phosphorothioate had reacted. A solution of 20 g (82 mmole) barium chloride dihydrate and 2.2 g (55 mmole) of sodium hydroxide in 75 ml of water was then added under stirring at 0°. The precipitate was filtered off and washed with 50 ml of ice cold water and with 100 ml of ethanol. 13.1 g (61 %) of substance were obtained after drying in vacuum. (Found: C 5.5; H 1.7; P 7.1; Ba 48.5. Calc. for $(BaPO_3SCH_2COO)_2Ba,6H_2O$ (858.34): C 5.6; H 1.9; P 7.2; Ba 48.0.) The prepared substance hydrolyzes spontaneously upon storage.

Acid hydrolysis of the prepared compounds. 7.61 mmole of $Na_2PO_3SCH_2CH_2COOCH_3$, 1.5 H_2O were hydrolyzed for 30 min in 50 ml of 1 M hydrochloric acid under nitrogen at 100°. Hydrogen sulfide or phosphorothioate were not detected after hydrolysis. The hydrolysate consumed 7.46 matom of iodine as titrated with a standardized iodine solution (98 % of theoretical amount). The oxidized hydrolysate was extracted with peroxide free ethyl ether giving 0.8 g of 3,3'-dithiodipropionic acid, m.p. 154—155° (from 4-methyl-2-pentanone), undepressed by admixture with an authentical sample.

3.37 mmole of $(BaPO_3SCH_2COO)_2Ba$, $6H_2O$ were hydrolyzed as described above. The hydrolysate consumed 6.41 matom of iodine (95 % of theoretical amount). Hydrogen sulfide or phosphorothioate could not be detected in the hydrolysate, which was then tested for glycolic acid by the two tests described by Feigl [5]. These were both negative. The thiol present in the hydrolysate was identified as mercaptoacetic acid by the paper chromatographic method recently described [6]. For chromatography the solvents Nos. 3 and 4 in Ref.[6] were used.

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The Isolation of S-Methylcysteinesulphoxide and *S-n*-Propylcysteinesulphoxide from Onion *(Allium cepa)* and the Antibiotic Activity of Crushed Onion

ARTTURI I. VIRTANEN and E. J. MATIKKALA

Laboratory of the Foundation for Chemical Research, Biochemical Institute, Helsinki, Finland

Fresh homogenized onions *(Allium cepa)* have a strong antimicrobial effect. 50 to 100 mg of crushed onions in 1 ml of nutrient solution inhibits the growth of the *Staphylococcus aureus* strain used in our experiments completely, and 15 mg still has a retarding effect on the growth. When the enzymes in whole onions *(Allium cepa)* are inactivated using different methods (heating to about 100°C in a sealed glass tube, or keeping in boiling ethanol for a shorter time, or homogenizing with ethanol after freezing with CO_2 ice) the antimicrobial effect of the extracts is again very low. A total inhibition is not then yet achieved with extracts corresponding to 1 g of onion in 1 ml of nutrient solution. Most of the antimicrobial activity is thus formed in onion through enzymatic reactions.

In a lecture the senior author [1] earlier mentioned that according to investigations in this laboratory onion contains both S-methylcysteinesulphoxide (MCSO) and S-*n*-propylcysteinesulphoxide (PCSO) from which the corresponding thiolsulphinates are formed enzymatically. These have a strong antibiotic effect against many microbes. This effect is generally somewhat weaker than that of allylthiolsulphinate formed from S-allylcysteinesulphoxide present in garlic *(Allium sativum)*, but nevertheless of the same order of magnitude [2].

PCSO was isolated from onion in the following way: 3 kg of chilled onions grown in Finland were extracted with cold methanol (added methanol + the water contained in the onion = 80 % methanol). The free amino acids were separated on an Amberlite IR-120 column and fractionated with water on a 2.2 × 95 cm column filled with Dowex 1 resin (the resin in acetate form was washed with water).

When 100 ml of solution had been let through, fractions of 8 ml each were taken.

Fractions 15—42 were combined, evaporated, and the extract obtained was fractionated on a column (3 × 105 cm) containing Dowex 50 resin in the H-form (1 N HCl). Fractions of 20 ml each were taken. PCSO emerged in fractions 172—218 together with some other amino acids. These fractions were combined, the amino acids separated in an Amberlite IR-120 column and refractionated in a 3.3 × 80 cm column containing Dowex 50 resin in equilibrium with 1.5 N HCl. 470 ml of the eluant emerging first from the column were discarded, then fractions of 20 ml each were taken. PCSO emerged in fractions 205—230. These fractions were combined and the amino acids separated in an Amberlite IR-120 column. Besides PCSO the solution still contained valine. The solution, freed from ammonia, was therefore fractionated on a cellulose powder column (3.2 × 34 cm) using water-saturated butanol as solvent. PCSO emerged in pure form in fractions 67—85 (12 ml/30 min). It was crystallized from a water-acetone solution. The crystals (30 mg) were small needles. When this substance and synthetic PCSO were run on a two-dimensional chromatogram (butanol-acetic acid-water and phenol-water-NH_3) the spots covered each other completely. (Found: C 40.63; H 7.71. Calc. for $C_6H_{13}O_3NS$: C 40.20; H 7.31.)

The thioether formed from PCSO on reduction was also isolated in crystalline form. On paper chromatographic comparison with synthetic S-*n*-propylcysteine both compounds showed to be identical. (Found: N 8.60. Calc. for $C_6H_{13}O_2S$: N 8.58.)

In order to ascertain that the PCSO isolated from onion is an *n*-propyl and not an *iso*-propyl derivative, S-*iso*-propylcysteine was prepared from L-cysteine and *iso*-propylbromide according to Stoll and Seebeck [3]. It was further oxidized with H_2O_2 to the sulphoxide. When comparing the travelling of PCSO with that of the obtained S-*iso*-propylcysteinesulphoxide on a one-dimensional paper chromatogram (solvent butanol-acetic acid-water), the *iso*-propyl compound was found to move so much slower than PCSO that when the substances had reached the middle of a Whatman sheet the spots separated from each other. Synthetic S-*n*-propylcysteinesulphoxide moved with the same speed as the PCSO isolated from onion. On the basis of this the structure of PCSO can be established as S-*n*-propylcysteinesulphoxide.

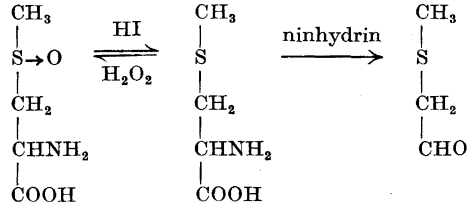
$$CH_3 \cdot CH_2 \cdot CH_2 \cdot SO \cdot CH_2 \cdot CHNH_2 \cdot COOH \underset{H_2O_2}{\overset{HI}{\rightleftharpoons}} CH_3 \cdot CH_2 \cdot CH_2 \cdot S \cdot CH_2 \cdot CHNH_2 \cdot COOH$$

S-*n*-propylcysteinesulphoxide (PCSO) or dihydroalliin

S-*n*-propylcysteine (PCS)

The isolation of MCSO. A methanol extract was prepared from 3 kg of onions in the same way as when PCSO was isolated. Amino acids were also separated on an Amberlite IR-120 column as before. The first fractionation was performed on a Dowex 1 resin (column 2.5 × 83 cm) in 0.5 N acetic acid form. When 150 ml of the eluant had emerged, fractions of 9 ml/20 min were taken. Fractions 3—30 were evaporated and then fractionated with a solvent of butanol-acetic acid-water on a cellulose powder column (5.7 × 80 cm). Because of the thick syrupy consistence the evaporated solution had to be placed on the column in 100 ml, and hence the separation of amino acids was not good. Fractions which contained MCSO and *cyclo*-alliin [4] were evaporated and fractionated on Dowex 50 resin in 0.5 N HCl form (column 3.1 × 112 cm). Fractions of 10 ml/25 min were first taken and then 350 fractions of 15 ml/20 min, *i. e.* 570 fractions in all. The solvent was still 0.5 N HCl. MCSO emerged in pure form in fractions 419—445. It was crystallized with ethanol from water solution. Yield 149 mg. It was identified by paper chromatography with S-methylcysteinesulphoxide which had earlier been isolated in this laboratory from *Capsella bursa pastoris* and chemically characterized. (Found: N 9.23. Calc. for $C_4H_9NSO_3$: N 9.27.) *Cyclo*alliin emerged in fractions 529—560. 4.6 g were obtained as crystals.

The structure of MCSO isolated from onion was confirmed by first reducing it to S-methyl-



S-Methylcysteine-sulphoxide S-Methyl-cysteine Methylthio-acetaldehyde

R_F-Values (descending chromatograms, Whatman No. 1 paper).

	Butanol-acetic acid-water	Phenol-water (NH_3-atm.)
S-Methylcysteinesulphoxide, synth.	0.10	0.73
» from onion	0.10	0.73
S-Methylcysteine	0.30	0.74
S-*n*-Propylcysteinesulphoxide, synth.	0.26	0.81
» from onion	0.26	0.81
S-*n*-Propylcysteine	0.56	0.86
S-*iso*Propylcysteinesulphoxide, synth.	0.23	0.81
S-*iso*Propylcysteine	0.54	0.88
Alanine	0.20	

cysteine and oxidizing this with ninhydrin to S-methylthioacetaldehyde according to Virtanen *et al.*[5]

30 mg of MCSO were added to 1 ml of 57 % HI. The solution was allowed to stand for 3 h after which a small amount of water was added. After extraction with ether the water solution was passed through a column containing Dowex 1 in acetate form (1.3 × 13 cm, resin 200—400 mesh). S-Methylcysteine was eluted with 100 ml of water. The solution was evaporated to dryness *in vacuo*, and the residue washed with 20 ml into a distillation flask which contained 3 g of KH_2PO_4, 4.5 g of NaCl, and 75 mg of ninhydrin. 10 ml of water was added, and the mixture was distilled with steam for 40 min. The distillate, 380 ml, was taken up in 20 ml dimedone solution. The crystals formed during 24 h were separated by filtration. They were recrystallized two or three times from an alcohol-water solution. The crystals melted at 126—127°C. The m. p. of the methylthioacetaldehyde, obtained in a similar way from synthetic S-methylcysteinesulphoxide, was the same, and so was the mixed m. p. The IR-spectra also confirmed the identity of the compounds.

Semi-quantitative estimation of MCSO and PCSO. 100 g of chilled, peeled onions were extracted in 85 % methanol. After 24 h the extract was filtered, and the procedure was repeated twice. The residue was pressed as dry as possible. The combined methanol solutions were passed through an Amberlite IR-120 column, and amino acids were eluted from the resin by 1 N ammonia. Two-dimensional chromatograms were run (butanol-acetic acid-water and phenol-water-NH_3) with different amounts of the evaporated extract. Parallel chromatograms were run with different concentrations of MCSO and PCSO. By comparing the colour of the spots it was possible to calculate that the onions used had contained about 50 μg of PCSO per 1 g of fresh material. The error of determination hardly exceeds ± 10 %. Using the above solvents MCSO travels in much the same way as does glutamine. Another solvent system had therefore to be used in the determination of MCSO. Substituting butanol-acetic acid-water with the solvent butanol-benzylalcohol (1:1)-water, the time of running being 9 days, MCSO could be separated from glutamine. 200 μg of MCSO were then found in 1 g of fresh onion. In another batch of onion 60 μg of PCSO and 240 μg of MCSO were found.

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