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Short Communications

Synthesis of Disodium S-[2-(methoxycarbonyl)ethyl] Phosphorothioate and a Barium Salt of S-(carboxymethyl) Phosphorothioic Acid

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Recently [1] it was shown that trisodium phosphorothioate can be used for the preparation of a S-substituted phosphorothioic acid. Thus 2-bromoethylamine was found to react quantitatively with trisodium phosphorothioate in the presence of N,N-dimethylformamide to yield a sodium S-(2-aminoethyl) phosphorothioate.

To investigate the usefulness of trisodium phosphorothioate as a starting material for the preparation of other S-substituted phosphorothioic acids the reaction between this compound and acrylic acid methyl ester and bromoacetic acid has now been investigated. In both cases a rapid reaction was observed without the presence of a catalyst. Bromoacetic acid was found to react with trisodium phosphorothioate in a manner analogous to that described for 2-bromoethylamine [1], whereas acrylic acid methyl ester adds the phosphorothioate to the double bond forming a S-[2-(methoxycarbonyl) ethyl] phosphorothioate:

$$CH_2 = CHCOOCH_3 + H_2O + Na_3SPO_3 \rightarrow Na_2PO_3SCH_2CH_2COOCH_3 + NaOH$$

The addition takes place in contradistinction to Markownikoff's rule. This behaviour is similar to that observed by Dahlbom [2] in the diethylamine catalyzed reaction between acrylic acid and hydrogen sulfide.

The prepared substances are easily hydrolyzed by acids to the corresponding thiols. Thus these compounds constitute intermediates in a route for the preparation of thiols.

Materials and methods. Trisodium phosphorothioate was prepared according to Yasuda and Lambert [3]. Acrylic acid methyl ester (stabilized with *p*-methoxyphenol) and bromoacetic acid were products of Eastman Kodak Company. Phosphate determinations were performed according to Gomori [4] after hydrolysis of the samples in 1 M hydrochloric acid at 100° for 30 min. When barium ions were present, the hydrolysis was carried out in the presence of sulfuric acid and the precipitated barium sulfate was removed before analysis.

Melting points are corrected.

Preparation of disodium S-[2-(methoxycarbonyl)ethyl] phosphorothioate. 10 g (56 mmole) of trisodium phosphorothioate were dissolved in 75 ml of water and 10 ml (110 mmole) of acrylic acid methyl ester were added. The mixture was vigorously stirred* for 5 min after which time all phosphorothioate had reacted (no black precipitate obtained with silver ions). 200 ml of ethanol were added and the precipitated oil was separated. 100 ml of methanol and 100 ml of ethanol were added to the oil in the order indicated under stirring. The precipitated substance was filtered off and dried in vacuum. Yield 10.2 g (68 %). (Found: C 17.6; H 3.7; P 11.4. Calc. for $Na_2PO_3SCH_2CH_2COOCH_3$, 1.5 H_2O (271.16): C 17.7; H 3.7; P 11.4.)

Preparation of barium S-(carboxymethyl) phosphorothioate. 9.0 g (50 mmole) of trisodium phosphorothioate were dissolved in 60 ml of

* The methoxycarbonyl group was found to be easily hydrolyzed when the reaction was carried out at temperatures above *ca.* 25°.

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

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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.