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# REACTION OF METHIONINE WITH BORON TRIBROMIDE AND BORON TRIIODIDE

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Cleavage of ethers with boron trihalides has been well studied (1, 2, 3). Boron trihalides and ethers form complexes which easily disproportionate leading to cleavage of the ether. The purpose of the present work was to investigate the applicability of this reaction to cleavage of the thioether side-chain of methionine as a possible approach for modification of methionine residues in proteins.

Methionine (4 mg) was stirred with $BBr_3$ (0.5 ml) for 88 hr at 0°. Examination by thin layer chromatography (Eastman Chromatogram Sheet; Silica Gel) in n-butanol-acetic acid-water (4:1:5 v/v) and by high voltage electrophoresis (3000 volts, 50 min; at pH 3.65 in pyridine-acetic acid-water 3:30:867 v/v) showed that little or no methionine had remained and at least three new reaction products had appeared in the reaction mixture. To test the selectivity of the reaction for methionine modification, each of leucine and valine was reacted with $BBr_3$ at 50° for 5 days and the reaction mixtures were examined by high voltage electrophoresis and quantitative amino acid analysis. A trace (less than 0.5%) of a single reaction product appeared in each case. Reaction of methionine with 5 molar excess of $BBr_3$ or $BI_3$ in chloroform was therefore examined under various conditions using internal standards of valine and leucine. The rate of disappearance of methionine was quantitatively determined by amino acid analysis. In reaction with $BBr_3$ at 22°, methionine contents in reaction mixture were: 0 time, 100%; 46 hr., 84%; 128 hr., 63%. On reaction with $BI_3$ at 22°, methionine contents were: 0 time, 100%; 46 hr., 79%; 167 hr., 6%. The rate of disappearance of Met at 50° was about twice as rapid as in the 22° reaction. By quantitative amino acid analysis, reaction products (from reaction of methionine with 5 molar excess of $BI_3$ at 50°) and their elution times were as follows: III, 22%, elution time, 19 min (superimposes with homocysteic acid); V, 20%, elution time, 9 min after aspartic acid (superimposes with homoserine); VI, 38%, elution time, 8 min after lysine (superimposes with homoserine lactone). The identity of these compounds was also confirmed by thin layer chromatography and by high voltage electrophoresis.

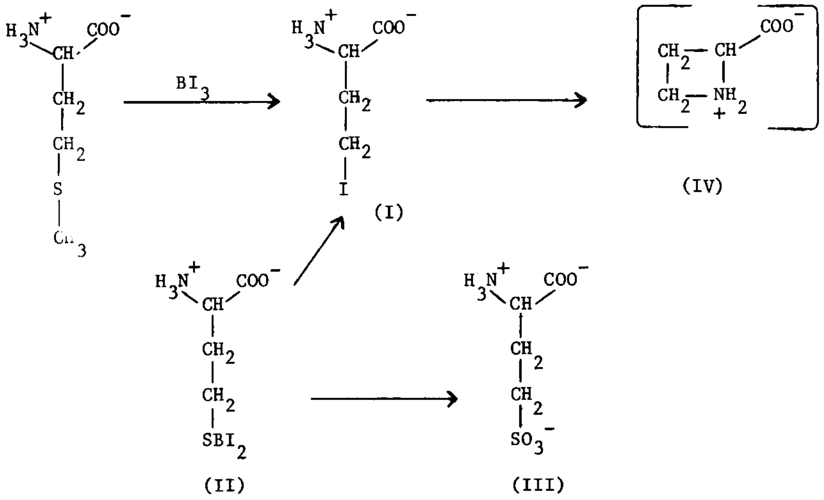
The reaction was examined for methionine modification in proteins. For reaction, duplicate samples of the protein (2-3 mg each) were reacted with 0.5 ml of $BBr_3$ or 0.5 ml of saturated $BI_3$ solution in $CHCl_3$. Mixtures were stirred magnetically at room temperature for about 100 hr., after which the liquid was evaporated by a stream of nitrogen followed by vacuum distillation or sublimation. Cold (0°) water was then added (1 ml) to each sample and the material dialyzed extensively against distilled water and freeze-dried. Acid and alkaline hydrolysates of the protein samples were subjected to duplicate amino acid analysis as described elsewhere (4). On reaction of sperm whale myoglobin with $BBr_3$, the methionine content decreased from 2.0 to 0.25 residues per mole. With human hemoglobin, by reaction with $BBr_3$ or with $BI_3$ the methionine content decreased from 3.0 to 1.50 residues in each case. Finally, reaction of lysozyme with $BI_3$ led to decrease of the methionine content from 2.0 to 1.1 residues per mole. With each protein, the contents of all other amino acids remained unchanged. However, in one single case out of five replicate reactions of myoglobin with $BBr_3$, modification of tyrosine was encountered and the tyrosine content decreased from 3.0 to 2.2. This might have been caused by destruction due to incomplete removal of salts in the dialysis step. In each case, the reduction in the methionine content was entirely accounted for by the appearance of homoserine lactone.

In an analogy to ethers, boron trihalides will cleave thioethers as follows when reactants are in 1:1 molar rates:

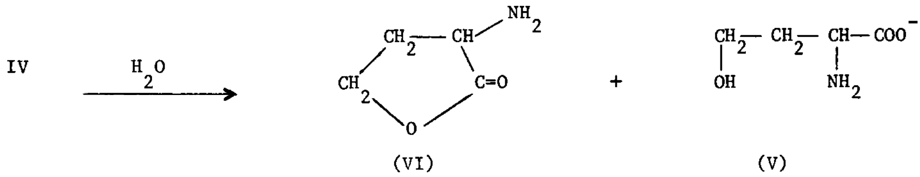
$$\text{R-S-R} + BI_3 \longrightarrow RSBI_2 + RI$$

$$3RSBI_2 \longrightarrow BI_3 + B_2S_3 + 3RI$$

With methionine the reaction will be more complex. The first reaction product, 1-amino-3-iodobutyric acid (I), is either produced directly or through 1-amino-3-thioxydiiodoborane butyric acid (II).



In addition compound II may oxidize in air to give homocysteic acid (III). Compound (I) will give the cyclic intermediate (IV). The opening of the ring structure of IV will give rise to homoserine (V) and homoserine lactone (VI)



Compounds (III), (V) and (VI) account for the major reaction products. Other reaction products may be explained by bimolecular condensation mechanisms and will account for the complexity of the reaction.

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