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AMINO ACIDS AND PEPTIDES. IV.[1]
SELECTIVE REDUCTION OF PEPTIDE-ESTER GROUPS[2]

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Chemical modification of carboxyl groups in protein or enzyme has been a very important subject in chemical studies of protein. The recent reports by Atassi and Rosenthal on the reduction of carboxyl groups in proteins[3] and peptides[4] with diborane prompted us to publish the results we have obtained concerning some model experiments on the reduction of peptides and peptide esters.

Reduction of Peptides with Diborane

Ac.DL-PheOH (Ia) or Bz.DL-AlaOH (Ib) was treated with diborane in tetrahydrofuran at room temperature as shown in Table I. Beside the expected amino alcohol (IIa or IIb), the fully reduced product (IIIa or IIIb) in which acyl bond was converted to the amine, together with the additional product (IVa or IVb) to which oxazolidine structure was tentatively assigned, were isolated in considerable amounts. Because of these accompanying reductive side-reactions, yield of the reduction of carboxyl group was unsatisfactory.

Further, Bz.Gly.DL-AlaOH was subjected to reaction followed by acid hydrolysis and the resultant products were converted to a mixture of N-trifluoroacetylamino acid butyl esters, which was analyzed by gas chromatography.[5] The recovery of amino acids

Table I. Reduction Products

From Ia			From Ib		
IIa	IIIa	IVa	IIb	IIIb	IVb
49%	23	27	35	32	30

reagent, 3 moles (BH_3) ; time, 2 hr.

$R\text{-}NH\text{-}\underset{\displaystyle CH_2R'}{\underset{|}{CH}}\text{-}CH_2OH$

IIa : $R=CH_3CO$, $R'=C_6H_5$

IIb : $R=C_6H_5CO$, $R'=H$

IIIa : $R=CH_3CH_2$, $R'=C_6H_5$

IIIb : $R=C_6H_5CH_2$, $R'=H$

Table II. Recovery of Amino Acids

Mole (BH_3)	Gly	Ala	Time (min)	Gly	Ala
1	93%	66%	10	83%	64%
3	54	15	30	76	50
5	50	14	60	67	43

time, 2 hr. reagent, 3 moles

thus obtained was listed in Table II. As amount of the reagent increases, not only the recovery of the terminal Ala, but that of Gly also decreases apparently as a result of non-specific reduction.

In view of the above data as well as the fact that diborane is a good reducing agent for amide,[6] and that such an organic solvent as tetrahydrofuran is usually used for the reaction,[7] it may be suggested that careful selection of reaction conditions is required for specific reduction of protein-carboxyl groups with diborane.[4]

Selective Reduction of Peptide Esters with Sodium Borohydride

Several attempts were made to effect reduction of protein-esters with lithium aluminum hydride or lithium borohydride in organic solvents, more or less accompanied by simultaneous reduction of peptide bonds.[8]

It has been generally accepted that carboxylic esters are unaffected by sodium borohydride. However, Rapoport et al. recently showed that large excess of the reagent can bring about the reduction,[9] while Yamada et al. reported that amino acid esters can be reduced in water-containing media.[10] Since we also occasioned to have related observations in the course of our studies on the reaction of peptide in aqueous solutions, a study was undertaken to explore the desired selective reaction.

N-Acyldipeptide esters (1 mMol/L) were treated with ten-fold excess of sodium borohydride in aqueous solution at room temperature. Preliminary experiments using paper chromatography indicated that terminal amino acid disappeared as a function of reaction time, whereas non-terminal one stayed almost unchanged. In order to confirm this, a reaction mixture was hydrolyzed and resultant amino alcohol was converted to corresponding TNP-amino alcohol with trinitrobenzenesulfonic acid,[11] which was separated from TNP-amino acid and determined spectroscopically. All TNP-amino alcohols were identified by comparison of TLC with the authentic specimen prepared by independent synthsis. Thus, from Bz.DL-Ala.GlyOEt, Bz.Gly.L-PheOEt, Bz.Gly.L-IleuOEt, and Bz.Gly.L-ValOEt, glycinol (77%), phenylalaninol (75%), isoleucinol (65%), and valinol (45%) were respectively obtained by the reaction for 5 hr.

Ac.Gly.DL-AlaOEt and Ac.Gly.L-ValOMe were subjected to the reaction and the recovery of amino acids as determined gas-chromatographically is shown in Fig.1. Terminal Ala and Val decreased as reaction proceeds though the rate for the latter is rather slow, whereas non-terminal Gly was invariably recovered in nearly quantitative yield.

Recently, sodium borohydride has been used for the reduction of disulfide groups in proteins,[12a,b,13] and in some cases, it was reported that some peptide bonds were reduced by the reagent,[14,15] though Crestfield et al. showed that this was minimized in the presence of EDTA.[12b]

The above results now demonstrate that by the use of sodium borohydride in aqueous solution under specified conditions, certain model peptide esters can be reduced to corresponding alcohols without any appreciable side-reaction. A study of the application of this method to larger peptides and proteins is in progress.

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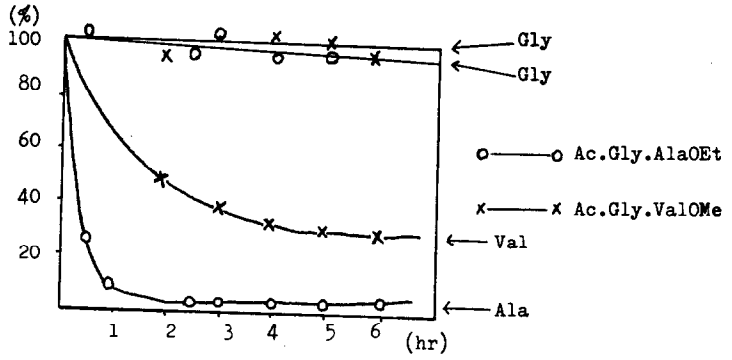
Fig.1. Recovery of Amino Acids



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