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TWO-STEP FRAGMENTATION OF THREONINE-CONTAINING PEPTIDES

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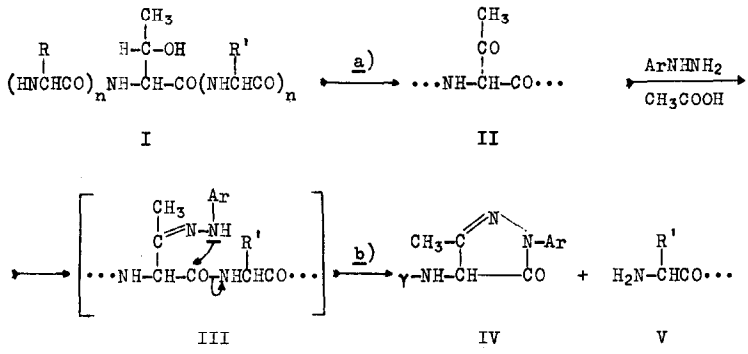
We have been investigating a novel procedure for the selective fragmentation of peptide chains, based on the modification of β-hydroxy-α-amino acids, followed by mediated interaction with the adjacent peptide bond.

Threonine-containing peptides (I) and model compounds (VI) are selectively oxidized to β-carbonyl-derivatives (II). The β-carbonyl function is then caused to react with phenyl hydrazine to give, through intramolecular cyclization, the two fragments IV and V, as depicted in the following scheme.



I II III IV V

Step a - The oxidation is performed by using the dimethylsulphoxide (DMSO)-dicyclohexylcarbodiimide(DCC)-phosphoric acid system, which is finding increasing use to convert primary and secondary alcohols into aldehydes and ketones, in mild conditions. Equat. c depicts the oxidation scheme suggested by the Authors (1) to whom reference is made for previous literature and a discussion on the mechanism.

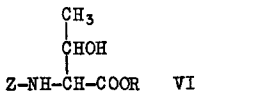
c) $R\text{-}CH(OH)\text{-}R' + C_6H_{11}N{=}C{=}NC_6H_{11} + CH_3SOCH_3 \xrightarrow{(H^+)}$

$\longrightarrow R\text{-}CO\text{-}R' + C_6H_{11}NH\text{-}CO\text{-}NHC_6H_{11} + CH_3\text{-}S\text{-}CH_3$

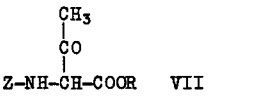
While our early search for suitable oxidizing agents gave only occasional conversion to the desired products (2), the use of the DMSO-DCC-H_3PO_4 system resulted in the consistent oxidation of threonine-containing compounds to β-ketoacid derivatives.

In ex., N-benzyloxycarbonyl-threonine esters (VI) and peptides (VIII) were converted at room temperature into β-carbonyl derivatives such as α-benzyloxycarbonylamino-acetoacetic acid methyl

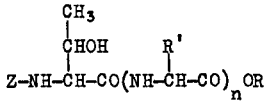
ester (VIIa), 80% yield, thick oil: 2,4-Dinitrophenyl-hydrazone, m.p. 137-9° (Found %: C 50.91; H 4.43; N 15.68; Calc.: C 51.23; H 4.27; N 15.73), and α-benzyloxycarbonylamino-acetoacetyl-glycine, ethyl ester (IXa; R'= H; n = 1): colorless prisms; m.p. 109-110°; 70% yield: 2,4-Dinitrophenyl-hydrazone, m.p. 219-22° (Found %: C 51.55; H 4.56; N 16.45; Calc.: C 51.16; H 4.68; N 16.27).



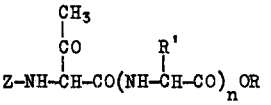
VI



VII

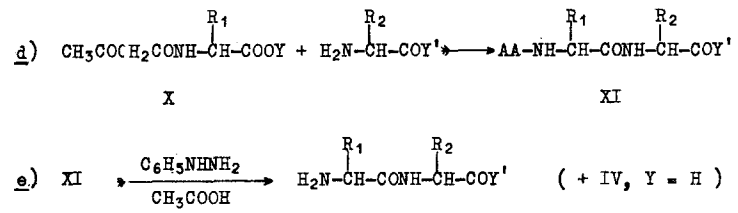


VIII



IX

Step b - The cleavage of the peptide bond adjacent to the oxidized threonine is achieved by reacting the β-carbonyl derivative formed, with phenyl hydrazine, few hours at room temperature or 20-40 minutes at 40-70°. The cycloaddition - elimination reaction occurs as in the pyrazolone Knorr synthesis (3) to yield in the cases here described, the pyrazolone moiety (IV) and the amine terminus (V). The splitting of the chain has been followed through chromatography and/or ninhydrine spot tests; the products are isolated in the same experimental conditions, as we used for the removal of the acetoacetyl (AA) group, that we reported as a potential amino-protecting group for peptide synthesis (equat. d, e) (4).



d) $CH_3COCH_2CONH-\underset{R_1}{CH}-COOY + H_2N-\underset{R_2}{CH}-COY' \longrightarrow AA-NH-\underset{R_1}{CH}-CONH-\underset{R_2}{CH}-COY'$

X XI

e) XI $\xrightarrow[CH_3COOH]{C_6H_5NHNH_2}$ $H_2N-\underset{R_1}{CH}-CONH-\underset{R_2}{CH}-COY'$ (+ IV, Y = H)

From α-benzyloxycarbonylamino-acetoacetyl-glycyl-glycine ethyl ester (IXb; R' = H; n = 2), we obtained the pyrazolone moiety 1-phenyl-3-methyl-4-benzyloxycarbonylamino-pyrazoline-5-one (IV; Y = $C_6H_5CH_2OCO$); colorless tablets, m.p. 184-6°. (Found %: N 12.56; Calc.: N 13.0) and the dipeptide fragment glycyl-glycine ethyl ester.

The research progress concerns i) the behaviour of serine peptides in the conditions described; ii) the fate of labile groups in the oxidation step; iii) the utilization of both fragments for the elucidation of the primary structure of polipeptide chains.

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