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THE ACETOACETYL GROUP, AN AMINO PROTECTIVE GROUP OF POTENTIAL USE IN PEPTIDE SYNTHESIS

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We wish to report our first studies on the possibility of applying the acetoacetyl group CH_3COCH_2CO-(AA), to the protection of amino groups in peptide synthesis.

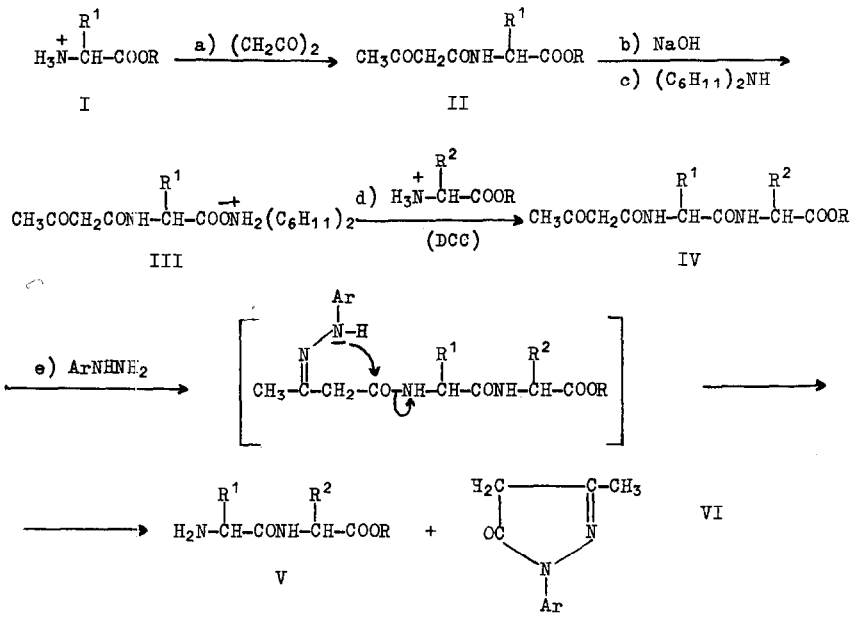
The AA-group meets most of the requirements needed for protection of the amino groups of amino acids and peptides[2]; in ex. i) it can be linked to the free amino groups in very mild conditions; ii) it offers the possibility of a selective removal after the condensation step; iii) it allows retention of optical activity, as far as it was indicated in the preliminary esperiment outlined below.

The overall conversion is depicted in Table 1.

The free amino group of an amino acid ester can be acetoacetylated by using diketene, which can be prepared by pyrolysis of acetone and subsequent dimerization[3]. Diketene is reacted at $0-5^0$ with the amino acid ester hydrochloride (I)(step a), in ethanol, in the presence of equimolecular amounts of sodium ethoxide or of a tertiary amine[4]. The acetoacetyl amino acid esters(II) are in many instances crystalline compounds, soluble in most organic solvents, and can be easily purified. Furthermore, they can be identified by

the blue or violet spots given on paper or thin layer chromatograms, when a 2% ferric chloride solution is used, even after a ninhydrine spray.

TABLE 1



The specificity of $-NH_2$ over -OH acetoacetylation was confirmed by obtaining a ninhydrine negative, monoacylated derivative, upon reaction of excess diketene with threonine ethyl ester hydrochloride. M.p. 76-7°. Found %: C 51.22; H 7.20; N 6.14; Calc.: C 51.94; H 7.36; N 6.06.

The condensation of the protected aminoacid or peptide occurs by the usual techniques. For example (steps b, c), acetoacetyl-L-leucine

ethyl ester (II; R^1= $CH_2CH(CH_3)_2$; R = C_2H_5) (oil) was hydrolyzed to the free acid (II; R^1= $CH_2CH(CH_3)_2$; R = H) m.p. 116° (Found %: N 6.91; Calc.: 6.51). Its dicyclohexylamine salt (III) (m.p. 185-6°) was condensed *via* dicyclohexyl carbodiimide (DCC) with glycine ethyl ester hydrochloride, yielding acetoacetyl-L-leucyl-glycine ethylester (IV; R^1= CH_2-$CH(CH_3)_2$; R^2 = H) m.p. 88-9°.(Found %: C 55.70; H 7.92; N 9.38; Calc.: C 56.01; H 8.05; N 9.33).

The possibility of obtaining the acetoacetyl derivatives of the free aminoacids and of activated esters is currently under investigation.

The selective removal of the acetoacetyl group (step *e*) is being studied by applying the long known Knorr synthesis[5], widely employed to obtain pyrazole derivatives from esters, amides, hydrazides[6]. To a solution of the acetoacetyl peptide (IV) in acetic acid, an equimolecular amount of phenyl hydrazine is added and the resulting mixture is kept at a suited temperature till maximum formation of the free amino derivative. The effectivness of the procedure and the time needed have been hitherto followed mainly by chromatography. Samples from the reaction mixtures have been developed using the solvent *n*-BuOH- 3% aqueous ammonia (1:1) which was shown not to allow the reaction to proceed further. The identity of the liberated peptide, as well as that of the secondary product 1-phenyl-3-methyl-5--pyrazolone (VI) could be demonstrated by comparison with samples of the pure compounds.

The research is continued with the aim of reaching a quantitative conversion and of finding optimum conditions for the recovery of the free amino-peptide derivatives. The possibility of using substituted aryl hydrazines is also taken into account.

The retention of configuration during protection, hydrolysis and condensation was checked[7] by comparing the optical activity of acetoacetyl-L-leucyl-glycine ethyl ester (IV) obtained as depicted in

Table 1, with that of the same compound obtained as follows. Carbobenzoxy-L-leucyl-glycine ethyl ester was synthesized via DCC and decarbobenzoxylated using hydrogen bromide in acetic acid; the obtained dipeptide ester was acetoacetylated with diketene. A compound was obtained, which showed the same melting point, 88-9°, with no depression on admixture, and had the same optical activity, $[\alpha]_D^{20}$ - 48°.

To check the stability of the acetoacetyl group in conditions of removal of other protecting groups, acetoacetyl-glycine ethyl ester was treated i) with trifluoro-acetic acid (15' at room temp.) and ii) with hydrogen bromide in acetic acid (2,5 molar; 1 h at 50°). No ninhydrine positive spots were present on the chromatograms of the reaction mixtures, after the times indicated.

Full details on this research will be described in subsequent papers.

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