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4-NITROSO-5-AMINO-PYRAZOLE DERIVATIVES IN CARBOXYL-GROUP ACTIVATION FOR PEPTIDE SYNTHESIS

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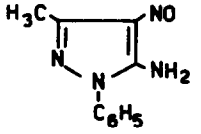
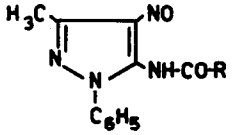
We investigated previously the behaviour of 1-phenyl-3-methyl-4-nitroso-5-amino-pyrazole (I) towards acylating agents [1].

Some acyl derivatives appear to be of interest with respect to the activation of the carboxyl groups for peptide synthesis.

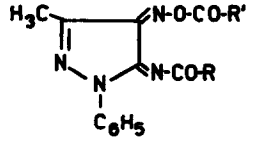
Compound I behaves as a di-nucleophile yielding mono- or di-acyl derivatives, when reacting respectively, with one or two moles of N-protected amino acid in the presence of dicyclohexylcarbodiimide (DCCI). The following structures nitroso-acylamine (II) and acyloxime-acylimine (III), confirmed through elemental and spectroscopic analyses, are assigned to the products.



I



II



III

The diacyl-derivatives (III) are crystalline, stable compounds, displaying strong acylating properties towards amino acid esters and other nucleophyles.

For example, the derivative of a benzyloxycarbonyl(Z)-aminoacid (III, R=R'= = -CHR"-NH-Z), or of a t.butyloxycarbonyl (BOC) aminoacid (III, R=R'= -CHR"-NH--BOC), reacts with an aminoacid ester yielding the N-protected peptide (eq. 1); the nitroso acylamine II is formed as a secondary product. The yields are very high and the reactions occur very rapidly at room temperature.

The coupling reaction can be followed from the discoloration of the solution; the acylating reagents (III) are in fact red, whereas the nitroso-acylamines (II) are green.

$$\text{III (red)} + H_2N\text{-CHR"-COOR'''} = \text{R'CONH-CHR"-COOR'''} + \text{II (green)} \qquad \text{eq. 1)}$$

Furthermore, the disappearance of the carbonyl function of the acyloxime at 1810 cm^{-1} can be followed by I.R. spectroscopy.

Satisfactory results have been obtained when I was first condensed with Z-gly-

cine The resulting product IIa (R = $-CH_2-NH-Z$; m.p. 139-140°, 80%) was then allowed to react, in the presence of DCCI, with a series of N-protected amino acids, to yield the corresponding pyrazolyl-activated derivatives (IIIa). Some examples of reactions of compounds IIIa with amino acid ethyl esters or with a peptide ester, used as free bases in chloroform are recorded below (R' is given).

1) IIIa (R' = $-CH(CH_3)-NH-Z$; m.p. 120-122°) reacts with H-Lys(Z)-OMe, to give, in 5 min., 91% yield of Z-Ala-Lys(Z)-OMe [2].

2) Z-Val-Phe-OEt, which is known to involve amino acids which are strongly sterically hindered [3], was obtained from IIIa (R'= $-CH(isoC_3H_7)-NH-Z$; m.p. 112-114°) and H-Phe-OEt in 95% yield within 45 min.

3) The tetrapeptide Z-Lys(Z)-Val-Phe-Gly-OEt [4], corresponding to the 1-4 N-terminal sequence of hen egg-white lysozyme, has been prepared by step-wise procedure starting from the C-terminal residue; the following IIIa were used: (R'= = $-CH(CH_2C_6H_5)-NH-Z$; m.p. 140°; R'= $-CH(isoC_3H_7)-NH-Z$; m.p. 112-114° and R'= = $-CH(n.C_4H_8-NH-Z)-NH-Z$; m.p. 99-100°. The overall yield was 70%.

No racemisation occurs in the condensation step on the basis of the Izumia's [5] and Weygand's tests [6].

The reaction by product IIa can be easily extracted from the reaction mixtures with aqueous sodium carbonate, and recovered upon acidification of the alkaline extracts. No acylation at the hydroxyl function of either serine, threonine or tyrosine or incorporation of the aminoacyl residue linked at position 5 of IIIa by the nucleophile were observed in the experiments hitherto carried out.

Some encouraging results have been obtained when representative compounds III (R=R'= $-CHR"-NH-BOC$) were used in the solid phase peptide synthesis (where R"= = H, CH_3, $CH_2C_6H_5$).

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