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O- AND N-ACYLUREAS IN PEPTIDE SYNTHESIS BY DCC METHOD. NEW OBSERVATIONS

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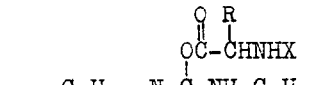
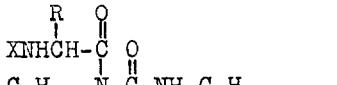
Dicyclohexylcarbodiimide /DCC/ as the coupling reagent for peptide synthesis was proposed in 1955 [1] and till now it has been the most used reagent for this purpose. The peptide synthesis by DCC method gives many advantages but the formation of N-acylurea as the side product is the main drawback of this method.

The mechanism of DCC action is not fully understood in spite of numerous investigations devoted to that subject. O-acylisourea postulated by Khorana [2] as the primary acylating reagent is still hypothetical and only indirect proofs indicate its formation and its role in the acylation process. Other intermediates, anhydride and azlactone are probably formed from O-acylisourea but its formation rate and its role in acylation has not been known yet. The side product, N-acylurea has generally been thought to be formed from O-acylderivative by O — N acyl shift. This reaction is possible in acidic medium [3], thought there are not proofs that it takes place in peptide synthesis.



dicyclohexyl-O-acylisourea



dicyclohexyl-N-acylurea

In our studies, we have employed radiochromatographic TLC/LSC method [4] of the unusual separating qualities appearing with the amounts of separated compounds much below the chemical detection limits but, at the same time, sufficient for the quantitatively LSC determination of their radioactivity. In the reaction of CHO-Phe* labelled tritium in the aromatic ring [5] with Leu-OMe in THF using DCC, at different stages and in various conditions we may determine the radioactive components: substrate 1, the main peptide 3 its epimer 4 and N-acylurea 5 which represent together 97% of total radioactivity of a sample.

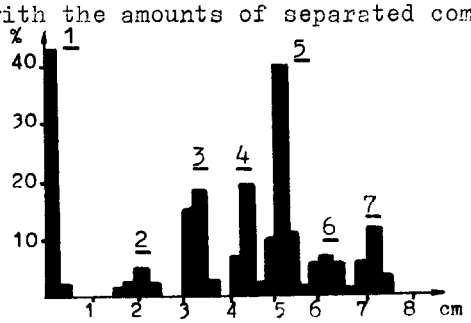


Fig. 1. The diagrame of radioactivity distribution on the chromarogram of reaction: CHO-Phe + DCC and then + Leu-OMe
The pre coated TLC plastic sheets and benzene -acetone 2:1 as the solvent system were used.

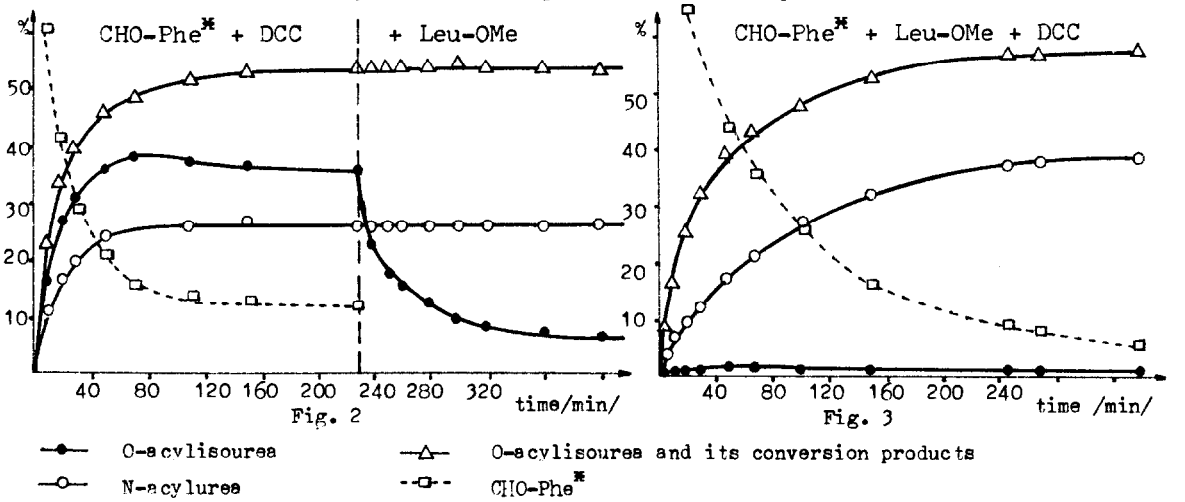
The incubation of CHO-Phe* with DCC for a few hours leads to the formation of intermediates which may be separated by TLC and determined quantitatively.

Thus, in an hour time we found 36% of compound 6, 17 % of compound 2 and 4 % of compound 7. The compound 2 was identified as anhydride of formylphenylalanine and the compound 7 as its azlactone on basis of comparison with standards.

The compound 6, relatively stable in condition of incubation reacts slowly with CHO-Phe yielding anhydride but after the addition of Leu-OMe it is quickly aminolysed forming both peptides. As it is neither anhydride nor azlactone and that it is a good acylating reagent and its R_f value is like R_f of N-acylurea, therefore the compound 6 is supposed to be O-acylisourea.

Fig. 2 represents the curves of concentration change of N-acylurea 5 and O-acylisourea 6. The character of this courves and the constant value of rate constants ratio /k_6/k_5 = 2/ prove that isomeric acylureas are formed in parallel reactions from the same substrates so N-acylurea is not the product of consecutive reaction of O-acylisourea transformation and that amines do not catalyze 6 — 7 transformation. However, we have observed that amines catalyze the formation of N-acylurea from carboxylic acid and DCC.

Fig. 3 shows that O-acylisourea may be also detected in normal conditions of peptide synthesis by DCC method. Its relative concentration then is low almost equal to about 1,5 % of a sample radioactivity.



A new approach to the problem of N-acylurea formation in peptide synthesis by DCC method makes the basis for the rational investigation of such a synthesis procedure that would decrease or exclude this side reaction.

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