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## Abstract

## Full Text

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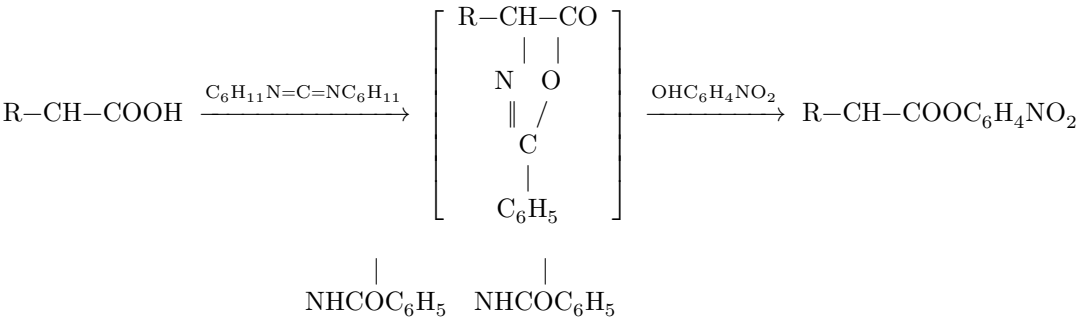
# INVESTIGATION BY IR SPECTROSCOPY OF THE MECHANISM OF FORMATION OF *p*-NITROPHENYL ESTERS OF BENZOYLAMINO ACIDS AND ACYLPEPTIDES BY THE CARBODIIMIDE METHOD

*(Presented by Academician A. N. Nesmeyanov, February 25, 1963)*

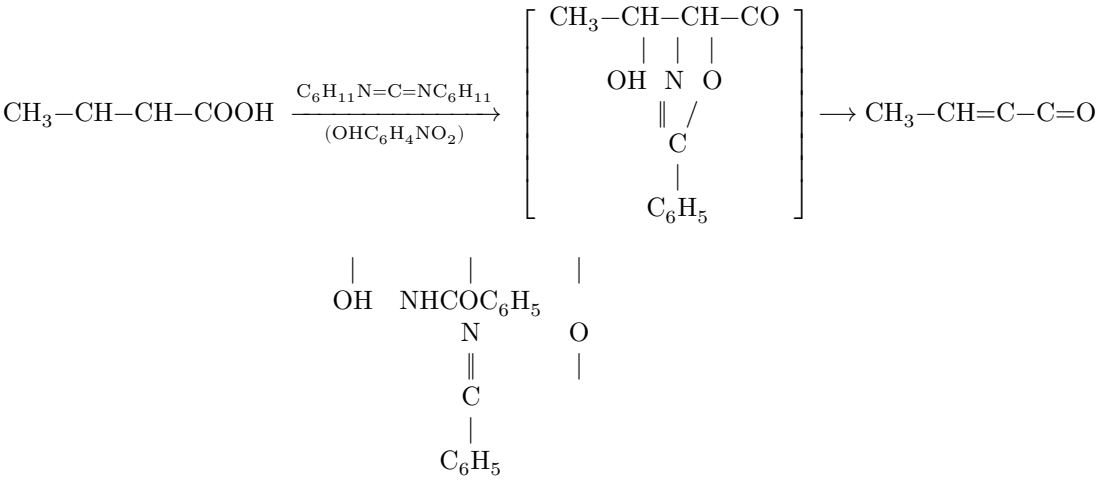
In the synthesis of nitrophenyl esters of benzoyl-*L*-valine and benzoyl-*L*-phenylalanine from benzoyl derivatives of amino acids and nitrophenol under the action of dicyclohexylcarbodiimide, the corresponding racemates were obtained by us instead of optically active compounds.* In this connection it was suggested that the reaction under consideration proceeds through an intermediate stage involving the formation of azlactones, which then react with nitrophenol.



$$R = (CH_3)_2CH-$$

$$R = C_6H_5CH_2-$$

This assumption was supported by the fact that, when an attempt was made to obtain by the same method the nitrophenyl ester of N-benzoyl-*D,L*-threonine, 2-phenyl-4-ethylidene-5-oxazolone was isolated; formation of the latter occurred upon interaction of N-benzoyl-*D,L*-threonine with dicyclohexylcarbodiimide even in the absence of nitrophenol.



It has already been noted in the literature that dicyclohexylcarbodiimide is capable of causing azlactonization of a number of benzoyl, acetyl, and formyl derivatives of amino acids ([4–8]); however, in the presence of nitrophenol this direction of the reaction could have been suppressed by esterification of the acylamino acid.

To elucidate the mechanism of the carbodiimide synthesis of nitrophenyl esters of benzoylamino acids, the method of IR spectroscopy was used; with its aid, the reaction of formation of the nitrophenyl ester of benzoyl-*D,L*-phenylalanine was followed in time (see Fig. 1). For comparison, IR spectra were recorded of the nitrophenyl ester of benzoyl-*D,L*-phenylalanine and of the azlactone of benzoyl-*D,L*-phenylalanine. The nitrophenyl ester had

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* In obtaining, by the carbodiimide method under the same conditions, nitrophenyl esters of optically active carbobenzoxyamino acids, racemization does not occur ([1–3]).

maximum at a frequency of 1776 $cm^{-1}$, due to absorption by the carbonyl of the ester group; the azlactone was characterized by the frequency of the stretching vibrations of its carbonyl, equal to 1826 $cm^{-1}$. In the IR spectrum of the reaction mixture recorded 10 minutes after the start of the reaction, an intense absorption band characteristic of the azlactone, with a frequency of 1826 $cm^{-1}$, was found; after 60 minutes, together with this band, a weak absorption band with a frequency of 1776 $cm^{-1}$ was detected, indicating the appearance of the nitrophenyl ester. In the course of the reaction the absorption band with a frequency of 1826 $cm^{-1}$ gradually decreased, while the intensity of the absorption band with a frequency of

Figure 1: IR spectra of the reaction mixture formed in the synthesis of the nitrophenyl ester of benzoyl-*D*, *L*-phenylalanine after 10 min. (a), 60 min. (b), 2 hours (c), and 24 hours (d) from the start of the reaction.

Figure 1: Figure 1: IR spectra of the reaction mixture formed in the synthesis of the nitrophenyl ester of benzoyl-*D*, *L*-phenylalanine after 10 min. (a), 60 min. (b), 2 hours (c), and 24 hours (d) from the start of the reaction.

**Fig. 1.** IR spectra of the reaction mixture formed in the synthesis of the nitrophenyl ester of benzoyl-*D*, *L*-phenylalanine after 10 min. (*a*), 60 min. (*b*), 2 hours (*c*), and 24 hours (*d*) from the start of the reaction.

1776 $cm^{-1}$ increased. After 24 hours, an intense absorption band with a frequency of 1776 $cm^{-1}$ was found in the IR spectrum, and absorption of the azlactone at a frequency of 1826 $cm^{-1}$ was practically absent. These data confirm the validity of the proposed reaction mechanism.

In the case of *N*-benzoyl-*D*, *L*-threonine the reaction stops at the stage of azlactone formation, since the azlactone of $\alpha$-benzoylaminocrotonic acid is only slightly reactive ([9]).

Recently, using formyl-*D*, *L*-valyl-*D*, *L*-norleucine and benzoylglycylglycine as examples, it was shown that dicyclohexylcarbodiimide is also capable of causing azlactonization of peptides ([7]). The data we obtained on the mechanism of the carbodiimide synthesis of nitrophenyl esters of benzoylamino acids made it possible to suggest that azlactonization may also occur during the synthesis of nitrophenyl esters of acylated peptides. This proposition was tested by IR spectroscopy using, as an example, the preparation of the nitrophenyl ester of carbobenzoxyglycyl-*D*, *L*-alanine from carbobenzoxyglycyl-*D*, *L*-alanine and nitrophenol (Fig. 2).

$$C_6H_5CH_2OCONHCH_2CONHCH(CH_3)COOH \xrightarrow[OHC_6H_4NO_2]{C_6H_{11}N=C=NC_6H_{11}} \left[C_6H_5CH_2OCONHCH_2C^{N}CH{-}CH_3O{-}CO\right.$$

Just as in the experiment described earlier, during the first 30 min after the start of the reaction a band corresponding to the azlactone was detected in the reaction mixture.

an absorption maximum at a frequency of 1826 $cm^{-1}$, which then gradually decreased and disappeared completely after 24 hours. At the same time, an increase occurred in the intensity of the absorption band at 1770 $cm^{-1}$, due to the formation of the nitrophenyl ester of carbobenzoxypeptide. The same maximum at 1826 $cm^{-1}$ was also found in the IR spectra of the reaction mixture formed in the interaction of carbobenzoxyglycyl-*D*, *L*-alanine and dicyclohexylcarbodiimide in the absence of nitrophenol.

IR spectra diagram

Figure 2: IR spectra diagram

**Fig. 2.** IR spectra of the reaction mixture formed in the synthesis of the nitrophenyl ester of carbobenzoxyglycyl-*D*, *L*-alanine after 30 min. (a), 2 hours (b), and 24 hours (c) after the start of the reaction.

Thus it was shown that, in the course of the synthesis of the nitrophenyl ester of carbobenzoxyglycyl-*D*, *L*-alanine, azlactonization of the peptide occurs. The data obtained lead to the conclusion that it is precisely azlactonization that is the cause of the racemization observed in the synthesis of nitrophenyl esters of carbobenzoxypeptides by the carbodiimide method ([10,11]).

By means of IR spectroscopy it was shown that the formation, by the carbodiimide method, of nitrophenyl esters of benzoylamino acids is preceded by azlactonization, which is the cause of racemization. The nitrophenyl ester of N-benzoyl-*D*, *L*-threonine is not formed under the synthesis conditions, since the reaction stops at the stage of azlactone formation. In obtaining the nitrophenyl ester of carbobenzoxyglycyl-*D*, *L*-alanine by the carbodiimide method, azlactonization of the peptide occurs.

## Experimental Part

### 1. Reaction of benzoylamino acids with nitrophenol in the presence of dicyclohexylcarbodiimide

To an ethyl acetate solution of a benzoylamino acid (1-2 mmol) and nitrophenol (20% excess), an equimolar amount of dicyclohexylcarbodiimide is added at 0°. After 24 hours (~20°), dicyclohexylurea is filtered off, the solution is evaporated in vacuo, and the residue is recrystallized.

Data on the compounds obtained are given below.

*p*-Nitrophenyl ester of benzoyl-*D*, *L*-valine (from benzoyl-*D*, *L*- and *L*-valine). Yield 75%; mp 114-115° (acetone/water); $[\alpha]_D^{20}$ 0° (*C* 0.5, dioxane).

$C_{18}H_{18}N_2O_5$. Found, %: C 62.69; H 5.50; N 8.17
Calculated, %: C 63.14; H 5.29; N 8.18

*p*-Nitrophenyl ester of benzoyl-*D*, *L*-phenylalanine (from benzoyl-*D*, *L*- and *L*-phenylalanine). Yield 84%; mp 157-158° (alcohol); $[\alpha]_D^{20}$ 0° (*C* 0.5, dioxane).

$C_{22}H_{18}N_2O_5$. Found, %: C 67.57; H 4.76; N 7.11
Calculated, %: C 67.60; H 4.69; N 7.17

2-Phenyl-4-ethylidene-5-oxazolone (from N-benzoyl-$D,L$-threonine a) in the presence and b) in the absence of nitrophenol). Yields respectively 74 and 95%; m.p. 84–88° (alcohol) [12]. The substance is identical with 2-phenyl-4-ethylidene-5-oxazolone [9,12].

**2. Infrared spectroscopic studies.** The IR spectra were recorded on an IKS-12 instrument (NaCl prism, layer thickness 0.1 mm). All reactions studied spectroscopically were carried out in chloroform analogously to the experiments described above (1) (the suspension of the starting substances in chloroform soon after the addition of dicyclohexylcarbodiimide turned into a solution); immediately before each recording of an IR spectrum, the precipitate of dicyclohexylurea was filtered off. The IR spectra of the nitrophenyl ester of benzoyl-$D,L$-phenylalanine and of the azlactone of benzoyl-$D,L$-phenylalanine were recorded in chloroform solution (0.5% solution).

We consider it our pleasant duty to express our gratitude for assistance in the work to the staff members of the Laboratory of Physicochemical Methods for the Study of Organic Compounds, L. A. Kozitsina, N. B. Kupletskaya, and L. D. Ashkinadze.

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named after M. V. Lomonosov

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*Note: Figure translations are in progress. See original paper for figures.*