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Source / Izvornik: *Croatica Chemica Acta*, 2003, 76, 229 - 233

Journal article, Published version

Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

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IR Spectroscopy Study of the Amidation Reaction of *N*-(1-Azolecarbonyl)amino Acids on L-Phenylalanine Derivative Models

Marijana Zovko, Igor Kalčić,* Branka Zorc, and Ivan Butula**

Faculty of Pharmacy and Biochemistry, A. Kovačića 1, 10 000 Zagreb, Croatia

RECEIVED DECEMBER 4, 2002; REVISED FEBRUARY 20, 2003; ACCEPTED FEBRUARY 21, 2003

Key words
N-(1-azolecarbonyl)amino acid
amino acid *N*-carboxy anhydride
polymerization
IR spectroscopy

Behaviour of *N*-(1-azolecarbonyl)-L-phenylalanine (Azc-Phe-OH, **1a-d**) and L-phenylalanine *N*-carboxy anhydride (L-Phe-NCA, **6**) in the presence of triethylamine (TEA) or cyclohexylamine was followed by IR spectroscopy. IR spectra were recorded in acetonitrile solutions. Attention was focused on characteristic absorptions of CO₂ (2340 cm⁻¹) and Azc, COOH or the anhydride group (1850–1600 cm⁻¹). The obtained results corroborate *N*-carboxy anhydride as an intermediate in amidation reactions of Azc-amino acids.

INTRODUCTION

In our previous paper¹ preparation of 1-azolecarbonyl chlorides, their reactivity and application in the synthesis of numerous derivatives of carbonic acid and amino acid derivatives were described. Reactions of *N*-(1-azolecarbonyl)amino acids (Azc-amino acids), notably *N*-(1-benzotriazolecarbonyl)amino acids (Btc-amino acids), were studied as well.² It was shown that Btc-amino acids reacted with alcohols³ (methanol, benzyl alcohol), amines⁴ (benzylamine, cyclohexylamine, L-methioninol) or other amino compounds^{4,5} (amino acids, hydroxylamine) to give, after elimination of benzotriazole (BtH) and carbon dioxide, amino acid esters, amide, di- and tripeptide and hydroxamic acids (Schemes 2 and 3 in Ref. 1 and Scheme 1 in this paper). Formation of amino acid esters occurred at room temperature if the reaction was performed in the presence of triethylamine (TEA). Reactions with amino compounds also proceeded faster and more completely

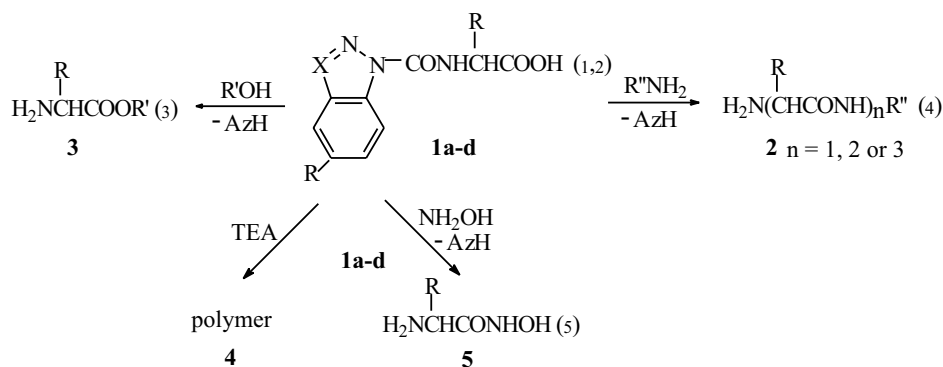
in the presence of TEA or in excess of amine. If the reaction proceeded at room temperature, without TEA or excess of amine, the released benzotriazole reacted with the starting amine and the resulting benzotriazoleammonium salt partially precipitated from the reaction mixture. No amine excess was necessary at elevated temperature.

In *N*-(1-benzotriazolecarbonyl)amino acids, the 1-benzotriazolecarbonyl group had a double function – it was both an N-protecting and a C-activating group. Although it was presumed that these reactions proceeded through the *N*-carboxy amino acid anhydride (NCA) intermediate,⁵ there was no evidence of it.

Kopple⁶ considered NCA-amino acids to be stable forms of α -isocyanate acids. However, the presence of isocyanate was proven only in the case of NCA-aminoisobutyric acid. The IR spectrum of this compound taken in solution showed a typical isocyanate absorption band at 2230 cm⁻¹. However, no isocyanate band was detected in the IR spectra of NCA derivatives of other amino ac-

* Present address: Belupo, Ltd. Pharmaceuticals & Cosmetics, 48 000 Koprivnica, Croatia.

** Author to whom correspondence should be addressed. (E-mail: ibutula@inet.hr)



Numbers in brackets are references.

AzH – azole a – benzotriazole (X = N, R = H)
 b – indazole (X = CH, R = H)
 c – 5-nitroindazole (X = CH, R = NO₂)
 d – 5-methylbenzotriazole (X = N, R = Me)

TEA – triethylamine

For designation of R, R' and R'', see references 1-5.

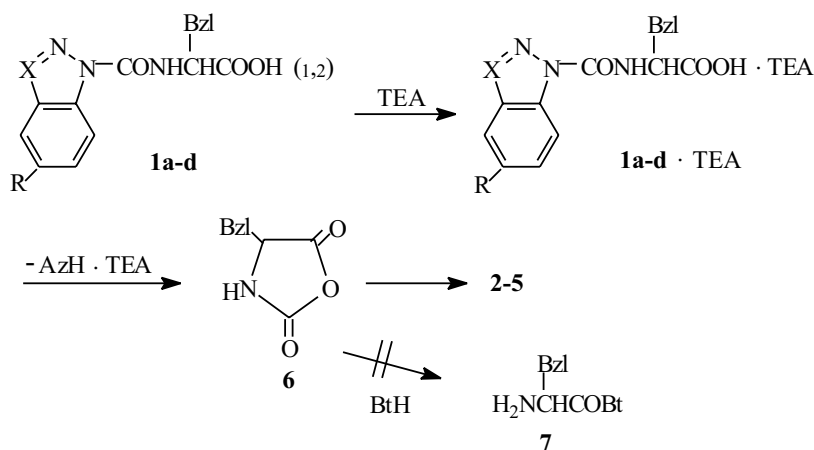
Scheme 1.

ids – anhydride absorption bands at 1850 and 1790 cm⁻¹ decreased gradually as the polymerization reaction progressed, while the carbon dioxide absorption band enlarged at 2340 cm⁻¹. At the same time, precipitation of poly(amino acids) occurred. In several papers, initiators, kinetics and the mechanism of such polymerization reactions of NCA were studied (see, for example, the paper of Schwarz⁷). It was also shown that esterification and amidation reactions of amino acids NCA proceeded by nucleophilic attack, predominantly at position 5 of 2,5-dioxo-1,3-oxazole, accompanied by the release of carbon dioxide from a carbamic acid intermediate.⁸

In order to estimate whether reactions of *N*-Azc-amino acids occurred through the amino acid NCA intermediate, amidation reactions of *N*-Azc-L-phenylalanine derivatives as model compounds were studied by IR spectroscopy.

RESULTS AND DISCUSSION

In the present study the following *N*-Azc-amino acids were used: *N*-(1-benzotriazolecarbonyl)-L-phenylalanine (**1a**), *N*-(1-indazolecarbonyl)-L-phenylalanine (**1b**), *N*-(5-nitro-1-indazolecarbonyl)-L-phenylalanine (**1c**) and *N*-(5-methyl-



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BtH – benzotriazole

Bzl – benzyl

TEA – triethylamine

Numbers in brackets are references.

Scheme 2.

1-benzotriazolecarbonyl)-L-phenylalanine (**1d**). Their behaviour in the presence of an equivalent amount (mole ratio 1:1) of triethylamine or cyclohexylamine as a model amine was followed by IR spectroscopy and compared with the behaviour of L-phenylalanine *N*-carboxy anhydride (L-Phe-NCA, **6**) after addition of triethylamine. The spectra of **1a-d** or **6** in acetonitrile solutions before, immediately after addition of the corresponding amine and at determined time intervals were recorded *in situ* and analyzed. The spectrum of acetonitrile was used as the background.

The following facts were observed:

(i) The $\nu(\text{CO})$ absorption band of Btc and COOH groups, present in the KBr spectrum at 1741 and 1707 cm^{-1} * (Figure 1a) appeared as one single band at 1742 cm^{-1} if the spectrum was taken in acetonitrile (Figure 1b). The intensiveness of that band decreased after addition of the equivalent amount of TEA (only Btc carbonyl at 1738 cm^{-1} remained) and new absorption of COO^- at 1628 appeared.** After a few minutes, a typical NCA absorption band at 1850w and 1790s cm^{-1} appeared (Figure 1c). One hour later, only NCA bands remained, together with CO_2 absorption at 2340 cm^{-1} (Figure 1d). After that period, CO_2 absorption gradually increased, while NCA bands decreased, with concomitant precipitation of poly-L-phenylalanine. In the IR spectrum of the isolated polymer taken in KBr, absorption bands at 3210 (NH) and 1660 (CO) cm^{-1} were present.

(ii) In the spectrum of L-Phe-NCA (**6**) in acetonitrile (Figure 1e), the same absorptions at 1850w and 1790s cm^{-1} were present. In order to initiate the polycondensation of NCA, 0.1 equivalent of TEA was added.⁷ Immediately, NCA^- absorption at 1650 cm^{-1} appeared. With the progression of time, NCA bands at 1850w and 1790s cm^{-1} gradually decreased and the CO_2 band at 2340 cm^{-1} increased. The overnight-precipitated polymer gave an identical spectrum as the polymer prepared from Btc-L-Phe-OH. Literature data⁹ for the polymer derived from D,L-Phe-NCA was 3290 (NH) and 1665 (CO) for α -helix and 3290 and 1640 cm^{-1} for β -helix.

(iii) The fact that L-Phe-NCA immediately reacted with cyclohexylamine was in agreement with the literature data.⁸ The anhydride absorption bands at 1850w and 1790s cm^{-1} disappeared and a band of high intensity at 1650 (L-Phe-NHC₆H₁₁ plus NCA^-) and 2340 (CO_2) cm^{-1} appeared. The amide product was detected by TLC as well.

(iv) When an equivalent amount of cyclohexylamine was added to Btc-L-Phe-OH in acetonitrile, the bands at

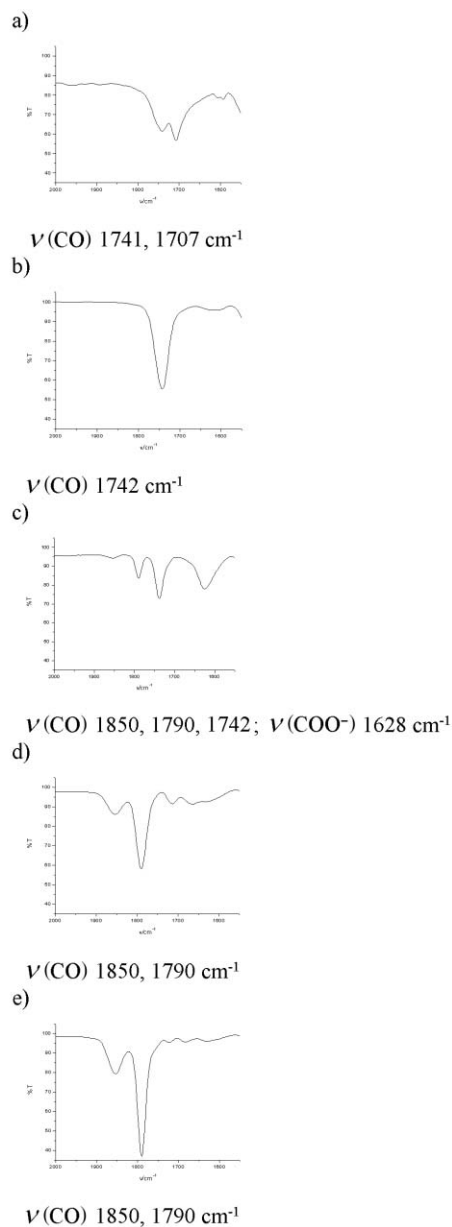


Figure 1. IR spectra: a) Btc-L-Phe-OH in KBr, b) Btc-L-Phe-OH in acetonitrile, c) Btc-L-Phe-OH in acetonitrile, 10 min after addition of an equivalent amount of TEA, d) Btc-L-Phe-OH in acetonitrile, 45 min after addition of the equivalent amount of TEA, e) L-Phe-NCA in acetonitrile.

1740 (Btc carbonyl) and 1650 cm^{-1} (COO^- plus CONH carbonyl) were present. As the time progressed, the first absorption decreased and the second increased concurrently with the CO_2 band. No NCA absorption bands were observed, since L-Phe-NCA formed from Btc-L-Phe-OH immediately reacted with cyclohexylamine.

* By mistake, only the absorption at 1700 cm^{-1} was reported in our previous paper.²

** For comparison, a spectrum of phenylacetic acid was taken: $\nu(\text{CO})$ in KBr at 1710 shifted in acetonitrile to 1750 cm^{-1} and after addition of TEA, carboxylate absorption band at 1630 cm^{-1} appeared.

IR spectra of the Btc-L-Phe-OH reactions with other amines were similar.

(v) IR spectra of *N*-(5-methyl-1-benzotriazolecarbonyl)-L-phenylalanine (MBtc-L-Phe-OH, **1d**) with TEA showed the same NCA and polymer absorption bands as in the experiment with **1a**.

According to our previous findings,¹ indazole derivatives *N*-(1-indazolecarbonyl)-L-phenylalanine (Inc-L-Phe-OH, **1b**), and *N*-(5-nitro-1-indazolecarbonyl)-L-phenylalanine (NInc-L-Phe-OH, **1c**), were less reactive. The spectrum of **1b** in acetonitrile showed COOH and Inc carbonyl absorptions at 1755 and 1725 cm⁻¹. After addition of TEA, the absorption of Inc remained at 1725 and the absorption at 1755 shifted to 1630 cm⁻¹ (COO⁻). This spectrum remained unchanged for 24 h without any signs of NCA and CO₂ formation. This was in agreement with the experimental data – **1b** did not react with amines and alcohols.¹

Absorption bands of the carboxy group at 1750 of nitroindazole derivative **1c** in acetonitrile shifted to 1630 (COO⁻), while the NInc band at 1730 cm⁻¹ remained unchanged after addition of an equivalent amount of TEA. NCA absorption appeared after 1 hour, and after 4 h the NCA band at 1790 cm⁻¹ was equally strong as the starting NInc band. According to IR, the reaction was not completed even 20 h later, but it finished after a short (40 min) reflux, affording poly-L-phenylalanine.

(vi) Addition of an equivalent amount of benzotriazole to a solution of L-Phe-NCA did not change the characteristic anhydride absorptions, so one could exclude benzotriazolide **7** formation, the starting compound in amidation of *N*-acylbenzotriazoles.^{10–12} The analogous imidazolide intermediate was proposed in oligopeptide formation from amino acids and 1,1'-carbonyldiimidazole.¹³

All the above facts strictly corroborate NCA as an intermediate in amidation reactions of Azc-amino acids (Scheme 2). One could expect the analogous esterification reactions to proceed through the same NCA intermediate.

EXPERIMENTAL

Infrared spectra of **1a-d** or **6** in dry acetonitrile were measured in 0.2 mm sodium chloride cells on a FT-IR Perkin Elmer Paragon 500 spectrometer (Perkin Elmer, UK). Attention was focused on absorptions close to 2340 cm⁻¹ (CO₂)

and in the range of 1850–1600 cm⁻¹ (Azc, COOH or the anhydride group). The used *N*-(1-azolecarbonyl)amino acids **1a-d** were prepared according to the previously published procedures from L-phenylalanine and the corresponding 1-azolecarbonyl chloride.^{1,2} L-Phe-NCA was prepared following the procedure of Daly and Poché.¹⁴ Acetonitrile, triethylamine and cyclohexylamine were dried and freshly distilled prior to use.

ABBREVIATIONS

Azc – 1-azolecarbonyl; AzH – azole; Bzl – benzyl; Bt – 1-benzotriazolyl; Btc – 1-benzotriazolecarbonyl; BtH – benzotriazole; Inc – indazolecarbonyl; MBtc – 5-methylbenzotriazolecarbonyl; NCA – *N*-carboxy anhydride; NInc – 5-nitro-1-indazolecarbonyl; TEA – triethylamine.

Acknowledgement. – This work was supported by Grant 0006543 of the Ministry of Science and Technology of the Republic of Croatia.

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SAŽETAK

Praćenje reakcije amidiranja *N*-(1-azolkarbonil)-aminokiselina na derivatima L-fenilalanina kao modelnim spojevima IR spektroskopijom

Marijana Zovko, Igor Kalčić, Branka Zorc i Ivan Butula

Proučavano je ponašanje *N*-(1-azolkarbonil)-L-fenilalanina (Azc-Phe-OH, **1a-d**) i *N*-karboksi anhidrida L-fenilalanina (L-Phe-NCA, **6**) u prisutnosti trietilamina (TEA) ili cikloheksilamina IR spektroskopijom. IR spektri studiranih spojeva snimljeni su u acetonitrilu. Praćene su apsorpcijske vrpce karakteristične za CO₂ (2340 cm⁻¹) i Azc, COOH ili anhidride (1150–1600 cm⁻¹). Dobiveni rezultati ukazuju na *N*-karboksi anhidrid kao intermedijer u reakcijama amidiranja Azc-aminokiselina.