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Reactions with *N*-(1-benzotriazolylcarbonyl)amino acids. V. Reactions with hydroxylamine

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Synthesis of 2-(*N*'-hydroxyureido)alkaneamides **5**, new hydroxyurea derivatives, starting from *N*-(1-benzotriazolylcarbonyl)-(Btc)-amino acid amides and hydroxylamine, is described. The products are tested for antibacterial, antifungal and mitodepressive activities. The preliminary tests show no significant bacteriostatic and antifungal activities, however, an important mitodepressive activity has been observed. In addition, a new synthetic way to 2-aminohydroxamic acids **3** is proposed.

Keywords: 2-aminohydroxamic acids, hydroxyurea, 2-(*N*'-hydroxyureido)alkaneamides, *N*-(1-benzotriazolylcarbonyl)amino acids, hydroxylamine, mitodepressive activity

The current interest in the chemistry of hydroxamic acids is related to the variety of their pharmaceutical and industrial applications, as well as to their role as siderophores and model systems for natural siderophores (1). Hydroxamic acids have a number of diverse pharmacological activities, including antibacterial, antifungal and cytostatic activities. A hydroxamic acid related compound is hydroxyurea, an antineoplastic agent which is used in the treatment of chronic myeloid leukemia, malignant melanoma and inoperable tumors of the ovary (2).

In this paper, synthesis and preliminary biological tests of 2-(*N*'-hydroxyureido)alkaneamides, new hydroxyurea derivatives, are reported. In addition, a new synthetic way to 2-aminohydroxamic acids is proposed.

EXPERIMENTAL

Melting points are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 457 spectrophotometer (Perkin-Elmer, Norwalk, CT, USA). ¹H-NMR spectra were recorded on a Jeol FX-100 instrument (Jeol, Tokyo, Japan). Specific rotation data were taken on an Opton polarimeter. For thin-layer chromatography, silica gel sheets Kieselgel 60 F254 Merck (Merck, Darmstadt, Germany) were used. Solvent systems were dichlo-

* Correspondence

romethane/methanol in ratio 95:5, 2-butanol/formic acid/water, 75:15:10 and dioxane/water, 9:1. For spot detection FeCl₃ solution or iodine were used. Column chromatography was performed on silica gel 0.063–0.200 mm. The *N*-(1-benzotriazolylcarbonyl)-(Btc)-amino acids **1** and the corresponding amides **4** were synthesized according to the literature (3, 4). The solutions of 2-(*N'*-hydroxyureido)alkaneamides **5** were screened for their antibacterial activity against *Staphylococcus albus*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella sp.*, *Candida albicans*, *Candida krusei* and *Candida parapsilosis* by disc diffusion test. Phytobiological tests were performed following the literature (5).

2-Aminopropiohydroxamic acid (*H*-DL-Ala-NHOH) (**3a**)

To the solution of 0.117 g (0.5 mmol) *N*-(1-benzotriazolylcarbonyl)-DL-alanine (*N*-Btc-Ala-OH) (**1a**) and 0.035 g (0.5 mmol) hydroxylamine hydrochloride in 2 mL *N,N*-dimethylformamide (DMF), 0.1 g (1 mmol) of triethylamine (TEA) is added. The reaction mixture was stirred for 4 h at room temperature. The separated TEA · HCl was filtered and the filtrate was evaporated at reduced pressure. The residue was washed several times with hot benzene and then with small amount of methanol in order to remove benzotriazole. In that way pure hydroxamic acid **3a** was obtained. Yield: 0.021 g (40%); m.p. 160–162 °C, lit. (6) m.p. 162–163 °C. IR spectrum is identical to that of the original sample.

2-(*N'*-Hydroxyureido)alkaneamides (**5a–g**). General procedure

To the solution of 5 mmol of the corresponding *N*-Btc-amino acid amide **4** in 20–50 mL dichloromethane, solution of hydroxylamine (5 mmol)* in 20 mL of methanol is added. The reaction mixture was stirred for 12 h at room temperature. The reaction was monitored by TLC (dichloromethane/methanol, 95:5). The solvent was evaporated *in vacuo* and the corresponding product **5** was isolated as follows:

N-Benzyl-2-(*N'*-hydroxyureido)-3-phenylpropionamide (**5a**). – The residue was dissolved in a mixture of 15 mL acetone and 5 mL water and the obtained solution was made acidic (pH 3–4) with diluted HCl. After evaporation of acetone the product **5a** precipitated. Two recrystallizations from benzene gave the pure product.

N-Butyl-2-(*N'*-hydroxyureido)-3-phenylpropionamide (**5b**). – The residue was dissolved in ethylacetate, the solution was extracted three times with 2 per cent sodium hydroxide solution (removal of benzotriazole) and then with water. The organic layer was dried over Na₂SO₄ and evaporated to give **5b**. The crude product was recrystallized from chloroform.

N-Benzyl-2-(*N'*-hydroxyureido)phenylacetamide (**5c**). – The same isolation procedure as for **5b** was applied. The crude product **5c** was recrystallized from ether.

N-Cyclohexyl-2-(*N'*-hydroxyureido)phenylacetamide (**5d**). – The residue was dissolved in a mixture of 15 mL acetone and 5 mL water and the obtained solution was made

* Hydroxylamine was prepared from equimolar amounts of hydroxylamine hydrochloride and sodium methoxide.

acidic (pH 3–4) with diluted HCl. After evaporation of acetone the product **5d** precipitated. The product was washed several times with hot benzene.

$^1\text{H-NMR}$ (DMSO- d_6), δ (ppm): 1.2–2 (m, 11H, C_6H_{11}), 5.45 (d, 1H, CHCO), 6.95 (d, 1H, $\text{CONHC}_6\text{H}_{11}$), 7.53–7.36 (m, 5H, C_6H_5), 8.249 (d, 1H, CHNHCO), 8.645 (s, 1H, NHOH), 8.883 (s, 1H, OH).

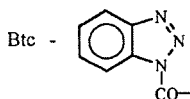
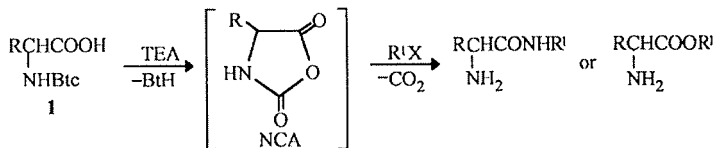
N-Cyclohexyl-2-(*N'*-hydroxyureido)propionamide (**5e**). – The reaction was performed in methanol. The solvent was evaporated and the residue was worked out analogously as for **5b**. The crude product was treated with hot toluene until the pure product **5e** was obtained.

N-Butyl-2-(*N'*-hydroxyureido)propionamide (**5f**). – The residue was worked out with 15 mL chloroform. The insoluble inorganic salt was filtered and the filtrate was evaporated. The crude product **5f** was purified on a silica gel column using chloroform/methanol, 7:3 as eluent.

N-Butyl-2-(*N'*-hydroxyureido)acetamide (**5g**). – Two reaction products in approximate molar ratio 1:1 were obtained: hydroxyureido derivative **5g** and 1-butylhydantoin. The residue was diluted with 10 mL of water and extracted twice with ethylacetate. The hydantoin partially crystallized from aqueous layer, but also from the concentrated organic layer. After evaporation of ethylacetate the crude product **5g** was recrystallized three times from chloroform.

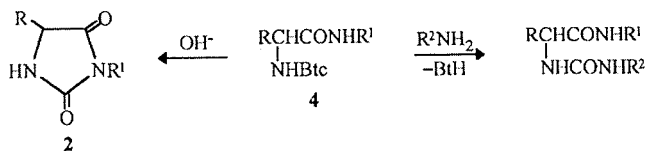
RESULTS AND DISCUSSION

In our previous papers (3, 7) the synthesis and use of *N*-Btc-amino acids **1** in amino acid amides and low peptides synthesis have been described. The amide (peptide) bond formation was achieved by means of 1-benzotriazolylcarbonyl (Btc) group both as an *N*-protecting and *C*-activating group. This process is accompanied with separation of benzotriazole and carbon dioxide. In similar reactions, *N*-Btc-amino acids and alcohols give amino acid esters (**8**). It has been confirmed (9) that the reactions of amide or ester bond formation proceed through the cyclic intermediate product *N*-carboxy anhydrides (NCA):



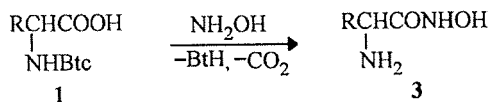
BtH - benzotriazole
TEA - triethylamine
X - NH_2 or OH

On the other hand, *N*-Btc-amino acid amides **4** under basic conditions cyclize to hydantoins **2** or react with amines yielding hydantoic acid amides (**4**):



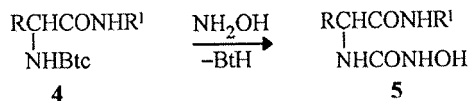
These products are results of direct nucleophilic attack of the amine on the carbonyl group activated by benzotriazole (10).

The use of hydroxylamine as an aminolyzing agent in the above reactions offers the opportunity for synthesis of hydroxamic acids and hydroxyureas, interesting and potentially useful amino acid derivatives. In this paper the reactions of *N*-Btc-amino acids 1 and *N*-Btc-amino acid amides 4 with hydroxylamine are described. The first reaction follows the scheme:



The reactions are performed in dry acetonitrile, DMF or methanol using sodium methoxide or triethylamine for transformation of hydroxylamine hydrochloride to hydroxylamine base. The preliminary experiments show that the reactions proceed unambiguously and the products are 2-aminoalkanehydroxamic acids 3. Thus, 2-aminopropiohydroxamic acid 3a (H-DL-Ala-NHOH) is prepared. The analogous *L*-phenylalanine and phenylglycine derivatives were identified on TLC only. Since the isolation of the corresponding hydroxamic acid from benzotriazole and sodium chloride or triethylamine hydrochloride is rather complicated, any further efforts to optimize the reactions have been stopped.

The reactions of *N*-Btc-amino acid amides 4 with hydroxylamine are in accordance with some previously described reactions of hydantoic acid amide formation (4). The reactions proceed according to the following scheme:



The products of the reactions are 2-(*N*'-hydroxyureido)alkaneamides 5a–g. In some cases hydantoin formation occurs also as a result of the presence of a small amount of sodium methoxide which is able to catalyze the cyclization (11). Yields and properties of compounds 5a–g are summarized in Table I.

The synthesized 2-(*N*'-hydroxyureido)alkaneamides 5a–g have been tested for antimicrobial and mitodepressive activity. The disc diffusion tests have shown no significant bacteriostatic activity against several Gram-positive and Gram-negative bacteria and somewhat higher, but also insignificant antifungal activity (the lowest inhibitory concentration, MIC = 0.25%, has been found against *Candida albicans*). In further experiments

Table I. Synthesis of 2-(*N*'-hydroxyureido)alkaneamides 5a–g

Starting compound 4	5	R	R ¹	Yield (%)	m.p. (°C)	[α] _D ²⁰ (°)	Molecular formula (M _r)	Analysis: calc. found		IR (KBr, cm ⁻¹)	
								C	H		N
								$\begin{array}{c} \text{RCHCONHR}^1 \\ \\ \text{NHCONHOH} \end{array}$			
Btc-L-Phe-NHCH ₂ C ₆ H ₅	a	C ₆ H ₅ CH ₂	C ₆ H ₅ CH ₂	87	158–160	+3.83 (c 0.67 MeOH)	C ₁₇ H ₁₉ N ₃ O ₃ (313.35)	65.15 65.41	6.11 6.30	13.41 13.28	3415, 3280, 1640, 1540
Btc-L-Phe-NHC ₄ H ₉	b	C ₆ H ₅ CH ₂	C ₄ H ₉	80	116–118	+11.12 (c 1.7 MeOH)	C ₁₄ H ₂₁ N ₃ O ₃ (279.33)	60.19 60.50	7.57 7.96	15.04 14.83	3415, 3300, 1660, 1620, 1550
Btc-DL-Pgly-NHCH ₂ C ₆ H ₅	c	C ₆ H ₅	C ₆ H ₅ CH ₂	85	128–130	–	C ₁₆ H ₁₇ N ₃ O ₃ (299.32)	64.19 64.32	5.72 5.99	14.03 14.37	3360–3280, 1630, 1550
Btc-DL-Pgly-NHC ₆ H ₁₁	d	C ₆ H ₅	C ₆ H ₁₁	77	258–260	–	C ₁₅ H ₂₁ N ₃ O ₃ (291.34)	61.81 61.78	7.27 7.06	14.48 14.30	3410, 3300, 1650, 1625, 1535
Btc-DL-Ala-NHC ₆ H ₁₁	e	CH ₃	C ₆ H ₁₁	65	188–190	–	C ₁₀ H ₁₉ N ₃ O ₃ (229.27)	52.38 52.49	8.35 8.23	18.32 18.33	3405, 3290, 1655, 1635, 1580
Btc-DL-Ala-NHC ₄ H ₉	f	CH ₃	C ₄ H ₉	50	110–112	–	C ₈ H ₁₇ N ₃ O ₃ (203.24)	47.27 47.84	8.43 8.91	20.67 20.45	3405, 3290, 1655, 1635, 1575
Btc-Gly-NHC ₄ H ₉	g	H	C ₄ H ₉	a	173–175	–	C ₇ H ₁₅ N ₃ O ₃ (189.20)	44.31 44.91	7.99 7.62	22.21 21.90	3360, 3280, 1645, 1565

^a 5g and 1-butylhydantoin are the reaction products in approximate molar ratio 1:1.

the compounds 5a–g were evaluated for their mitodepressive activity on *Lepidium sativum* L. seeds (5). Since 2-(*N'*-hydroxyureido)alkaneamides are sparingly soluble in water, phytobiological tests have been made in one per cent aqueous solution of methylcellulose. The mitodepressive activities of 5a–g are compared with the activity of antineoplastic agent hydroxyurea (12). The results are presented in Table II. All compounds exhibit important mitodepressive activities. The activity of 5d is similar to the activity of hydroxyurea in the same concentration and all other products show significantly higher activities. To evaluate their pharmacological effects and potential application as antineoplastic agents, further studies are required.

Table II. Mitodepressive activity of 2-(*N'*-hydroxyureido)alkaneamides (5a–g) on *Lepidium sativum* L. seeds

Compound 5	Inhibition (%)	
	γ (mg mL ⁻¹)	
	0.5	1.0
a	48	47
b	44	49
c	44	48
d	16	31
e	43	45
f	23	33
g	32	40
Hydroxyurea	15	24
	16 ^a	20 ^a

^a Literature data (12).

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S A Ž E T A K

Reakcije s *N*-(1-benzotriazolilkarbonil)aminokiselinama. V. Reakcije s hidroksilaminom

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Opisana je sinteza amida 2-(*N*-hidroksiureido)alkan kiselina 5 iz odgovarajućih amida *N*-(1-benzotriazolilkarbonil)aminokiselina i hidroksilamina. Ispitan je antibakterijski, antimikotski i mitodepresivni učinak sintetiziranih spojeva. Preliminarni rezultati pokazuju neznatno antibakterijsko i antimikotsko djelovanje, ali značajno mitodepresivno djelovanje. U radu su opisani i preliminarni pokusi za pripravu 2-aminohidroksamskih kiselina 3.

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