SYNTHESIS AND ANTIOXIDANT PROPERTIES OF α- AND β-HETERYLAMIDES AND N-HETERYLIMIDES OF (±)-1,2,2-TRIMETHYLCYCLOPENTANE-1,3-DICARBOXYLIC ACID

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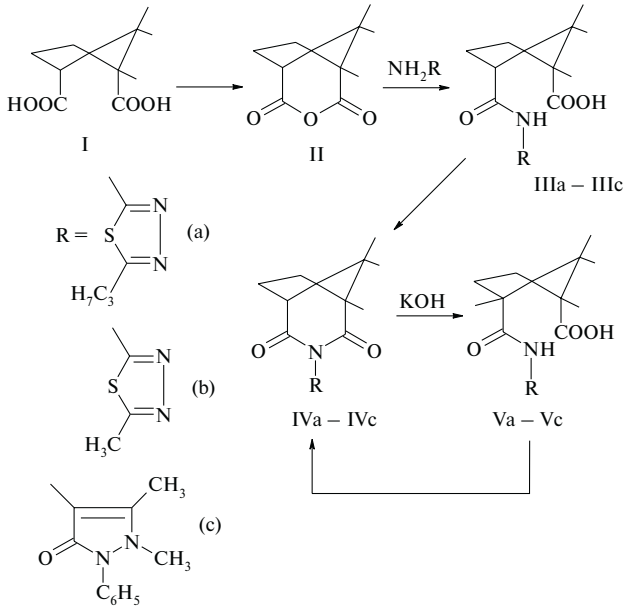
It was experimentally established that hyperglycemia is accompanied by increased intensity of lipid peroxidation (LPO). The administration of antioxidants leads to normalization of the LPO process and decreases the blood glucose level [1, 2]. In continuation of the search for new substances possessing antidiabetic activity [3, 4], we have synthesized α- and β-heterylamides and the corresponding N-imides – the derivatives of (±)-1,2,2-trimethylcyclopentane-1,3-dicarboxylic acid (I):



The synthesis proceeded from heterylamines and *d*,*l*-camphor anhydride (II) obtained by boiling acid I with a double excess (by weight) of acetic anhydride. α-Amides (IIIa – IIIc) were synthesized by interaction of anhydride II with equimolar amounts of the corresponding heterylamines in DMF. The reactions were carried out by heating the reaction mixtures to 150°C for 30 min and the products were obtained with a yield of up to 60%.

Imides IVa – IVc were synthesized by heating the corresponding heterylamides for 30 min in the presence of acetyl chloride (in a 2 : 1 ratio) in glacial acetic acid. The final products were obtained with a yield of about 50%. The proposed structures were confirmed by countersynthesis using cyclization of the corresponding β-heterylamides Va – Vc under analogous conditions. β-Heterylamides Va – Vc were obtained by heating imides IVa – IVc for 2 h in a 10% aqueous KOH solution. These products were obtained with a yield of up to 45%.

The synthesized α-amides IIIa – IIIc and β-amides Va – Vc are soluble in alcohols, acetone, and aqueous alkali solutions. Imides IVa – IVc are soluble in alcohols and acetone.

In order to confirm the proposed structures of the synthesized compounds, we studied their ^{13}C and ^{1}H NMR spectra. The ^{13}C NMR spectrum of acid I (dissolved in CD_3OD) exhibits two signals in the region corresponding to carbon atoms of the carbonyl groups. The low-field signal (δ = 179.54 ppm) is related to the carbon atom of a carboxy group at the quaternary carbon atom C_1, while the high-field signal (δ = 177.70 ppm) is assigned to the carbon atom of a carboxy group at C_3 (Table 1). The passage to amides is usually ac-

TABLE 1. Chemical Shifts in the ^{13}C NMR Spectra of the Synthesized Compounds

Compound	δ, ppm			
	3-CO	1-CO	C_3	C_1
I	177.70	179.54	53.8	57.30
IIIa	173.70	179.17	54.8	57.64
IVa	173.35	177.04	55.02	56.46
Va	177.15	175.90	53.85	57.73

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TABLE 2. Parameters of the ^{1}H NMR Spectra of the Synthesized Compounds*

Compound	δ (ppm), multiplicity, J (Hz)			
	1 – CH_3, 2.2 – $(CH_3)_2$	$C_{(3)}H$	$C_{(5)}H$**	$C_{(4)}H_2$***
I	0.86 s, 1.28 s, 1.22 s	2.83 q (9.0; 9.8)	2.50 t 1.47 t	2.10 q 1.81 q
IIa	0.88 s, 1.29 s, 1.28 s	2.98 t (7.3)	2.6 m 1.6 m	2.2 m 1.9 m
IVa	1.06 s, 1.25 s, 1.15 s	2.87 d (6.8)	2.3 m	2.2 m 2.1 m
Va	0.85 s, 1.36 s, 1.35 s	2.88 q (8.0, 10.0)	2.6 t, 1.69 t	2.23 q 1.93 q

* Solvent CD_3OD (for I, IVa, Va) and $CDCl_3$ (for IV).
** $J_2 = 12.0 \pm 0.2$ Hz.
*** $J_2 = 13.7 \pm 0.1$ Hz.

companied by a decrease in the chemical shift for the carbon atoms of carboxy groups [5]. In the spectrum of amide IIIa, the chemical shift of carbon in the carboxy group at C_1 remains virtually unchanged (δ = 179.17 ppm), while the signal due to carbon of the carbamide group at C_3 exhibits a shift by 4 ppm toward higher fields: from δ = 177.70 ppm for acid I to 173.70 ppm for compound IIIa.

The ^{13}C NMR spectrum of imide IVa is characterized by a decrease in the chemical shift of carbon atoms in both carbonyl groups relative to the value for the initial acid I, which confirms cyclization of the α- and β-amides with the formation of imide structures. In the spectrum of β-amide Va, the chemical shift of the carbon atom in the carboxy group at C_3 (in contrast to the analogous value for the α-amide IIIa) remains almost unchanged (cf. δ = 177.70 ppm for acid I and 177.15 ppm for compound Va), whereas the signal due to carbon of the carbamide group at C_1 significantly shifts (Δδ = 3.64 ppm) toward higher fields: from δ = 179.54 ppm for acid I to 175.90 ppm for compound Va, which confirms the β-amide structure.

The ^{1}H NMR spectra of the α- and β-amides also exhibit characteristic distinctions when compared to the spectrum of imide IVa. The main difference is that the signal due to a $C_{(3)}H$-methine proton in the spectrum of imide IVa has the form of a quartet or triplet, while the signal of analogous protons in α- and β-amides IIIa and Va appear as doublets (Table 2).

The IR absorption spectra most clearly reveal a difference between the structures of amides (III, V) and imides (IV). The spectra of the latter compounds display characteristic bands with the ν^s and ν^{as} frequencies of the carbonyl groups (Table 3) significantly reduced as compared to the analogous values for the initial anhydride II (1760 and 1800 cm^{-1}). The data of our spectroscopic measurements confirm the results of calculations [4] showing evidence of a regioselective character of the opening anhydride and N-imide cycles in acid I.

The results of TLC analyses confirmed the purity of the synthesized compounds. The proposed structures are consistent with the results of elemental analyses and IR spectroscopic measurements (Table 3).

It was a established that compounds IIIa and IVc possess antioxidant properties comparable to those of vitamin E. Tested under analogous conditions, the synthesized β-heterylamides exhibited no antioxidant activity.

TABLE 3. Yields and Physicochemical Characteristics of the Synthesized Compounds

Compound	Yield, %	M.p., °C	Empirical formula	R_f in solvent system*			IR spectrum: ν, cm^{-1}
				(1)	(2)	(3)	
IIIa	45	216 – 219	$C_{15}H_{23}N_3O_3S$	0.33	0.65	…	1677 (ν_{CONH}); 1554 (δ_{NH}); 1718 ($\nu_{C=O}$); 3202 (ν_{NH})
IIIb	48	257 – 260	$C_{13}H_{19}N_3O_3S$	0.24	0.44	0.29	1541(δ_{NH}); 1692($\nu_{C=O}$); 3185 (ν_{NH})
IIIc	45	238 – 240	$C_{21}H_{27}N_3O_4$	0.26	0.29	…	1670 (ν_{CONH}); 1575 (δ_{NH}); 3260 (ν_{NH})
IVa	58,** 56***	82 – 83	$C_{15}H_{21}N_3O_2S$	0.33	0.54	…	1680 ($\nu^{as}_{C=O}$); 1731 ($\nu^{s}_{C=O}$)
IVb	59,** 56***	112 – 114	$C_{13}H_{17}N_3O_2S$	0.50	0.41	0.28	1681 ($\nu^{as}_{C=O}$); 1732 ($\nu^{s}_{C=O}$)
IVc	59,** 54***	223 – 225	$C_{21}H_{25}N_3O_3$	0.41	0.24	…	1701($\nu^{as}_{C=O}$); 1742($\nu^{s}_{C=O}$)
Va	60	118 – 120	$C_{15}H_{23}N_3O_3S$	0.33	…	0.44	1675 (ν_{CONH}); 1555 (δ_{NH}); 3204 (ν_{NH})
Vb	59	137 – 138	$C_{13}H_{19}N_3O_3S$	0.21	…	0.41	1688 (ν_{CONH}); 1537 (δ_{NH}); 1722 ($\nu_{C=O}$); 3205 (ν_{NH})
Vc	59	215 – 216	$C_{21}H_{27}N_3O_4$	0.26	0.40	…	1659 (ν_{CONH}); 1574 (δ_{NH}); 1680 ($\nu_{C=O}$); 3271 (ν_{NH})

* R_f determined in solvent systems (1) chloroform – acetone, 2 : 1; (2) chloroform – ethanol – hexane, 1 : 1 : 1; (3) carbon tetrachloride – acetone, 6 : 4.
** Yield from amides IIIa – IIIc.
*** Yield from amides Va – Vc.

EXPERIMENTAL CHEMICAL PART

The ^1H and ^{13}C NMR spectra were measured on an XL-400 (Varian) spectrometer using CD_3OD as the solvent and TMS as the internal standard. The IR absorption spectra were recorded on a Specord M-80 spectrophotometer using samples pelletized with KBr. The course of reactions was monitored and purity of the reaction products was checked by TLC on Silufol UV-254 plates (Czech Republic). The plates were developed by treatment with Bromocresol Blue, revealing blue spots of imides and yellow spots of amides. The melting temperatures were determined with the aid of a Boetius heating table. The yields and physicochemical characteristics of the synthesized compounds are listed in Table 3. The data of elemental analyses (S, N) of compounds III – V agree with the results of analytical calculations performed according to their empirical formulas.

1,2,2-Trimethylcyclopentane-1,3-dicarboxylic acid (±)-α-N-(5-propyl-1,3,4-thia-2-diazolyl)amide (IIIa). A mixture of 1.82 g (0.01 mole) of anhydride II, 1.15 g (0.01 mole) of 2-amino-5-propyl-1,3,4-thiadiazole, and 2 ml of DMF was boiled for 30 min, cooled, and diluted with two volumes of water. The precipitate was separated by filtration, dried, and crystallized from methyl alcohol. Analogous procedures were used to obtain amides IIIb and IIIc.

1,2,2-Trimethylcyclopentane-1,3-dicarboxylic acid (±)-N-(5-propyl-1,3,4-thia-2-diazolyl)imide (IVa).

Method A. A mixture of 3.25 g (0.01 mole) of amide IIIa, 4 ml of glacial acetic acid, and 2 ml of acetyl chloride was heated on a water bath for 30 min, cooled, and diluted with two volumes of water. The precipitate was separated by filtration, dried, and crystallized from methyl alcohol.

Method B. A mixture of 3.25 g (0.01 mole) of amide Va, 4 ml of glacial acetic acid, and 2 ml of acetyl chloride was heated on a water bath for 30 min, cooled, and diluted with two volumes of water. The precipitate was separated by filtration, dried, and crystallized from methyl alcohol.

Analogous procedures were used to obtain imides IVb and IVc.

1,2,2-Trimethylcyclopentane-1,3-dicarboxylic acid (±)-β-N-(5-propyl-1,3,4-thia-2-diazolyl)amide (Va). A mixture of 3.07 g (0.01 mole) of imide IVa and 11.2 ml (0.02 mole) of a 10% aqueous KOH solution was heated on a water bath for 2 h, cooled, and acidified with diluted (1 : 1) HCl to pH 4. The precipitate was separated by filtration, dried, and crystallized from methyl alcohol. Analogous procedures were used to obtain amides Vb and Vc (Table 3).

EXPERIMENTAL BIOLOGICAL PART

The antioxidant activity of the synthesized compounds was studied on Wistar rats weighing 175 – 180 g with a model of acute toxic hepatitis induced by intragastric introduction of tetrachloromethane (1 ml of 50% oil solution per 100 g body weight). The compounds studied (in a dose of 25 mg/kg) and the reference drug (vitamin E, 50 mg/kg) were administered perorally 2 h before and 2 h after introduction of the hepatotoxic agent.

TABLE 4. Acute Toxicity and Antioxidant Activity of Compounds IIIa and Va

Compound	LD_{50} (mice)		Dose, mg/kg	Antioxidant effect: MDA content, mM
	intragastric administration	intraperitoneal administration		
Vitamin E + tetrachloromethane			50	115.0 ± 5.31**
IIIa + tetrachloromethane	10000	2300	25	91.3 ± 7.0**
IVc + tetrachloromethane	9000	2000	"	108 ± 8.20**
Intact control				85.8 ± 3.22
Tetrachloromethane				145.7 ± 6.11*

Notes. Each test group contained five animals.
* $p < 0.05$ relative to intact control.
** $p < 0.05$ relative to tetrachloromethane.

The antioxidant effect was evaluated by the decrease in the malonic dialdehyde (MDA) content in rat liver homogenates determined according to [6]. The acute toxicity (LD_{50}) was determined in mice according to [7] for the substances introduced (in the form of a 3 – 5% aqueous suspension stabilized with Tween-80) intragastrically or intraperitoneally in a volume not exceeding 1 ml (Table 4).

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