SYNTHESIS AND ANTIVIRAL ACTIVITY OF N-[2-HYDROXYIMINOALKYL[, N-[2-OXOALKYL]-, AND N-[2-HYDROXYALKYL]-β-HYDROXYAMINOACID ESTERS

A. Ya. Tikhonov, N. N. Voinova,
T. I. Reznikova, L. B. Volodarskii,
G. V. Vladyako, E. I. Boreko,
and L. V. Korobchenko

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α-Hydroxyaminoacids have aroused interest as potential biologically active compounds and as metabolites of α-aninoacids [5]. Little is known, however, about β-hydroxyaminoacids and their derivatives [3, 4]. In continuation of work on organic derivatives of hydroxylamine [7] in a search for compounds with biological activity, we have synthesized the β-hydroxyaminoacid (Ia) and esters of N-substituted β-hydroxyaminoacids containing an oxime group (Ib-s), a keto-group (II), (III), and a hydroxyl group (IV), and tested them for antiviral activity.

3-[(2-Hydroxyiminocycloheptyl)hydroxyamino]propanoic acid (Ia) was obtained from 2-hydroxyaminocycloheptanone oxime (V, $R^1 + R^2 = (CH_2)_5$, $R^3 = H$) and acrylic acid. The N-(2-hydroxyiminoalkyl) (Ib-s), N-(2-oxoalkyl]- (II, III), and N-(2-hydroxyalkyl- (IV) β-hydroxyaminoacid esters were obtained from the appropriate 1,2-hydroxyamino-oximes (V), 1,2-hydroxyaminoketones, or 1,2-hydroxyaminoalcohols with esters of α,β-unsaturated acids (VI). All the products (Ia-q, II-IV), as in the reactions of N-alkyl (or aryl)-hydroxylamines with α,β-unsaturated acid esters [3], were the Michael addition products involving the hydroxyamino-nitrogen in 1,2-hydroxyamino-oximes (V), 1,2-hydroxyiminoketones, and the hydroxyiminoalcohol, even when the hydroxyimino-group is bonded to a tertiary carbon atom (R^2, $F^3 \neq H$). Accordingly, the PMR spectrum of (Iq) (Table 2) in DMSO-d_6 shows singlets for the oxime and hydroxyimino protons at 10.60 and 7.57 ppm respectively. The IR spectra of (II) and (III) in CCl_4 (Table 1) show absorption for stretching vibrations of the hydroxyimino OH group at 3570-3580 cm^{-1}. The UV spectra of (Io) and (Ip) show absorption maxima at 230 nm (log ε 3.81) and 242 nm (log ε 3.96) respectively, showing that the oxime group has the E- and Z-configurations, but that the configuration of the oxime group is unaffected on reaction of 1,2-hydroxyimino-oximes with α,β-unsaturated acid esters. (see scheme on next page.)

EXPERIMENTAL (CHEMICAL)

The IR spectra of crystalline solids were obtained in KBr disks, and those of the oils (II) and (III), in CCl_4, PMR spectra were recorded on a Varian A56/60A (60 MHz) in $(CD_3)_2SO$ (for Ic, g, h, q, II, and III), or in $(CD_3)_2CO$ (Ii) or pyridine (In), using HMDS as internal standard. The reactions were followed by TLC on Silufol UV-254 plates in the system chloroform–methanol, visualized by UV and iodine vapor. The elemental analyses were in agreement with the calculated values. Melting points were determined on a Boetius hot block.

Preparation of Esters of β-Hydroxyaminoacids (Ib, d-g, i-q), (II)-(IV), and β-Hydroxyaminoacid (Ia. To a suspension or a solution of the 1,2 hydroxyaminooxide [2, 7], 1,2-hydroxyaminoketone [7] or 1,2-hydroxyaminoalcohol [6] in ethanol was added with stirring an equimolar amount of the appropriate ester (VI) ($R^6 \neq H$) or the acrylic ester (VI, $R^4 = R^5 = R^6 = H$), and the mixture kept until the starting material was no longer present. The solution was evaporated, the residue treated with dry ether, and the solid filtered off. Compound (Ii), which separated from the reaction mixture, was filtered off and washed with alcohol. Compounds (II) and (III) were isolated by chromatography of the residue on silica gel using chloroform as eluent.

Institute of Organic Chemistry, Siberian Branch, Russian Academy of Sciences, Novosibirsk. Novosibirsk University, Belorussian Research Institute for Epidemiology and Microbiology, Minsk. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 26, No. 5, pp. 45-47, May, 1992. Original article submitted July 10, 1991.

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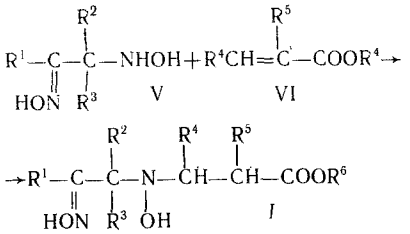
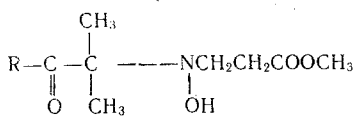
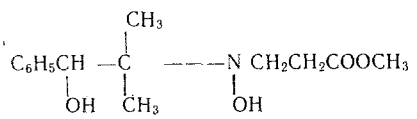


R1=CH_3 (Ik-n), C_6H_5 (Io-q); R^2=CH_3 (Ij,m-q); R^3=CH_3 (Id,i,j,n,q);
R_4=CH_3 (If); R^5=CH_3 (Ie,g,h,l); R^6=CH_3 (Ib,d,g,i-k, m-q);
C_2H_5 (Ie,f), $CH_2CH(CH_3)_2$ (Ic,h,l); R^1+R^2=$(CH_2)_5$ (Ia,e-h);
R^1+R^2=$(CH_2)_4$ (Ib-d), R^1+R^2=1,2-bismethyleno-3,3-dimethylcyclopropane (Ii), when not given, R=H



R=CH_3 (II), C_6H_5 (III)



IV

TABLE 1. Properties of Compound Obtained

Compound*	Yield, %	Mp, °C**	Empirical formula	IR spectrum, cm^{-1}
Ia	80	131—133	$C_{10}H_{18}N_2O_4$	1710
Ib	83	123—126	$C_{10}H_{18}N_2O_4$	1740
Ic	50	104—107	$C_{14}H_{26}N_2O_4$	1730
Id	83	129—131	$C_{11}H_{20}N_2O_4$	1725
Ie	79	100—103	$C_{12}H_{22}N_2O_4$	1735
If	80	124—126	$C_{13}H_{24}N_2O_4$	1740
Ig	70	116—117	$C_{12}H_{22}N_2O_4$	1740
Ih	40	129—131	$C_{15}H_{28}N_2O_4$	1720
Ii	77	195—196	$C_{14}H_{24}N_2O_4$	1730
Ij	75	74—75	$C_8H_{16}N_2O_4$	1730
Ik	70	110—112	$C_7H_{14}N_2O_4$	1730
Il	70	120—122	$C_{11}H_{22}N_2O_4$	1725
Im	60	125—126	$C_8H_{16}N_2O_4$	1745
In	92	161—163	$C_9H_{18}N_2O_4$	1740
Io	80	101—103	$C_{13}H_{18}N_2O_4$	1730
Ip	80	65—68	$C_{13}H_{18}N_2O_4$	1730
Iq	85	132—135	$C_{14}H_{20}N_2O_4$	1745
II	60	Oil	$C_9H_{17}NO_4$	1725, 1745, 3580
III	60	Oil	$C_{14}H_{19}NO_4$	1690, 1745, 3570
IV	80	96—100	$C_{14}H_{21}NO_4$	1705

*The PMR spectra of (Ic, g, h) show signals for a single isomer (although it is possible that small amounts of a second isomer are present).

**Compounds (Ib, d-g, l, o, q) were crystallized from ethyl acetate, (Ic, h) and (IV) from a 2:1 mixture of hexane and ethyl acetate, (Ia, k, m, n) from alcohol, (Ii) from DMF, (Ij) from ether, and (Ip) from a 1:1 mixture of ether and hexane.

TABLE 2. PMR Spectra of Esters of N-Substituted-β-hydroxyaminoacids

Compound	R'	$C(CH_3)_2$	$-\underset{|}{N}-CH_2$	$CH_2C(=O)$	OCH_3	OH
Il	7,45 s	1,22 s	2,88 m	2,51 m	3,62 s	7,03
In	2,12 s	1,43 s	3,06 m	2,71	3,49 s	
Iq	7,1—7,4 m	1,14 s	2,8—3,1 m	2,3—2,7 m	3,54 s	7,57 10,60
II	2,10 s	1,07 s	2,4—2,9 m		3,55 s	7,79
III	7,1—7,4, 8,0—8,3 m	1,28 s	2,3—2,9 m		3,50	7,80

TABLE 3. Antiviral Spectrum of Esters of N-Substituted-β-hydroxyaminoacids

Compound	MTC, μg/ml	Antiviral activity against							
		HSV	VVV	CAP	VSV	RSV	VEV	ECHO-6	RV
Ia	400	—	—	—	—	+	—	—	—
Ib	100	—	+	—	—	—	—	—	—
Ic	400	++	—	+	—	—	—	++	—
Ie	800	—	—	+++	—	+	—	—	—
Ig	400	—	—	—	—	—	+	—	—
Ik	400	—	—	—	—	—	—	—	++
Il	400	—	++	—	—	—	—	—	—
Im	400	—	—	+	—	—	—	—	—
In	800	—	—	—	++	—	—	—	—
IP	400	—	—	++	+	—	—	—	—
IV	800	+	—	—	—	+	—	—	—

Notes. Absence of activity; +, ++ weak activity (CTR = 1 or 2); +++ moderate activity (CTR = 4).

Isobutyl 3-[(2-Hydroxyiminocyclohexyl)hydroxyamino]- (Ic) and Isobutyl 3-[(2-hydroxyiminocycloheptyl)hydroxyamino]- (Ih) 2-methylpropanoates. A mixture of 16 mmole of the appropriate 1,2-hydroxyaminooxime and 8 ml of isobutyl methacrylate was stirred at 70°C until starting material was no longer present. The mixture was then treated with a mixture of ether and hexane (2:1), and the precipitated (Ic) or (Ih) filtered off.

EXPERIMENTAL (BIOLOGICAL)

Antiviral activity was assessed in tissue culture against the viruses herpes simplex type I (HSV), variola vaccine (VVV), classical avian plague (CAPV), vesicular stomatitis (VSR), Venezuelan equine encephalomyelitis (VEV), ECHO-6, and rotavirus (RV) by a screening test and platelet reduction under an agar cover.

The criteria for antiviral activity were the presence of platelet formation inhibition zones, and a decrease in viral titer as compared with the untreated controls.

The chemotherapeutic ratio (CR) was calculated as the ratio of the maximum concentrations tolerated by the tissue culture (MTC) to the mimimum active concentration.

Details of the method and calculation of results have been reported previously [1].

Eleven out of the twenty compounds tested were found to possess some degree of activity against both DNA and RNA viruses (Table 3). None of the compounds were toxic towards the CEF culture, the maximum tolerated concentrations ranging from 100 to 800 μg/ml. It will be seen that four of the compounds inhibited the multiplication of classical avian plague virus, with (Ie) having a chemotherapeutic ratio of 4-8.

The observed antiviral activity of these N-alkyl-β-hydroxyaminoacid esters therefore encourages a further search for antiviral drugs.

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