SYNTHESIS, SPATIAL STRUCTURE, AND BIOLOGICAL ACTIVITY OF 1-AZA-4-OXABICYCLO[4.1.0]HEPTAN-5-ONES

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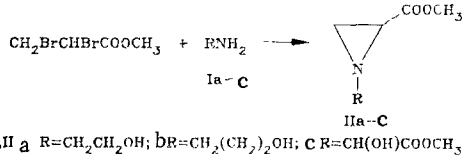
It has been found that the reaction of β- and γ-amino alcohols with methyl 1,2-dibromopropionate results in the formation of esters of 1-(hydroxyalkyl)aziridine-2-carboxylic acids or 1-aza-4-oxabicyclo[4.1.0]pentan-5-ones, depending on the nature of the original reactants. An x-ray structural analysis of this new bicyclic system has been carried out. The hepatoprotector activity of the new compounds has been studied.

Continuing the work on the synthesis and study of derivatives of aziridine-2-carboxylic acids and peptides containing fragments with three-membered rings, we obtained esters of 1-(hydroxyalkyl)aziridine-2-carboxylic acids by reacting methyl 1,2-dibromopropionate with amino alcohols.

An analysis of the PMR and IR spectra of the reaction products (Tables 1 and 2) unequivocally confirms the formation of methyl 1-(hydroxyalkyl)aziridine-2-carboxylates in the case of the use of initial amino alcohols such as 2-aminoethanol, 3-aminopropanol, and the methyl ester of serine.



I, II a R=CH_2CH_2OH; b R=$CH_2(CH_2)_2OH$; c R=CH(OH)$COOCH_3$

The PMR spectra of the compounds synthesized are characterized by values for the spin–spin coupling constants which are typical of 1,2-substituted aziridines. The assignment of the signals in the aziridine ring was determined on the basis of the known law governing the variation of the spin–spin coupling constants $J_{AB} > J_{AC} >> J_{BC}$ [1].

TABLE 1. Proton-Magnetic-Resonance Spectra of IIa-c, IIIa, b in $CDCl_3$

Compound	δ, ppm							J, Hz		
	H_A	H_B	H_C	CH_2OH	CH_2	$N-CH_2$	OCH_3	H_AH_B	H_AH_C	H_BH_C
IIa[a]	2,42	1,89	2,16	3,73 (t)	—	2,53 (t)	3,71 (s)	7,8	4,0	0,7
IIb[b]	2,09	1,62	2,21	3,81 (t)	1,81 (m)	2,53 (m)	3,72 (s)	6,5	3,0	0,8
IIc	1,88	2,22—2,62 (m)		3,93 (d)	—	—	3,71 (s)			
IIIa[c]	2,78	2,27	2,58	3,87, 3,69 (J=11,1 Hz)	4,32, 4,14 (J=12,7 Hz)	—	—	6,2	3,0	0,7
IIIb[d]	2,74	2,14	2,36		3,96, 3,83 (J=12,0 Hz)	—	—	6,5	2,8	0,8

[a] In D_2O. [b] δ_{CH} 2.40 ppm (m). [c] δ_{OH} 2.1 ppm. [d] δ_{CH_3} 1.32 and 1.16 ppm.

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TABLE 2. Characteristic Frequencies (cm^{-1}) in IR spectra of IIa-c, IIIa, and IIIb

Compound	νCH (aziridine ring)	δ (aziridine ring)	νC=O	νOH
IIa	3070	1200	1740	3350
IIb	3070	1200	1750	3360
IIc	3080	1210	1750	3250—3470
IIIa	3070	1230	1720	3370
IIIb	3080	1210	1740	—

TABLE 3. Carbon-13 Chemical Shifts of IIIa and b

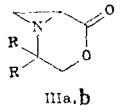
Compound	Solvent	$C_{(2)}$	$C_{(3)}$	$C_{(5)}$	$C_{(6)}$	$C_{(7)}$	$C_{(R)}$
IIIa	$CDCl_3$	56,0	62,9	168,7	29,1	25,4	25,2 and 23,4
IIIb	DMSO-d_6	49,4	69,9	165,2	31,3	26,3	62,9 and 64,4

TABLE 4. Influence of Compounds on the Prooxidant Effect of Carbon Tetrachloride in *in Vitro* Experiments

Scheme of experiment	Number of animals	Malonic dialdehyde, nM/mg protein·min	
		$M \pm m$	%
Control	30	6,0±0,6	100
CCl_4	30	18,9±1,0[a]	314
CCl_4+ vitamin E	5	4,1±0,7b	69
CCl_4+ cysteamine	5	4,7±0,3b	81
CCl_4+IIIa	5	6,3±0,7[b]	105
CCl_4+IIIb	5	13,9±1,5[b]	231

[a]The difference are statistically significant in comparison to the control at $P < 0.05$. [b]The differences are statistically significant in comparison to the series with the addition of CCl_4 at $P < 0.05$.

The use of amino alcohols in which the carbon atom adjacent to the amino group is bonded to a methyl or hydroxymethyl group, tris(hydroxymethyl)aminomethane and 2-amino-2-methylpropanol, as starting compounds results in the formation of a new heterocyclic system, viz., 1-aza-4-oxabicyclo[4.1.0]heptan-5-ones (III).



IIIa,b

a R=CH_2OH; b R=CH_3

In the PMR spectra of compounds IIIa and b the protons in the aziridine ring form an ABC system, and, in contrast to esters IIa-c, there is no singlet for the esteric group. The signals of the CH_2OH fragments differ with respect to the values of the chemical shifts from the signals of the CH_2 fragments of the morpholine ring. The values of J also differ accordingly (Table 1).

The ^{13}C NMR data for compounds IIIa and b correspond completely to the data in the PMR spectra (Table 3). The ^1H NMR spectrum of IIIa remains unchanged over a broad range of temperatures (from −50°C to +150°C) in various solvents ($CDCl_3$ and DMSO-d_6).

An x-ray diffraction investigation of IIIa was carried out for the purpose of determining the spatial structure of the new bicyclic system. The geometric characteristics of the aziridine ring in IIIa (Fig. 1) are close to those for other aziridine derivatives [2-4]. In the morpholine ring the $C_{(2)}$—$O_{(3)}$ bond is a single bond, while the $C_{(4)}$—$O_{(3)}$ bond is shortened, indicating the presence of conjugation of the lone pairs of the $O_{(3)}$ atom with the π system

TABLE 5. Activity of Alanine and Aspartate Aminotransferase and the Serum Bilirubin Concentration 24 h after the Introduction of CCl_4 and the Preliminary Introduction of the Compounds Investigated

Scheme of experiment	Number of animals	Alanine aminotransferase		Aspartate aminotransferase		Total bilirubin	
		M±m, units	%	M±m, units	%	M±m, mg %	%
Control	14	1,64±0,11	100	2,25±0,07	100	0,184±0,031	100
CCl_4	14	4,67±0,07[a]	285	3,96±0,10[a]	176	0,358±0,040[a]	194
CCl_4+ vitamin E	5	3,70±0,58[b]	226	3,18±0,18[b]	141	—	—
CCl_4+ cysteamine	5	1,91±0,16[b]	116	2,35±0,16[b]	104	—	—
CCl_4+IIIa	5	3,89±0,52[b]	237	2,96±0,40[b]	131	0,233±0,43[b]	127
CCl_4+IIIb	5	4,82±0,18[a]	294	3,56±0,31[a]	158	—	—

[a]The differences are statistically significant in comparison to the control at $P < 0.05$. [b]The differences are statistically significant in comparison to the group which was given CCl_4 at $P < 0.05$.

TABLE 6. Concentration of Protein and Cytochrome P-450 in the Liver 24 h after the Introduction of CCl_4 and the Preliminary Introduction of the Compounds Investigated

Scheme of experiment	Number of animals	Protein		Cytochrome P-450	
		M ± m, mg/g liver	%	M ± m, nm/mg protein	%
Control	9	158,4±9,2	100	0,39±0,04	100
CCl_4	9	123,2±6,8[a]	78	0,17±0,04[a]	44
CCl_4+ vitamin E	5	143,0±3,3[b]	90	0,34±0,02[b]	86
CCl_4+ cyasteamine	5	140,6±6,2[b]	89	—	—
CCl_4+IIIa	5	130,2±13,7[a]	82	0,20±0,07[a]	51
CCl_4+IIIb	5	132,5±9,4[a]	84	0,15±0,03[a]	38

[a]The differences are statistically significant in comparison to the control at $P < 0.05$. [b]The differences are statistically significant in comparison to the group which was given CCl_4 at $P < 0.05$.

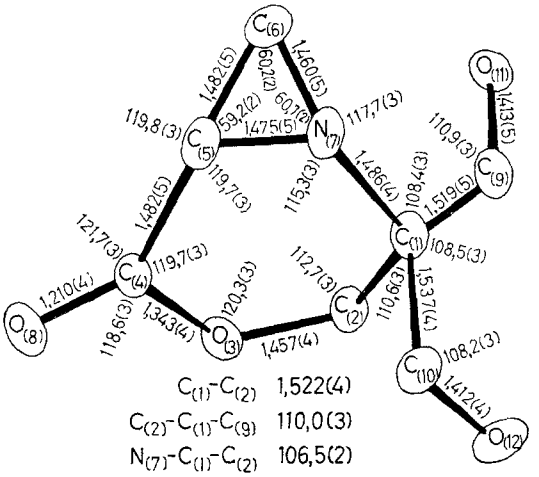


Fig. 1. Geometry of the molecule of 2,2-dihydroxymethyl-1-aza-4-oxabicyclo[4.1.0]-pentan-5-one (IIIa).

of the $C_{(4)}=O_{(8)}$ carbonyl group. The slope of the $C_{(1)}-N_{(7)}$ bond relative to the plane of the aziridine is 58.7°. The $N_{(7)}$ atom has pyramidal coordination, and the height of the pyramid is 0.671 Å.

TABLE 7. Physicochemical Characteristics of Compounds IIa-c, IIIa, and IIIb.

Compound	mp, °C	Found, %			Empirical formula	Calculated, %			Yield, %
		C	H	N		C	H	N	
IIa	—	49,9	7,6	9,5	$C_6H_{11}NO_3$	49,6	7,7	9,7	91
IIb	—	52,6	8,5	8,7	$C_7H_{13}NO_3$	52,8	8,3	8,8	87
IIc	—	47,7	6,5	7,1	$C_8H_{13}NO_3$	47,3	6,5	6,9	89
IIIa	103—105	48,7	6,1	8,0	$C_7H_{11}NO_4$	48,5	6,4	8,1	90
IIIb	67—69	59,3	8,0	9,8	$C_7H_{11}NO_2$	59,6	7,9	9,9	84

TABLE 8. Coordinates of Atoms ($\times 10^4$ and $\times 10^3$ for H)

Atom	x	y	z	Atom	x	y	z
$C_{(1)}$	1504 (2)	3811 (4)	0614 (3)	$H_{(1)}$	085 (2)	354 (4)	233 (4)
$C_{(2)}$	0815 (2)	3288 (4)	1370 (3)	$H_{(2)}$	036 (2)	375 (4)	118 (4)
$O_{(3)}$	0711 (1)	1591 (3)	1338 (2)	$H_{(3)}$	151 (2)	087 (4)	160 (3)
$C_{(4)}$	0895 (2)	0765 (4)	0226 (3)	$H_{(4)}$	091 (2)	314 (6)	258 (5)
$C_{(5)}$	1226 (2)	1580 (4)	0957 (3)	$H_{(5)}$	040 (2)	328 (4)	139 (4)
$C_{(6)}$	0858 (2)	2983 (5)	1537 (3)	$H_{(6)}$	201 (2)	594 (4)	016 (4)
$N_{(7)}$	1546 (1)	3160 (3)	0784 (3)	$H_{(7)}$	150 (2)	609 (5)	153 (4)
$O_{(8)}$	0795 (2)	0638 (3)	0228 (3)	$H_{(8)}$	216 (2)	206 (4)	161 (3)
$C_{(9)}$	1526 (2)	5591 (4)	0522 (3)	$H_{(9)}$	263 (2)	351 (4)	090 (4)
$C_{(10)}$	2199 (2)	3250 (5)	1361 (3)	$H_{(10)}$	262 (2)	370 (4)	306 (4)
$O_{(11)}$	0932 (2)	6163 (4)	0265 (3)	$H_{(11)}$	088 (3)	702 (7)	009 (5)
$O_{(12)}$	2240 (1)	4043 (3)	2618 (3)				

As is seen from Fig. 2, which presents the conformation of the morpholine ring with indication of the angles of torsion, the latter has a distorted boat conformation. The $C_{(1)}N_{(7)}$-$C_{(5)}C_{(4)}$ fragment is planar. The $C_{(1)}C_{(2)}N_{(7)}$ fragment forms a dihedral angle equal to 38° with the mean plane passing through the $C_{(2)}$, $O_{(3)}$, $C_{(5)}$, and $N_{(7)}$ atoms. The relative slope of the aziridine ring and the mean plane of the six-membered ring is equal to 96°. The hydroxymethyl groups and the $C_{(1)}$ atom lie in a single plane within a range of 0.25 Å.

The packing of the IIIa molecules in a crystal is stabilized by two intermolecular hydrogen bonds: $O_{(12)}H...N_{(7)}$ (2.81 Å, N...N = 1.95 Å, ∠O—H...N = 172.7°) and $O_{(11)}$—H...$O_{(8)}$ (2.78 Å, N...O = 2.03 Å, ∠O—H...O = 175.6°). The remaining intermolecular distances are no smaller than the sums of the van der Walls radii of the atoms in contact [5].

The hepatoprotector properties of IIIa and b were studied on a model of damage in rat liver due to carbon tetrachloride in *in vitro* and *in vivo* experiments. In the *in vitro* experiments drastic enhancement of the processes of the peroxide oxidation of lipids was observed when CCl_4 was added to an incubation medium consisting of the postmitochondrial supernatant and NDP-H (Table 4). The introduction of the control and experimental compounds into the incubation medium containing CCl_4 prevented the enhancement of the peroxide oxidation of lipids, the influence of compound IIIb being less pronounced than that of IIIa. In the *in vivo* experiments an abrupt increase in the transaminase activity and the concentration of the blood serum bilirubin is observed 24 h after the introduction of CCl_4, indicating damage to the hepatocyte membranes (Table 5). A decrease in the concentration of protein and cytochrome P-450 is observed in the liver (Table 6). The use of compound IIIa resulted in statistically significant prevention of the enhancement of the transaminase activity and the increase in the concentration of bilirubin in the blood serum. The data obtained attest to the protective effect of IIIa in the case of liver damage by carbon tetrachloride. At the same time, the activity of the compound does not fall short of the activity of the control compounds: vitamin E and cysteamine. Compound IIIb does not have a protective effect in the model of liver damage.

EXPERIMENTAL

The PMR spectra were obtained on Bruker WH-90 (90 MHz) and Bruker WM-360 (360 MHz) spectrometers in the case of compound IIb; 5% solutions in $CDCl_3$ and D_2O were used; the internal references were HMDS and DSS. The ^{13}C NMR spectra were obtained on a WH-90 spectrometer (22.63 MHz). the solvents were $CDCl_3$ and DSMO-d_6, and the internal reference was HMDS. The

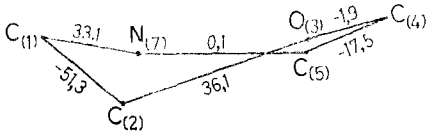


Fig. 2. Conformation of the morpholine ring in 2,2-dihydroxymethyl-1-aza-4-oxabicyclo[4.1.0]heptan-5-one (IIIa).

IR spectra were obtained on a Specord IR-75 spectrometer.

General Method for the Synthesis of IIa-c, IIIa, and IIIb. A 6.3-ml portion (0.05 mole) of methyl 1,2-dibromopropionate in 200 ml of absolute ethanol was given a dripwise addition of 7 ml (0.05 mole) of triethylamine over the course of 15 min at 0°C with stirrring. The temperature of the reaction mixture was gradually increased to room temperature, and 7 ml (0.05 mole) of triethylamine and 0.05 ml of the amino alcohol or the methyl ester of serine were added. Then the temperature of the mixture was adjusted to 50-60°C, and the mixture was stirred for 12-14 h. At the conclusion of the reaction (monitoring by TLC), the solvent was driven off in a vacuum, and 250 ml of ethyl acetate were added to the residue. The precipitate of the triethylamine salt was filtered out, and the filtrate was passed through a column with silica gel. Chromatographically pure reaction products were isolated after evaporation of ethyl acetate (Tables 1-3 and 7).

X-Ray Diffraction Analysis. The intensities of 1178 nonzero reflections were measured by the $\theta/2\theta$ scan technique to $2\theta_{max} = 150°$ on a Syntex $P2_1$ autodiffractometer (monochromatic Cu Kα radiation, graphite monochromator). The crystallographic characteristics of the orthorhombic single crystal with the composition $C_7H_{11}NO_4$ were: a = 18.161(2), b = 8.515(1), c = 9.843(a) Å, V = 1522.2(3) Å^3, d_{cal} = 1.51 g·cm^{-3}, μ(Cu Kα) = 10.8 cm^{-1}, Z = 8, space group P_{cab}, F_{ooo} = 736. The model of the molecule was found by direct methods. The structure was refined by the least-squares method in the full-matrix anisotropic approximation for the nonhydrogen atoms and in the corresponding isotropic approximation for the hydrogen atoms with the use of the weighting formula $W = 1/(\sigma_F^2 + 0.0001 \cdot F^2)$. The final R factor was 0.053. The maximum standard deviations of the bond lengths and angles are 0.005 Å and 0.5°. The coordinates of the atoms are given in Table 8.*

Study of Hepatoprotector Activity. The experiments were carried out on unbred white male rats weighing 180-220 g. The animals were deprived of food for 18 h before the beginning of the experiments. The *in vitro* experiments were carried out on intact animals according to the method in [6]. The estimate of the peroxide oxidation of the liver lipids was obtained by determining malonic dialdehyde. In the *in vivo* experiments CCl_4 was injected intraperitoneally in a 0.5-ml/kg dose in the form of a 10% solution in olive oil. The compounds investigated were introduced orally in two 100-mg/kg doses in the form of an aqueous suspension with an addition of Tween 80. The animals were killed by decapitation 24 h after the injection of CCl_4. The activities of the serum alanine aminotransferase (AlAT) and aspartate aminotransferase (AsAT) were determined according to the method in [7], and the serum bilirubin concentration was determined according to Jendrassik's method [8]. Cytochrome P-450 was determined in the liver homogenate on an Hitachi 557 spectrophotometer according to the method in [9], and the protein was determined with the use of the biuret reaction [10].

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CRYSTAL AND MOLECULAR STRUCTURE OF 2-BENZOLY-5-PHENYLPYRROLE

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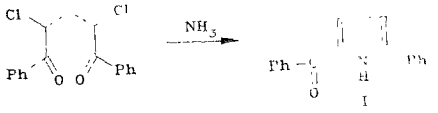
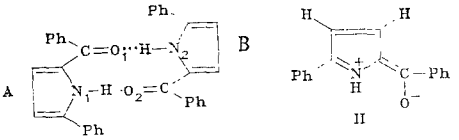
An x-ray diffraction investigation of 2-benzoyl-5-phenylpyrrole, whose molecules are combined as dimers in the crystal, has been carried out. The bond lengths and angles have been presented.

We previously [1] showed that 2,4-dichloro-3R-1,5-pentanediones undergo heterocyclization under the action of ammonia to form the corresponding 3-chloropyridines. The α,α'-dichlorodiketone, which does not have substituent in position 3, reacts with ammonia to form a compound, which is assigned the structure of 2-benzoyl-5-phenylpyrrole (I), according to the data from IR and PMR spectroscopy:



The PMR spectrum of I in $CDCl_3$ shows not only a multiplet for the aromatic protons at δ 7.21-7.97 ppm and a singlet for the >N—H proton at 10.18 ppm, but also signals in the form of two double doublets at 6.62 and 6.93 ppm, which can probably be assigned to the 3-H and 4-H protons of the pyrrole ring. It is difficult to explain the splitting of the signals of the protons indicated on the basis of the structure of phenylpyrrole I, and this prompted us to carry out an x-ray diffraction investigation of I for the purpose of rigorously substantiating its structure.

The x-ray diffraction investigation confirmed the hypothesis that compound I has the structure of 2-benzoyl-5-phenylpyrrole (see Experimental). In addition, in the crystal molecules A and B are joined as dimers by means of N—H...O hydrogen bonds:



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