ACYCLIC ANALOGS OF NUCLEOSIDES: SYNTHESIS AND IN VITRO ANTIVIRAL ACTIVITY OF BENZIMIDAZOLES AND BENZOTRIAZOLES

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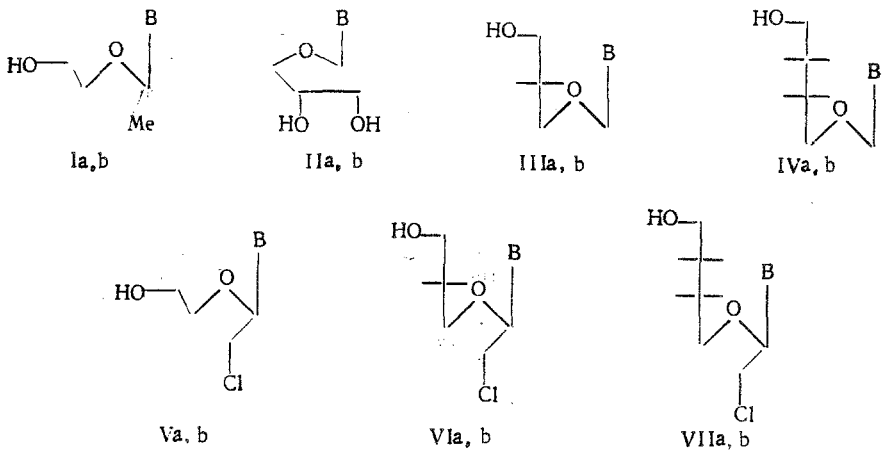
One of the most promising approaches to the development of novel antiviral drugs is the synthesis of acyclic analogs of nucleosides. This approach to the synthesis of biologically active compounds was predicted over ten years ago [2], and has been confirmed by the discovery of such highly active antiviral drugs as 9-(4-hydroxy-2-oxa-butyl)guanine (acyclovir), 9-(4-hydroxy-3-hydroxymethyl-2-oxabutyl)guanine (BIOLF-62), (S)-9-(2,3-dihydroxypropyl) adenine (DHPA) [6], and (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine (HPMPA) [7]. It is, however, necessary to point out that these antivirals basically inhibit the reproduction of DNA viruses. The only exceptions are (S)-DHPA and (S)-HPMPA, which have a wide spectrum of activity. There are no true chemotherapeutants at the present time for RNA viruses [1].

This investigation is a continuation of earlier studies [4, 5] on the relationship of structure to biological activity in a series of "twice modified" nucleosides, and describes the synthesis and assessment of antiviral activity against RNA viruses of some acyclic analogs of benzimidazole and benzotriazole nucleosides (Ia, b-VIIa, b).



B = benzimidazol-1-yl (a), benzotriazol-1-yl (b)

Reaction of acetates (XV-XXI) with trimethylsilyl derivatives of benzimidazole and benzotriazole in the presence of trimethylsilyl trifluoromethanesulfonate gave satisfactory yields of the protected nucleoside analogs (VIIIa, b-XIVa, b; method A). Alkylation was carried out at 20°C in dry acetonitrile. The use as catalyst of an equimolar mixture of trifluoromethanesulfonic acid and $ClSiMe_3$ in the presence of an excess of hexamethyldisilazane considerably simplified the method of synthesis. The yields of protected nucleosides (VIIIa, b-XIVa, b) rose by an average of 20-30% (method B). The yields and melting points of the products are given in Table 1.

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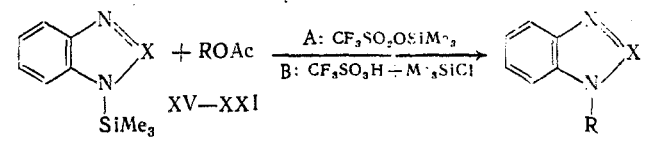
TABLE 1. Synthesis of Acyclic Analogs of Nucleosides

Method of preparation	Protected analogs*		Unprotected analogs		
	compound	yield, %, A (B)	compound	yield, %	mp, °C
A (B)	VIIIa	50 (70)	Ia	90	Oil
The same	VIIIb	60 (80)	Ib	90	»
A (B)	IXa	40 (60)	IIa	90	82—3**
The same	IXb	50 (65)	IIb	93	50—1**
A (B)	Xa	46 (55)	IIIa	89	84—5**
The same	Xb	58 (70)	IIIb	90	85—6***
A (B)	XIa	35 (50)	IVa	85	100—1***
The same	XIb	48 (60)	IVb	88	Oil
A (B)	XIIa	30 (45)	Va	85	»
The same	XIIb	42 (50)	Vb	80	»
A (B)	XIIIa	28 (40)	VIa	90	»
The same	XIIIb	33 (45)	VIb	85	»
B	XXII	(20)	—	—	—
A (B)	XIVa	27 (40)	VIIa	80	Oil
The same	XIVb	40 (58)	VIIb	90	»
B	XXIII	(28)	—	—	—

*All the compounds were oils.
**Ether–ethanol.
***Ether–ethyl acetate.

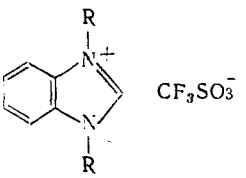
Scheme



VIIIa, b – XIVa, b

R = CH(Me)O(CH$_2$)$_2$OAc (XV, VIIIa, b), CH$_2$OCH$_2$(OBz)CH$_2$OAc (XVI, IXa, b), CH$_2$O(CH$_2$)$_3$OAc (XVII, Xa, b), CH$_2$O(CH$_2$)$_4$OAc (XVIII, XIa, b), CH(CH$_2$Cl)O(CH$_2$)$_2$OAc (XIX, XIIa, b), CH(CH$_2$Cl)C(CH$_2$)$_4$OAc (XX, XIIIa, b), CH(CH$_2$Cl)O(CH$_2$)$_4$OAc (XXI, XIVa, b);
X = CH (a), N (b).

Alkylation of the trimethylsilyl derivative of benzimidazole with acetates (XX) and (XXI) by method B gave, in addition to the expected products, significant amounts of the 1,3-dialkylbenzimidazoles (XXII) and (XXIII) as by-products.



R = CH(CH$_2$Cl)O(CH$_2$)$_3$OAc (XXII), CH(CH$_2$Cl)O(CH$_2$)$_4$OAc (XXIII).

When (XV-XIX) were used as the alkylating agents, no bisalkylated benzimidazoles were formed.

The acetyl groups in (VIIIa, b-XIVa, b) were removed by treatment with methanolic ammonia. The acyclic nucleoside analogs (Ia, b-VIIa, b) were obtained in near-quantitative yields (Table 1).

The structures of the products were confirmed by PMR spectroscopy (Table 2). The structure of the hydroxyalkyl substituent was clearly apparent in the multiplicity of the OH signals in the NMR spectra of the unprotected compounds in deuterodimethyl sulfoxide. These signals are readily identified, since they disappear on addition of D_2O to the sample. The doublet and triplet signals in the spectra of (IIa) and (IIb) show the presence of secondary and primary hydroxyl groups respectively. The single primary hydroxyl group in (Ia, b) and (IIIa, b-VIIa, b) is seen as a triplet of relative intensity 1H. Double resonance was employed to assign the signals of the protons of the chloromethyl groups in the PMR spectra of (Va, b), (VIa, b), and (VIIa, b) in deuterochloroform. The UV spectra of the

TABLE 2. PMR Spectra of Acyclic Nucleoside Analogs

Compound	Chemical shifts, δ, ppm (coupling constant, Hz)			
	1′-CH_2 or 1′-CH_3	2′-CH_2 or 2′-CH	CH_3CO	OH
VIIIa	1,80 d (6)	5,64 q	1,97 s	—
Ia	1,66d (6)	5,78 q	—	4,37 t
IXa	5,77 s	—	1,90 s	—
IIa	5,63 s	—		4,42 t
				4,58 d
Xa	5,56 s	—	1,90 s	—
IIIa	5,64 s	—	—	4,40 t
XIa	5,52 s	—	1,99 s	—
IVa	5,66 s	—	—	4,41 t
XIIa	3,91 dd	5,65 t (6)	2,00 s	—
	4,03 dd			
Va	4,20 d (6)	6,03 t (6)	—	4,73 t
XIIIa	3,97 m	5,60 t (6)	1,91 s	—
VIa	4,18 d (6)	5,95 t (6)	—	4,40 t
XIVa	3,97 m	5,59 t (6)	2,01 s	—
VIIa	4,19 d (6)	5,97 t (6)	—	4,37 t
VIIIb	1,90 d (6)	6,25 q (6)	1,93 s	—
Ib	1,82 d (6)	6,32 q (6)	—	4,61 t
IXb	6,00 s	—	1,91 s	—
IIb	5,07 s	—	—	4,46 t
				4,70 d
Xb	5,57 s	—	1,91 s	—
IIIb	6,04 s	—	—	4,35 t
XIb	6,11 s	—	1,95 s	—
IVb	6,06 s	—	—	4,42 s
XIIb	4,13 m	6,33 t (6)	1,91 s	—
Vb	4,73 d (6)	6,50 t (6)	—	4,74 t
XIIIb	4,10 m	6,23 t (6)	1,89 s	—
VIb	4,34 d (6,5)	6,43 t (6,5)	—	4,35 t
XIVb	4,10 m	6,21 t (6)	2,01 s	—
VIIb	4,37 d (6)	6,43 t (6)	—	

Note. The more characteristic signals are shown: the protons of the aromatic ring are present as a complex multiplet at 7-8 ppm. The spectra of the protected compounds were obtained in $CDCl_3$, and of the unprotected compounds, in DMSO-d_6.

TABLE 3. Effects of Acyclic Analogs of Nucleosides on the Reproduction of Coronaroviruses, Agents of Swine Transmissive Gastroenteritis, Purdue Strain, in Swine Embryo Kidney Virus Culture

Compound	Maximum tolerated concentration, μg/ml	Viral titer, log TCD_{50}/ml	Suppression of viral titer, log TCD_{50}/ml
Infected cells	—	5.5	—
Ia	250	4,5	1,0
Ib	125	4,0	1,5
IIa	62,5	4,0	1,5
IIb	125	4,0	1,5
IIIa	62,5	4,5	1,0
IIIb	62,5	4,5	1,0
2-(α-Hydroxybenzyl)benzimidazole	31,2	5,48	0,02

products correspond to 1-substituted benzimidazoles and benzotriazoles. Proof of the structures of the 1,3-dialkylbenzimidazoles (XXII) and (XXIII) is provided by the fact that the ratio of the integral intensities of the signals for the protons of the hydroxyalkyl substituent and the aromatic protons is twice as great as in the corresponding monosubstituted compounds. Further proof of the structure of the 1,3-dialkyl benzimidazoles is obtained by comparing the UV spectra of (XXII) and (XXIII) with those of known dialkylbenzimidazoles.

TABLE 4. Effect of Acyclic Analogs of Benzimidazole and Benzotriazole Nucleosides on the Reproduction of Swine Enterovirus B386/79 in Swine Embryo Kidney Virus Culture

Compound	Concentration, μg/ml	Viral titer, log TCD_{50}/ml	Inhibition of viral titer, log TCD_{50}/ml
Infected cells	—	7,0—7,33	—
Ia	250*	6,00	1,00
	31,2	6,76	0,57
IIb	125*	6,00	1,00
	31,2	7,00	0,33
IIa	62,5*	6,11	0,89
	31,2	6,40	0,93
IIb	125*	6,50	0,50
	31,2	5,48	1,85
IIIa	31,2	6,00	1,33
IIIb	31,2	5,48	1,85
VIa	31,2	6,00	1,33
Va	31,2	5,50	1,83
Vb	31,2	6,50	0,83
VIb	31,2	5,66	1,67
VIIb	31,2	7,33	0,00
2-(α-Hydroxybenzyl)benzimidazole	31,2	7,16	0,17

*Maximum tolerated concentration.

The antiviral activity of the compounds was assessed by the reduction in the infective titer of the virus. As an analog, the known inhibitor of RNA viruses 2-(α-hydroxybenzyl)-benzimidazole [1] was used. The biological tests showed that (Ia, b-Va, b) and (VIb-VIIb) had greater antiviral activity than 2-(α-hydroxybenzyl)benzimidazole against both coronarovirus and swine enterovirus in concentrations of 62.5 and 31.2 μg/ml. The effects of the test compounds on the accumulation of infective virus are shown in Tables 3 and 4. The results show that hydroxyalkyl derivatives of benzotriazole display relatively higher antiviral activity against enteroviruses than the benzimdiazole derivatives. The greatest activity is shown by (IIb) and (IIIb) (1.85 log TCD_{50}/ml), which contain hydroxyalkyl substituents which mimic fragments of the ribose ring with 2', 3', and 5'-hydroxy groups.

All the test compounds inhibited the infective process in coronarovirus by 1-1.5 log TDC_{50}/ml at a dose of 31.2 μg/ml.

EXPERIMENTAL (CHEMISTRY)

NMR spectra were obtained on a WP-100SV spectrometer (Brucker, West Germany), and UV spectra on a Specord UV-VIS instrument (East Germany). TLC was carried out on Silufol UV-254 silica plates (Czech SSR). The chromatograms were developed with a mixture of chloroform and methanol (9:1). The sorbent used in preparative column chromatography was silica gel L 40/100. The eluent was a mixture of methanol and chloroform (1-10% of methanol). The elemental analyses of all the compounds did not differ from the calculated values by more than 0.2%.

The synthesis of the alkylating agents (XV), (XVI), and (XIX) has been described ([4], [3], and [5] respectively).

1,3-Dioxane. A mixture of 144.7 ml (152 g, 2 moles) of propane-1,3-diol, 6 g (2 moles) of paraformaldehyde, and 6 g of toluene-p-sulfonic acid in 500 ml of chloroform was boiled in a Dean and Stark apparatus. When 40 ml of water had been removed, the chloroform was evaporated under reduced pressure, and the residue distilled to give 130 g (78%) of product, bp 105-106°C.

1,5-Diacetoxy-2-oxapentane (XVII). To a mixture of 48 ml (50 g, 0.56 mole) of 1,3-dioxane, 20 ml of glacial acetic acid, and 100 ml of acetic anhydride was added with stirring and ice-water cooling 0.5 ml of conc. sulfuric acid. The mixture was stirred for 12 h at

20°C, 5 g of anhydrous sodium acetate added, stirred for an additional 1 h, and the inorganic precipitate filtered off. The filtrate was distilled under reduced pressure to give 100 g (93%) of product, bp 132-133°C (12 mm). PMR spectrum, δ, ppm ($CDCl_3$): 5.26 (2H, s, CH_2), 4.16 (2H, t, J 6 Hz, CH_2), 3.72 (2H, t, J 6 Hz, CH_2), 1.92 (2H, m, CH_2), 2.07 ppm (6H, s, $2CH_3CO_2$).

1,3-Dioxepane was obtained from butane-1,4-diol as for 1,3-dioxane.

1,6-Diacetoxy-2-oxahexane (XVIII) was obtained from 1,3-dioxepane as for (XVII). Yield 87%, bp 148-149°C (12 mm). PMR spectrum, δ, ppm ($CDCl_3$): 5.27 (2H, s, CH_2), 4.09 (2H, t, J 5.9 Hz, CH_2), 3.66 (2H, t, J 5.9 Hz, CH_2), 1.70 (4H, m, $2CH_2$), 2.08 (6H, s, $2CH_3CO_2$).

2-Chloromethyl-1,3-dioxane. A mixture of 145 ml (152 g, 2 mole) of propane-1,3-diol, 228.4 ml (249 g, 2 mole) of 2-chloroacetaldehyde dimethyl acetal, and 1 g of toluene-p-sulfonic acid was heated in such a way that the methanol slowly distilled over. When 115 ml of methanol had been removed, the residue was distilled under reduced pressure to give a quantitative yield of product, bp 75-76°C (12 mm).

1-Chloro-2,6-diacetoxy-3-oxahexane (XX) was obtained from 2-chloromethyl-1,3-dioxane, as for (XVII). Yield 50%, bp 110-115°C (1.5 mm). PMR spectrum, δ, ppm ($CDCl_3$): 5.90 (1H, t, J 6 Hz, CH), 4.16 (2H, m, CH_2), 3.79 (2H, m, CH_2), 3.57 (2H, d, J 6 Hz, CH_2), 1.92 (2H, m, CH_2), 2.13 (3H, s, 2-$OCOCH_3$), 2.05 ppm (3H, s, 6-$OCOCH_3$).

2-Chloromethyl-1,3-dioxepane was obtained from butane-1,4-diol as for 2-chloromethyl-1,3-dioxane, yield quantitative, bp 88-90°C (12 mm).

1-Chloro-2,7-diacetoxy-3-oxaheptane (XXI) was obtained from 2-chloromethyl-1,3-dioxepane as for (XVII). Yield 66%, bp 125-130°C (1.5 mm). PMR spectrum, δ, ppm ($CDCl_3$): 5.91 (1H, t, J 6 Hz, CH), 4.09 (2H, m, CH_2), 3.70 (2H, m, CH_2), 1.69 (4H, m, $2CH_2$), 2.13 (3H, s, 2-$OCOCH_3$), 2.05 (3H, s, 7-$OCOCH_3$), 3.58 ppm (2H, d, J 6 Hz, CH_2).

Alkylation of Nitrogenous Bases. Method A: To a suspension of 0.5 g (4.24 mmole) of benzimidazole or 0.5 g (4.2 mmole) of benzotriazole in 5 ml of hexamethyldisilazane was added 1 ml of $ClSiMe_3$, and the mixture boiled for 2 h. It was then cooled to 40°C, and 5 mmole of the appropriate alkylating agent (Table 1), 10 ml of dry acetonitrile, and 0.78 ml (5 mmole) of trimethylsilyl trifluoromethanesulfonate added. The mixture was kept for 3 h at 40°C, cooled, and poured into 50 ml of saturated $NaHCO_3$ solution. The mixture was extracted with chloroform, the extracts dried over Na_2SO_4, and evaporated under reduced pressure. The residue was chromatographed on a column (3 × 15 cm) of silica gel, and eluted with a 1-10% solution of methanol in chloroform. The protected nucleoside analogs thus obtained were dissolved in 30 ml of half-saturated methanolic ammonia at 0°C, kept for 24 h at 20°C, and evaporated under reduced pressure. The residue was chromatographed on a column (2 × 20 cm) of silica gel, eluent 10% methanol in chloroform. The resulting deprotected nucleosides were treated with dry ether, and recrystallized. The yields and melting points of the products are given in Table 1.

Method B: To the solution of the silylated base (see method A), cooled to 20°C, was added 4.3 mmole of the appropriate alkylating agent (Table 1), 20 ml of dry acetonitrile, 0.253 ml (2 mmole) of $ClSiMe_3$, and 0.176 ml (0.3 g, 2 mmole) of trifluoromethanesulfonic acid. The mixture was boiled for 20 min, and cooled. The subsequent isolation and removal of the acetyl protecting group were carried out as in method A. The yields and melting points of the products are given in Table 1.

EXPERIMENTAL (BIOLOGY)

Antiviral activity was determined for swine enterovirus B386/79, with an infective titer of 7.00-7.33 log TCD_{50}/ml, and for swine coronarovirus Purdue-115, with an infective titer of 5.5 log TCD_{50}/ml. The viruses were titrated in a tube culture of swine embryo kidney virus according to their cytopathic effects. The toxicities of the cultures towards the culture were first established. The compounds were tested at the maximum tolerated concentrations (MTC).

After one hour's contact of tenfold dilutions of the virus and culture, the former was poured off, and 1 ml of supporting nutrient medium (50% N199 + 50% of 0.5% lactalbumin) containing the drug in solution added. The experimental results were assessed 120 h after infection. The antiviral activity was expressed as the inhibition of the cytopathic effects of the viruses.

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SYNTHESIS AND ANTIVIRAL ACTIVITY OF 1,2-OXAPHOSPHOL-3-ENES

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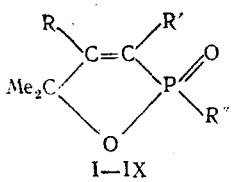
Organophosphorus compounds of many types are finding increasing application as antiviral drugs [5, 6, 13]. The antiviral drugs fosfonet (disodium phosphonoacetate), foscarnet (trisodium phosphonoformate), arylalkyl- and aryloxyalkylphosphonates, phosphorylated chloralureas, guanine thiophosphamides, vidarabin phosphate, and other organophosphorus compounds have been examined in detail [4, 9, 10, 14-21]. The attention of investigators has recently been drawn to heterocyclic organophosphorus compounds, which have shown activity against oncogenic or infective viruses [6, 11, 13, 19-21]. There is, however, no information on potential antiviral drugs in the 1,2-oxaphosphol-3-ene series.

Studies of the reactivity of 1,2-alkadienephosphonous acids, which contain two reactive sites (the allene system and the phosphorus-hydrogen bond), have shown that these unsaturated hydrophosphoryl compounds undergo heterocyclization to give 1,2-oxaphosphol-3-enes (I-IX) [1-3, 12].



I—IX

R = H (I, III—IX), Br (II); R′ = CH=CH$_2$ (I), H (II—IX); R″ = CH(OH)CCl$_3$ (III), C(OH)Me$_2$ (IV), CH(OH)C$_6$H$_4$Br-4 (V), CH(OH)C$_6$H$_4$Cl-4 (VI), CH(OH)C$_6$H$_4$NO$_2$-4 (VII), CH(Ph)NHPh (VIII), CH(C$_6$H$_4$Br-4)C$_6$H$_4$Br-4 (IX).

The aim of the present investigation was to synthesize novel 1,2-oxaphosphol-3-enes, and the examine the antiviral activity both of the novel compounds, and also previously-described heterocyclic compounds of this type.

We have found that hydrochlorination of 1,2-alkadienephosphonous acids containing a tertiary carbon atom in the 3-position of the allene system with gaseous hydrogen chloride in polar solvents (chloroform and nitromethane) results in cyclization involving the phosphoryl hydrogen with the formation of 1,2-oxaphosphol-3-enes containing the phosphorus—hydrogen bond (X-XV).

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