Alkyl Esters of 3-Adenin-9-yl-2-hydroxypropanoic Acid: A New Class of Broad-Spectrum Antiviral Agents

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A new class of acyclic adenosine analogues is described which exhibit broad-spectrum antiviral activity and are apparently targeted at S-adenosyl-L-homocysteine hydrolase. The compounds are all alkyl (i.e., methyl, ethyl, 1-propyl, 2-propyl, 1-butyl, 2-butyl, 2-methylpropyl, *tert*-butyl, 1-pentyl, 3-methylbutyl, 1-octyl, 2-hydroxyethyl, 2-methoxyethyl, furylmethyl, cyclohexyl) esters of (*RS*)-3-adenin-9-yl-2-hydroxypropanoic acid. They are inhibitory to a broad variety of viruses, including vesicular stomatitis, vaccinia, reo, parainfluenza, and measles, and, with one exception (the furylmethyl ester), nontoxic to the host cell at antivirally active concentrations. It is postulated that the alkyl esters are as such taken up by the cells and hydrolyzed within the cells to release the parent compound, 3-adenin-9-yl-2-hydroxypropanoic acid.

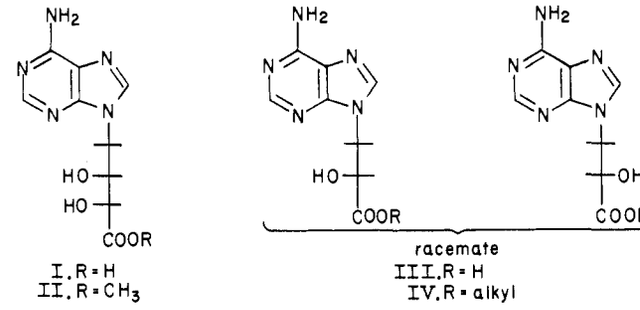
Two types of acyclic nucleoside analogues have been actively pursued for their antiviral potentials: (i) guanosine analogues in which the sugar moiety is replaced by a (2-hydroxyethoxymethyl group[1] (or aminoacyl ester thereof[2]), a (2-hydroxy-1-(hydroxymethyl)ethoxy)methyl group,[3-5] a 4-hydroxybutyl group,[6] or a 3,4-dihydroxybutyl group[7] and (ii) adenosine analogues in which the sugar moiety is replaced by an aliphatic side chain such as 2,3-dihydroxypropyl[8] and 2,3-dihydroxybutyl[9] or an alkanoic acid such as 2,3-dihydroxybutanoic acid (as in eritadenine[10]). Whereas the former are specifically active against herpes virus (herpes simplex virus,[1-7] cytomegalovirus,[11,12] and Epstein–Barr virus[13]), the latter are directed against a broad range of DNA and RNA viruses, including poxviridae (vaccinia), rhabdoviridae (rabies, vesicular stomatitis), paramyxoviridae (measles, parainfluenza), and reoviridae (reo, rota).

The mechanism of action of the aliphatic adenosine analogues[8-10] has not been rigorously established, but it is assumed that (*S*)-9-(2,3-dihydroxypropyl)adenine [(*S*)-DHPA][8,9] and (2*R*,3*R*)-4-adenin-9-yl-2,3-dihydroxybutanoic acid (D-eritadenine),[10] in analogy with other adenosine analogues such as 3-deazaadenosine (c^3Ado) and carbocyclic 3-deazaadenosine (C-c^3Ado),[14] owe their broad-spectrum antiviral activity to an inhibition of *S*-adenosyl-L-homocysteine (SAH) hydrolase. SAH is the product of *S*-adenosylmethionine (SAM) mediated transmethylation reactions and is itself a feedback inhibitor of these reactions. To permit the transmethylation reactions SAH has to be removed through the action of SAH hydrolase, but if SAH hydrolase is inhibited, SAH would accumulate and thereby impair all biological processes that require intensive methylations.[15] One such situation is the maturation of viral mRNA, i.e., 5′-cap formation. Therefore, inhibitors of SAH hydrolase may be expected to block virus replication.

For the di- or trihydroxyalkyl derivatives of adenine there is a close correlation between antiviral activity and inhibition of SAH hydrolase:[16,17] both depend on an intact adenine moiety, an alkyl chain bound at N-9, and a vicinal diol at the 2,3-position of the side chain with the 2-hydroxyl group in the *S* configuration. These structural features are present in (*S*)-DHPA, which is both a broad-spectrum antiviral agent[8,9] and a relatively potent reversible inhibitor of SAH hydrolase ($K_i/K_m = 5 \times 10^{-2}$ for the rat liver enzyme).[18]

During our investigations we discovered a novel group of SAH hydrolase inhibitors, namely, eritadenines,[19] which,

Chart I



I. (2*R*,3*R*)-4-adenin-9-yl-2,3-dihydroxybutanoic acid (D-eritadenine)
II. D-eritadenine methyl ester
III. (*RS*)-3-adenin-9-yl-2-hydroxypropanoic acid
IV. alkyl esters of (*RS*)-3-adenin-9-yl-2-hydroxypropanoic acid

in contrast with (*S*)-DHPA, inhibit the enzyme irreversibly. The prototype of this class of inhibitors, D-eritadenine (I:

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Table I. Alkyl Esters of (*RS*)-3-Adenin-9-yl-2-hydroxypropanoic Acid

no.	alkyl	method[a]	yield, %	mp, °C	R_f[b] S1	R_f[b] S2	mass spectrum (M^+)	mol formula[c] (M_r)
1	methyl	A	68	196–198	0.15	0.37	237	$C_9H_{11}N_5O_3$ (237.2)
2	ethyl	A	65	184	0.18	0.42	251	$C_{10}H_{13}N_5O_3$ (251.2)
3	1-propyl	A	72	164–165	0.30	0.45	265	$C_{11}H_{15}N_5O_3$ (265.3)
4	2-propyl	B	80	166	0.30	0.47	265	$C_{11}H_{15}N_5O_3$
5	1-butyl	A	66	178–179	0.42	0.62	279	$C_{12}H_{17}N_5O_3$ (279.3)
		B	84					
6	2-butyl	B	75	65–67	0.40	0.60	279	$C_{12}H_{17}N_5O_3$
7	2-methylpropyl	B	80	159–160	0.42	0.60	279	$C_{12}H_{17}N_5O_3$
8	*tert*-butyl	D	42	178–179	0.45	0.62	279	$C_{12}H_{17}N_5O_3$
9	1-pentyl	B	85	149–152	0.55	0.68	293	$C_{13}H_{19}N_5O_3$ (293.3)
10	3-methylbutyl	B	82	139	0.55	0.70	293	$C_{13}H_{19}N_5O_3$
11	1-octyl	C	66	136–137	0.70		335	$C_{16}H_{25}N_5O_3$ (335.4)
12	2-hydroxyethyl	C	69	129–130	0.05	0.20	267	$C_{10}H_{13}N_5O_4$ (267.3)
13	2-methoxyethyl	A	70	140–141	0.32	0.46	281	$C_{11}H_{15}N_5O_4$ (281.3)
14	2-furylmethyl	C	60	113	0.50	0.64	303	$C_{13}H_{13}N_5O_4$ (303.3)
15	cyclohexyl	B	77	178	0.57	0.70	305	$C_{14}H_{19}N_5O_3$ (305.3)

[a] See Experimental Section. [b] TLC in chloroform–methanol systems S1 (9:1), S2 (4:1). [c] C, H, N analyses correspond to calculated values.

Table II. Chemical Hydrolysis of Alkyl Esters of (*RS*)-3-Adenin-9-yl-2-hydroxypropanoic Acid

no.	purity,[a] %	t_R,[b] min	system[c]	pH 6.86[d] $10^{-5}k$	pH 6.86[d] $t_{1/2}$, h	pH 7.42[d] $10^{-5}k$	pH 7.42[d] $t_{1/2}$, h
1	99.9	3.7	I	21.0	55	52.8	22
2	100.0	5.2	I	9.1	127	26.2	44
3	98.5	8.4	I	1.9	595	4.4	261
4	99.4	8.3	I	2.7	430	3.8	300
5	99.5	4.6	II	5.3	218	13.4	86
6	99.6	4.4	II	1.6	714	3.0	385
7	97.3	4.7	II	5.7	202	13.0	89
9	99.8	6.9	II	8.9	130	12.8	90
10	100.0	6.5	II	5.1	226	11.8	98
12	98.7	3.2	I	33.0	35	74.1	15.5
13	99.3	4.5	I	32.1	36	53.6	21.5
14	97.7	8.4	II	169.6	6.8	216.9	5.3
15	99.7	6.9	II	1.9	600	4.3	268

[a] Analyzed by HPLC. [b] Retention time. [c] I, 30% aqueous methanol; II, 50% aqueous methanol. [d] See Experimental Section.

see Chart I), irreversibly inhibits SAH hydrolase from different sources with an IC_{50} of approximately 10^{-8} M and an half-life time of 1–2 min.[19-21] Although D-eritadenine is a much more potent inhibitor of SAH hydrolase than (*S*)-DHPA, it is not as active as an antiviral agent.[10] An obvious explanation for its weaker antiviral activity is the hindrance of D-eritadenine, an ionic (polar) compound, in penetrating into the cells. This difficulty can be circumvented by esterification of the carboxyl group of D-eritadenine, and, indeed, the methyl ester of D-eritadenine (II: see Chart I) is more potent as an antiviral agent than the acidic parent compound.[22]

However, the large-scale synthesis of D-eritadenine esters is rather appalling because of the complicated starting materials, multistep reactions, and extensive purifications.[10] It appeared much simpler to pursue the 3-adenin-9-yl-2-hydroxypropanoic acids (III: see Chart I), which are, like D-eritadenine, potent and irreversible inhibitors of SAH hydrolase.[22] The racemic mixture which is easily accessible[25] served as the starting material for the synthesis of a wide variety of alkyl esters (IV: see Chart I).

Chemistry. The *S* and *R* enantiomers of III (Chart I) can be prepared in small yield by Neff oxidation of 5-adenin-9-yl-deoxyaldopentoses[10,23] or, more specifically, by a two-step oxidation procedure.[24] The racemate III can be obtained in large amounts by successive treatment of adenine with bromoacetaldehyde diethyl acetal, acid hydrolysis, and cyanohydrin synthesis followed by acid hydrolysis.[25] The free acid III is poorly soluble in water, which allows its efficient purification.

From the racemic acid III (Chart I), a series of esters (IV) were prepared by an acid-catalyzed esterification with excess alcohol (this procedure was followed for the lower alcohols) or esterification mediated by *N,N'*-dicyclohexylcarbodiimide activation of the acid. While the latter procedure was compounded with traces of *N,N'*-dicyclohexylurea which were hard to remove, the main difficulty in utilizing the former procedure consisted in the removal of acid catalyst from the reaction mixture. The ion-exchange resin used for removing the acid catalyst substantially limited the yields. It was found more profitable to desalt the neutralized reaction mixture by chromatography on octadecyl-silica column in aqueous medium. This procedure proved particularly advantageous with esters derived from alcohols higher than C3. The only reaction products obtained by the above methods were compounds

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Table III. Antiviral Activity of Alkyl Esters of (*RS*)-3-Adenin-9-yl-2-hydroxypropanoic Acid against Different Viruses

	min cytotoxic concn, μg/mL			minimum inhibitory concentration,[b] μg/mL								
				PRK cells			Vero cells				HeLa cells	
compd	PRK cells	Vero cells	HeLa cells	vesicular stomatitis virus	vaccinia virus	herpes simplex virus-1 (KOS)	reo virus-1	parainfluenza virus-3	measles virus	sindbis virus	coxsackie virus-B4	polio virus-1
1	>400	>400	>400	3	7	>400	200	20	40	>400	>400	>400
2	>400	>400	>400	1	3	>400	40	20	20	>400	>400	>400
3	100	>400	>400	1–3	3–10	>100	200	100	100	>200	>400	>400
4	>400	>400	>400	3	3	>400	40	70	150	>400	>400	>400
5	200	200	400	3	3–30	>200	150	70	25	150	>400	>400
6	>400	>400	>400	1	3	>400	70	200	40	300	>400	>400
7	>400	>400	>400	1–3	3–30	>400	150	20	40	>400	>400	>400
8	>400	>400	>400	150	150	>400	150	150	150	>400	>400	>400
9	200	200	>400	10–70	70–100	>200	30	70	70	200	>400	>400
10	400	400	400	3	10–30	>400	30	>200	12	200	>400	>400
11	100	200	100	7	7	>100	7	15	70	30	>100	>100
12	>400	>400	>400	30	70	>400	>200	>200	30	>400	>400	>400
13	>400	>400	>400	7	30	>400	70	300	400	>400	>400	>400
14	40	40	40	15	10–30	>40	30	15	>40	>40	>40	>40
15	100	100	100	1	3	>100	7	>40	15	>100	>100	>100
(*S*)-DHPA	>400	>400	>400	15–30	30–70	>400	200	20	30	>400	>400	>400
(*RS*)-DHPA	>400	>400	>400	15–30	30–70	>400	100	20	20	>400	>400	>400

[a] Required to cause a microscopically detectable alteration of normal cell morphology, when incubated with cells for the same duration as required to measure antiviral activity. [b] Required to reduce virus-induced cytopathogenicity by 50%. Viral cytopathogenicity was recorded as soon as it reached completion in the control virus-infected cell cultures, i.e., at 1–2 days for vesicular stomatitis, at 2 days for coxsackie and polio, at 2–3 days for vaccinia, herpes simplex, and sindbis, and at 6–7 days for reo, parainfluenza, and measles. The multiplicity of infection (MOI) was invariably 100 × $CCID_{50}$, that is 100 times the virus dose needed to infect 50% of the cells.

of type IV (Chart I), homogeneous on TLC and HPLC analysis.

The *tert*-butyl ester of IV was synthesized by a procedure described for the preparation of amino acid *tert*-butyl esters:[26] i.e., by treatment of the acid III with *tert*-butyl acetate in the presence of perchloric acid. The product was isolated by chromatography on octadecyl-silica column (see above).

Results

The alkyl esters of (*RS*)-3-adenin-9-yl-2-hydroxypropanoic acid and their physical properties are reviewed in Table I. Most esters of the type IV are subject to hydrolysis in aqueous solutions not only at extreme pH values but also under conditions similar to those used for culturing the cells. Their rates of hydrolysis in sodium–potassium phosphate buffer at pH 6.86 or 7.42 are presented in Table II. The half-life of compounds **1**, **12**, **13**, and **14** was particularly short ($t_{1/2}$ less than 24 h at pH 7.42). We can assume that this hydrolysis will take place during the incubation on the cells, particularly because the cell culture medium contains a rather high concentration of serum proteins which could act both as (enzymatic) catalysts and nucleophilic agents. An exact analysis of the fate of the esters IV in the cell culture medium is complicated by their instability under the conditions required for removal of the proteins before HPLC analysis.

The antiviral activity spectrum exhibited by the alkyl esters of (*RS*)-3-adenin-9-yl-2-hydroxypropanoic acid was remarkably similar to that of (*RS*)- or (*S*)-DHPA (Table III), thus directed toward poxviruses (vaccinia), (−)RNA viruses (vesicular stomatitis, parainfluenza, measles), and (±)RNA viruses (reo). Herpes viruses (herpes simplex) and (+)RNA viruses (sindbis, coxsackie, polio) were virtually insensitive to the compounds (Table III). From Table III it is also clear that most alkyl esters, viz., **1–7**, **10**, **11**, and **15**, were about 10 times more potent in their antiviral activity (against vesicular stomatitis and vaccinia virus) than DHPA. These alkyl esters were inhibitory to vesicular stomatitis virus and vaccinia virus in the concentration range of 1–3 and 3–10 μg/mL, respectively. When assayed under similar conditions, the *S* and *R* enantiomers of the free acid III were inhibitory to vesicular stomatitis virus at 10–100 μg/mL and inhibitory to vaccinia virus at 100–200 μg/mL (data not shown), thus at significantly greater concentrations than those found inhibitory for the alkyl esters **1–7**, **10**, **11**, and **15**. The only compounds that did not show a greater potency than DHPA or the free acids III were the *tert*-butyl, 1-pentyl, 2-hydroxyethyl, and 2-furylmethyl esters.

With the exception of the 2-furylmethyl ester (**14**), the alkyl esters of (*RS*)-3-adenin-9-yl-2-hydroxypropanoic acid did not prove cytotoxic to the host cells at a concentration below 100 μg/mL (Table III). Most compounds were even devoid of cytotoxicity at a concentration of 400 μg/mL. This implies that for several compounds, i.e., **1**, **2**, **4**, **6**, **7**, **10**, the antiviral index, as defined by the ratio of the minimum inhibitory concentration (toward vesicular stomatitis virus) to the minimum cytotoxic concentration [for primary rabbit kidney (PRK) cells] was higher than 100-fold, if not higher than 400-fold. An antiviral index greater than 400 was noted for the ethyl, 2-butyl, and 2-methylpropyl esters. In contrast, the furylmethyl ester did not exhibit a specificity index greater than 2-fold.

The choice of the cell system was of prime importance in the determination of the activity against vesicular stomatitis virus (Table IV). While the minimum inhibitory concentrations of the compounds in E_6SM (human embryonic skin-muscle fibroblasts), T-21 [human fibroblasts trisomic for chromosome 21 (LR strain)], and HEp-2 (a human epithelial cell line) (Table IV) corresponded rather well with the minimum inhibitory concentrations recorded in PRK cells (Table III), little, if any, activity against vesicular stomatitis virus was observed in various other cell lines, such as CV-1 (a continuous cell line derived from African green monkey kidney cells), BS-C-1 (a simian epithelial cell line), and Balb/3T3 (murine fibroblasts derived from Balb/c mouse embryos). The minimum inhibitory concentrations denoted in RK13 (a continuous

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Table IV. Antiviral Activity of Alkyl Esters of (*RS*)-3-Adenin-9-yl-2-hydroxypropanoic Acid against Vesicular Stomatitis Virus in Different Cells

	minimal inhibitory concentration,[a] μg/mL									
compd	E_6SM	T-21	HEp-2	HeLa	HK	CV-1	Vero	BS-C-1	RK-13	Balb/3T3
1	30	ND[c]	3	70	>400	ND[c]	>400	150	1	300
2	7	1	7	4	200	>400	400	>400	2	>400
3	10	7	3	50	200	>400	150	300	2	>400
4	3	1	7	30	300	>400	200	>400	1	>400
5	7	2	2	15	50	300	30	150	100	200
6	1	1	7	30	>400	>400	150	>400	15	>400
7	5	1	1	10	20	200	30	100	30	300
8	150	15	40	150	>400	>400	>400	>400	30	>400
9	3	1	2	10	20	300	20	100	100	>200
10	2	1	1	3	10	200	15	70	100	200
11	7	0.5	2	1	15	>100	100	>100	30	100
12	70	30	3	70	200	>400	150	>400	100	>400
13	7	15	7	20	>400	>400	300	>400	70	>400
14[b]	40	7	4	7	>40	>40	>40	>40	>40	>40
15	7	1	2	30	>200	>200	>100	40	70	70
(*S*)-DHPA	15	20	4	30	50	>400	200	>400	150	>400
(*RS*)-DHPA	20	30	5	50	50	>400	250	>400	300	>400

[a] Required to reduce virus-induced cytopathogenicity by 50%. Viral cytopathogenicity was recorded as soon as it reached completion in the control virus-infected cell cultures, that is, at 1–2 days, irrespective of the cell type. [b] Cytotoxic at a concentration of ≥40 μg/mL (see also Table III). [c] Not determined. The multiplicity of infection (MOI) was invariably 100 × $CCID_{50}$, that is, 100 times the virus dose needed to infect 50% of the cells.

rabbit kidney cell line), human kidney (HK, another human epithelial cell line), and the well-established HeLa and Vero cell lines were also higher than those obtained in PRK, E_6SM, T-21, and HEp-2 cells. The striking dependence of the antiviral potency of the compounds on the nature of the cell substrate is not unique for the 3-adenin-9-yl-2-hydroxypropanoic acid esters, as a similar variation in antiviral potency from one cell line to another was also observed with DHPA (Table IV and ref 27). This variations can at least partially be attributed to differences in the uptake of the compounds by the cells.[27]

That the inhibitory effects of the 3-adenin-9-yl-2-hydroxypropanoic acid esters on virus-induced cytopathogenicity (Tables III and IV) truly reflected an inhibition of virus replication was ascertained by measuring the growth of vesicular stomatitis virus and vaccinia virus in the presence of the compounds (100 μg/mL). As shown in Figure 1, all compounds effected a marked reduction in virus yield: at an average log 1.5 for vesicular stomatitis virus and log 2 for vaccinia virus. However, the extent of virus yield reduction did not precisely correspond to the inhibitory activity of the compounds on viral cytopathogenicity. With the 2-furylmethyl ester (**14**), virus growth was completely suppressed, but this suppressive effect can be considered as the consequence of cytotoxicity, since compound **14** proved toxic for the host cells at a concentration of ≥40 μg/mL (Table III).

The alkyl esters were not inhibitory to host cell DNA or protein synthesis at concentrations below 100 μg/mL (Table V). They appeared somewhat more inhibitory to RNA synthesis, which was inhibited in the concentration range of 10–100 μg/mL (for compounds **7**, **9**, and **10**: Table V). This effect might be due to the general disorder in purine (and pyrimidine[28]) nucleoside metabolism caused by SAH hydrolase inhibitors.

Discussion

Considering the potent and irreversible inhibitory effect of the racemic 3-adenin-9-yl-2-hydroxypropanoic acid on SAH hydrolase,[22] it is conceivable that this acid and its alkyl esters achieve their antiviral effects by an inhibition of transmethylation reactions, e.g., those that are required for viral mRNA maturation.

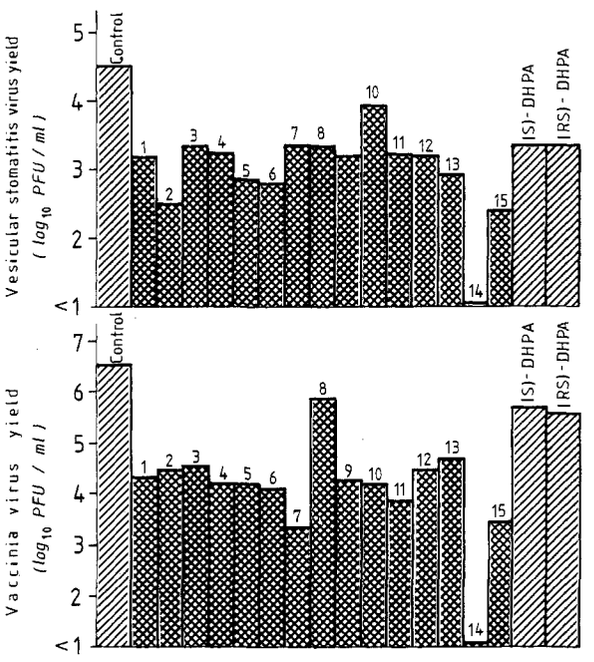

Figure 1. Inhibitory effects of the alkyl esters of (*RS*)-3-adenin-9-yl-2-hydroxypropanoic acid on the multiplication of vesicular stomatitis virus (VSV) (upper panel) and vaccinia virus (VV) (lower panel) in primary rabbit kidney cells. The cells were inoculated with $10^{4.5}$ PFU/0.5 mL per petri dish and, after 1 h of adsorption of the virus, further incubated with 100 μg/mL of the test compounds. Virus yield was measured at 24 h after VSV inoculation and 48 h after VV inoculation by plaque formation in murine L929 cells (VSV) or primary rabbit kidney cells (VV). PFU: plaque-forming units.

In general, the concentrations required for inhibition of DNA, RNA, or protein synthesis in normal uninfected PRK cells were at least 1, if not 2, orders of magnitude higher than the minimal antiviral concentrations (against vesicular stomatitis virus in PRK cells). This points to the specificity of the esters in their antiviral action.

The alkyl esters of 3-adenin-9-yl-2-hydroxypropanoic acid are poor inhibitors of SAH hydrolase.[22] If, as postulated above, their antiviral activity is due to an inhibition of SAH hydrolase within the virus-infected cell, it follows that the alkyl group merely serves as the protective moiety

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Table V. Inhibitory Effects of Alkyl Esters of (*RS*)-3-Adenin-9-yl-2-hydroxypropanoic Acid on DNA, RNA, and Protein Synthesis in PRK Cells

compd	ID_{50}[a] (μg/mL) for incorporation of		
	[*methyl*-^{3}H]dThd	[5-^{3}H]Urd	[4,5-^{3}H]leucine
1	>400	350 (300–400)	>400
3	276 (150–>400)	168 (131–200)	>400
5	253 (186–>400)	102 (37–182)	381 (327–>400)
7	242 (200–308)	34 (16–50)	277 (183–>400)
9	151 (90–229)	21 (12–32)	215 (100–347)
10	114 (64–179)	29 (9–44)	228 (98–400)
12	314 (200–>400)	>400	>400
(*S*)-DHPA	326 (275–>400)	>400	>400
(*RS*)-DHPA	311 (200–>400)	376 (297–>400)	>400

[a] ID_{50}: mean value for five to seven determinations (the range of individual values is indicated in parentheses).

to ensure the uptake of the ester by the cell. Within the cell, the ester would then be cleaved to release the free acid. The free acid III is, if added as such, only weakly active as an antiviral agent, probably because it is not readily taken up by the cells.

There appears to be an inverse correlation between the antiviral potency (against vesicular stomatitis virus) and the rate ($t_{1/2}$) of chemical hydrolysis: i.e., compounds **12–14**, which are the three most rapidly hydrolyzed compounds ($t_{1/2}$ = 5–22 h, see Table II), also rank among the least effective inhibitors of virus replication (Tables III and IV). Other compounds (**4**, **6**, **10**, **15**) with a $t_{1/2}$ of >80 h are clearly more effective as virus inhibitors. The compound (**14**) with the fastest hydrolysis rate ($t_{1/2}$ = 5.3 h) is also the one that is most cytotoxic. This implies that to achieve an optimal antiviral activity, the alkyl ester should remain intact in the cell culture medium so as to enable its effective uptake as such by the cell.

Experimental Section

Materials. The *R* and *S* enantiomers of 3-adenin-9-yl-2-hydroxypropanoic acids (III) have been described previously.[22] (*RS*)-3-Adenin-9-yl-2-hydroxypropanoic acid (III) was prepared as described elsehwere.[25]

N,N'-Dicyclohexylcarbodiimide was purchased from Fluka. The alcohols used were homogeneous on gas–liquid chromatography and were dried by the usual procedures before use. Triethylamine was distilled from sodium borohydride and dried over molecular sieves. Dimethylformamide was distilled from phosphorus pentoxide and stored over molecular sieves. Indicator-containing silica for preparative loose layers was purchased from Kavalier (Czechoslovakia). The octadecyl-silica (20 μm) was purchased from Laboratory Equipment, Praha (Czechoslovakia).

Methods. If not stated otherwise, the solutions were evaporated at 40 °C (2 kPa) and the substances were dried at 13 Pa over phosphorus pentoxide. UV-spectra were measured in methanol solutions on a Specord UV–vis apparatus (Carl Zeiss, Jena, G.D.R.), and the elution from the columns was monitored continuously with an Uvicord apparatus (LKB, Uppsala, Sweden). Thin-layer chromatography on silica-coated plates was done with Silufol UV 254 ready-made plates (Kavalier, Czechoslovakia). HPLC analysis was done on high-pressure glass columns (3.3 × 150 mm) of Separon SIX C 18 (5 μm) and LCD-254 UV detector and was recorded on an EZ 11 instrument (Laboratory Equipment, Praha, Czechoslovakia). The samples were applied by a sample valve injector (7-μL sample loop) and the elution (0.5 mL/min) was monitored by a high-pressure pump (Constametric I, LDC, USA) at 25 °C [detection at 254 nm, sensitivity 0.32 AUFS (absorption units for full scale)].

Preparation of (*RS*)-3-Adenin-9-yl-2-hydroxypropanoic Acid Esters. General Procedures. Method A. A suspension of (*RS*)-3-adenin-9-yl-2-hydroxypropanoic acid (5 mmol) in the corresponding alcohol (60 mL) was treated with concentrated sulfuric acid (0.6 mL) and the mixture was refluxed with stirring and exclusion of moisture till a complete reaction (followed by TLC in a chloroform–methanol mixture, 4:1), usually after 3–4 h. The mixture was cooled and poured into a suspension of Amberlite IR 45 (50 mL, prewashed with dioxane) in dioxane (200 mL). The slurry was stirred for 30 min and filtered; the resin was washed with dioxane (100 mL) and the filtrate evaporated in vacuo to dryness. The residue was chromatographed on two loose layers (45 × 16 × 0.3 cm) of silica (see above) in a chloroform–methanol mixture (9:1). The products were eluted with methanol (500 mL), the eluate was evaporated in vacuo, and the residue was crystallized from acetone (petroleum ether added to turbidity) to give the chromatographically pure products.

Method B. The esterification was performed as indicated in method A. After the reaction was completed, the mixture was cooled with ice and carefully neutralized by triethylamine. The solution was then taken down to dryness in vacuo, and the oily residue was dissolved in water (20 mL) and applied immediately onto a column (200 mL) of octadecyl-silica (see above), prewashed with water. The elution with water (6 mL/min) was continued till the drop of the conductivity of the eluate. The column was then washed with a stepwise gradient of aqueous dioxane (5%, 10%, 20%) (1 L each) until the elution of the UV-absorbing compound started and then continued with the appropriate eluent till the drop of the UV absorption. This eluate was evaporated in vacuo and codistilled with dioxane (2 × 50 mL) and the residue crystallized (acetone/petroleum ether or dioxane) to afford the chromatographically pure compounds.

Method C. A mixture of (*RS*)-3-adenin-9-yl-2-hydroxypropanoic acid (5 mmol), dimethylformamide (20 mL), and the corresponding alcohol (30 mL) was treated with *N,N'*-dicyclohexylcarbodiimide (10 mmol) and stirred overnight under the exclusion of moisture at room temperature. Water (100 mL) was then added, the suspension filtered, and the precipitate washed with water (100 mL). The filtrate was extracted with ether (3 × 100 mL) and the aqueous phase evaporated in vacuo [finally at 40 °C (13 Pa)]. The residue was redissolved in methanol (50 mL), filtered again, and concentrated in vacuo. Further purification was achieved as indicated in method A.

Method D. (*RS*)-3-Adenin-9-yl-2-hydroxypropanoic Acid *tert*-Butyl Ester. A suspension of III (2.23 g, 10 mmol) in *tert*-butyl acetate (100 mL) was treated with stirring with 70% perchloric acid (0.95 mL, 11 mmol) in a closed flask for 48 h at room temperature. After being cooled with ice, the mixture was neutralized with triethylamine and evaporated in vacuo. The residue was coevaporated with dioxane (2 × 50 mL), dissolved in water (100 mL), and applied on a column of octadecyl-silica (see method B); the elution was performed with water (41% starting compound III was recovered from this eluate after deionization) and the ester was eluted by 25% aqueous dioxane. After the usual workup, the product was obtained by crystallization from ethyl acetate.

Further details on the properties of the esters are given in Table I.

Chemical Hydrolysis of Esters IV. The reaction mixture consisted of either of the compounds listed in Table II (at 5 × 10^{-3} M) in sodium–potassium phosphate buffer, pH 6.86 or 7.42 (200 μL). The mixture was incubated at 37 °C, and 50-μL samples were taken in 16- and 35-h intervals and frozen to −60 °C. Zero-time samples were frozen immediately after the compounds were dissolved in the buffer solution. The samples were analyzed by HPLC with Lichrosorb C8 (5 μm) columns (3.3 × 150 mm) (purchased from Merck, F.R.G.) (see Table II). The integrated areas of peaks corresponding to compounds **3** and **4** were used for the computations. The results are summarized in Table II.

Biological Activity. The origin of the viruses and the techniques used for measuring inhibition of virus-induced cytopathogenicity and virus multiplication have been described previously.[14] Further details are provided in the footnotes to Tables III and IV and the legend to Figure 1.

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Thromboxane Synthetase Inhibitors (TXSI). Design, Synthesis, and Evaluation of a Novel Series of ω-Pyridylalkenoic Acids

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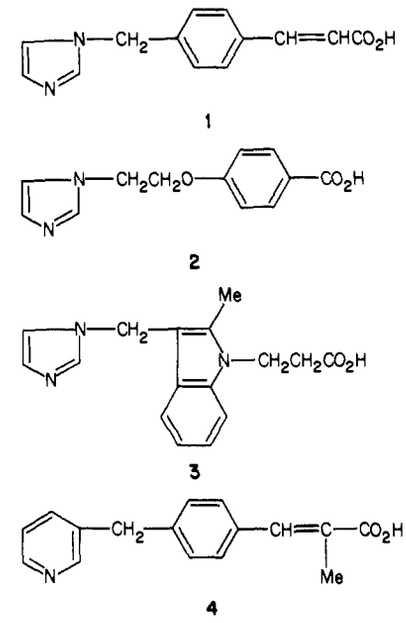
A novel series of ω-pyridylalkenoic acids has been prepared by applying the Wittig reaction. Modifications were made in the ω-aryl moiety, the alkylene chain length, the α-methylene group adjacent to the carbonyl group, and the carboxyl group of the molecule. The compounds were tested as inhibitors of thromboxane synthetase in an in vitro assay and in ex vivo experiments with the rat. Most members of this new class of thromboxane synthetase inhibitors (TXSI) showed good activity in both assay systems. (*E*)-7-Phenyl-7-(3-pyridyl)-6-heptenoic acid (**9c**; CV-4151) was one of the most potent compounds in in vitro enzyme inhibition ($IC_{50} = 2.6 \times 10^{-8}$ M) and, when orally administered, the most potent and long acting in the inhibition of blood thromboxane A_2 production in the rat. New conceptual models I–III for the enzyme–substrate (prostaglandin H_2, PGH_2) and the enzyme–TXSI interactions are proposed for understanding the molecular design and structure–activity relations.

Since the discovery of a thromboxane synthetase inhibitor (TXSI), imidazole, in 1977,[1] numerous analogues[2a-f] have been prepared in the hope of improving its pharmacological profile. In the search for potent inhibitors 1–4 (Chart I), many compounds of diverse structure, but all containing an imidazole or pyridine moiety, have been identified. The basic structural requirements for congeneric compounds inhibiting thromboxane synthetase are a 1-imidazolyl or a 3-pyridyl moiety at one end of the molecule and a carboxylic acid group at the other. Further, the distance between the carboxyl group and the nitrogen atom at the 3-position of the imidazole or pyridine moiety in potent compounds is concentrated between 8.5 and 10 Å. The distance appears to be similar to that between the carboxyl group and the endoperoxide moiety in prostaglandin H_2 (PGH_2), the substrate of thromboxane synthetase.

Recently, Ullrich and Haurand[3] reported that the cytochrome P-450 enzyme from human platelets possesses a kind of thromboxane synthetase activity and forms ligand complexes with TXSI. Kinetic studies indicate that TXSI appear to interact with the heme iron, the active site of the enzyme. The affinities are close to stoichiometric binding and correspond to the inhibitory action on biological activity. This investigation suggests that it is the 3-pyridyl nitrogen of TXSI that is oriented in close proximity of the heme iron. This finding enabled us to formulate the construction of new conceptual models I and II for the enzyme–substrate (PGH_2) and the enzyme–TXSI interactions (Figure 1). These models were constructed on the basis of reference data on cytochrome c[4] and cytochrome P-450 enzyme.[5] Since it is thought that enzymes that include a heme iron have a space in which the substrate molecule such as PGH_2 can be received, the so-called crevice between the substrate-binding site and the heme iron, the optimal molecular size of TXSI to reach the space through the entrance of the crevice, can be presumed to be nearly equal in size to the molecular size of PGH_2. The conceptual feature of the crevice would not be unexpected when one considers the lipophilic character of the substrate (PGH_2) that competes with a TXSI at the same active site.

Chart I



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