STRUCTURE-ACTIVITY STUDIES ON OPEN-CHAIN ANALOGUES OF NUCLEOSIDES: INHIBITION OF S-ADENOSYL-L-HOMOCYSTEINE HYDROLASE AND ANTIVIRAL ACTIVITY
2. ACID OPEN-CHAIN ANALOGUES*

Antonín Holý[a], Ivan Votruba[a] and Erik De Clercq[b]

[a] *Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6, Czechoslovakia and*
[b] *Rega Instituut, Katholieke Universiteit Leuven, B — 3000 Leuven, Belgium*

Received June 6th, 1984

Over 50 ω-carboxyalkyl derivatives of adenine and other purine bases were examined for their inhibitory effects on rat liver S-adenosyl-L-homocysteine hydrolase and their antiviral activity. To be an inhibitor of SAH-hydrolase the analogue must contain an adenine base substituted at the position 9 by an ω-carboxyalkyl (C_3—C_5) chain bearing at least one hydroxyl function. The absolute configuration at the side-chain is decisive for the dihydroxy and trihydroxy compounds, but less important for the monohydroxyalkanoic acids. D-Eritadenine (*1a*) and 3-(adenin-9-yl)-2-hydroxypropanoic acids (*12a*) are the most potent SAH-hydrolase inhibitors and the only compounds possessing an antiviral activity (against vesicular stomatitis, parainfluenza type 3, reovirus type 1, and vaccinia virus). All these compounds effect a rapid irreversible inactivation of SAH-hydrolase. The esters of *1a* and *12a* exhibit little, if any inhibitory activity toward the enzyme; they are, however, much more potent antiviral agents than the parent compounds *1* and *12a*, most probably acting as prodrugs of the latter. 2-Amino-D-eritadenine, (2*R*,3*R*)-5-(adenin-9-yl)-2,3-dihydroxypentanoic acid, 9-(dicarboxymethyl)adenine, 4(-adenin-9-yl)-2-hydroxybutanoic acid, 3-(8-bromoadenin-9-yl)-2-hydroxypropanoic acid and O-carboxymethyl derivatives of 9-(2,3-dihydroxypropyl)- and 9-(2,3,4-trihydroxybutyl)adenine are described as novel compounds.

In our preceding communication[1] we described the relationship between structure of neutral open-chain adenosine analogues and their inhibitory activity toward S-adenosyl-L-homocysteine hydrolase (SAH-hydrolase), and demonstrated a correlation between the inhibition of this enzyme and the antiviral activity of the compounds. Within the framework of our investigations in this area we have found some time ago that eritadenines, particularly the natural D-eritadenine (*1a*), are extremely potent inhibitors of SAH-hydrolase[2]. Eritadenines also show an antiviral activity, the spectrum of which conforms qualitatively to that of 9-(*S*)-(2,3-dihydroxypropyl)-adenine ((*S*)-DHPA), a neutral adenosine analogue[1], which also inhibits SAH-

* Part XIV of the series of Studies on S-Adenosyl-L-homocysteine Hydrolase; Part XIII: This Journal *50*, 245 (1985).

-hydrolase[3,4]. D-Eritadenine is equally inhibitory for SAH-hydrolases originating from rat liver[2], L-1210 murine leukemia cells[5], tobacco culture cells[6] and *Pyrrhocoris apterus* ovaria[7]. However, D-eritadenine differs from the neutral open-chain analogues in that the latter inhibit the enzyme competitively and reversibly, whereas D-eritadenine (*1a*) and its configurational isomers inactivate SAH-hydrolase rapidly and irreversibly. The IC_{50} value for D-eritadenine suggests that it forms an equimolar complex with the enzyme[2]. In the action of D-eritadenine on liver hepatocytes, the cellular SAH-hydrolase is irreversibly inactivated and the intracellular concentration of S-adenosyl-L-homocysteine (SAH) markedly increases[8].

A detailed study of D-eritadenine is prompted by its additional biological activities, such as hypocholesterolemic[9], aspermatogenic and chemosterilizing[10] effects. We therefore investigated the relationship between the structure of the eritadenine analogues, *i.e.* ω-carboxyalkyl derivatives of adenine, and their inhibitory effect on SAH-hydrolases. Since the mechanism of inhibition of SAH-hydrolase by neutral adenine analogues is different from that of the eritadenines, both classes of com-

TABLE I

Base-modified 9-(ω-carboxyalkyl)adenines[a]

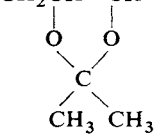
Compound	Base residue (ref.)	v_i/v_0[b]
	D-Eritadenines	
1a	Adenin-9-yl[3]	0
1b	2-Aminoadenin-9-yl[c]	0
1c	2-Methylthioadenin-9-yl[9]	1·00
1d	8-Bromoadenin-9-yl[9]	0·30
1e	N^6-Ethyladenin-9-yl[9]	0·64
1f	N^6-Dimethyladenin-9-yl[9]	0·74
1g	Hypoxanthin-9-yl[9]	0·54
1h	6-Methylthiopurin-9-yl	1·00
2	Adenin-3-yl	0
	(*RS*)-3-(Adenin-9-yl)-2-hydroxypropanoic acids	
12a	Adenin-9-yl	0
12b	2-Methyladenin-9-yl[14]	0·49
12c	2-Aminoadenin-9-yl[14]	0·49
12d	8-Bromoadenin-9-yl[14]	0·78

[a] Except for compound *1a* (*cf.*[3]) and *12a* (Table II), none of the compounds exhibited any antiviral activity against VSV and VV; [b] hydrolytic reaction, for adenine $v_i/v_0 = 0{\cdot}85$; [c] for preparation, see Experimental.

pounds are discussed separately. However, since the biological consequences of this inhibition may be the same for both series of compounds, we investigated to what extent the inhibitory effects of the eritadenines on SAH-hydrolase may be correlated with their antiviral effects.

TABLE II

Side-chain substituted 9-(ω-carboxyalkyl)adenines

Compound	Configuration	Substituent (ref.)	v_i/v_0	MIC_{50}, μg/ml[a]	
				VSV	VV
3	2*R*,3*R*	$-CH_2CH(OH)CH(OCH_3)COOH$[12]	0·32	—	—
4	2*R*,3*R*		0·97	—	—
5	2*R*,3*R*	$-CH_2CH_2CH(OH)CH(OH)COOH$[b]	0·07	—	—
6a	2*S*,3*S*,4*S*	$-CH_2CH(OH)CH(OH)CH(OH)COOH$[13]	0·60	—	—
6b	2*R*,3*R*,4*R*		0·67	—	—
6c	2*S*,3*S*,4*R*		0·07	—	—
6d	2*R*,3*R*,4*S*		0·60	—	—
6e	2*S*,3*R*,4*R*		0·07	—	—
6f	2*R*,3*S*,4*S*		0·14	—	—
6g	2*S*,3*R*,4*S*		0·92	—	—
6h	2*R*,3*S*,4*R*		0·10	—	—
7	—	$-CH_2COOH$[3]	1·00	NA	NA
8	—	$-CH(COOH)_2$[b]	1·00	NA	NA
9	—	$-CH{=}CH-CH_2COOH$[3]	0·83	NA	NA
10	*RS*	$-CH_2CH(OCH_3)COOH$[14]	0·88	—	—
11	*RS*	$-CH_2CH(NH_2)COOH$[15]	0·71	—	—
12a	*RS*	$-CH_2CH(OH)COOH$[14]	0	—	—
12a	2*S*	$-CH_2CH(OH)COOH$[3]	0	15	40
12a	2*R*	$-CH_2CH(OH)COOH$[3]	0	100	200
13	*RS*	$-CH(CH_3)CH(OH)COOH$[14]	0·45	NA	NA
14	*RS*	$-CH(n\text{-}C_6H_{13})CH(OH)COOH$[14]	0·92	NA	NA
15	*RS*	$-CH_2CH(OH)CH_2COOH$[14]	0·28	NA	NA
15	3*S*	$-CH_2CH(OH)CH_2COOH$[14]	0	—	—
16	*RS*	$-CH(CH_3)CH(OH)CH_2COOH$[14]	0·74	NA	NA
17	*RS*	$-CH_2CH_2CH(OH)COOH$[b]	0·26	—	—
18	*RS*	$-CH_2CH(OH)CH_2OCH_2COOH$[b]	1·00	NA	NA
17	2*S*,3*S*	$-CH_2CH(OH)CH(OH)CH_2OCH_2COOH$[b]	0·35	NA	300

[a] NA not active ($MIC_{50} > 400$ μg/ml); [b] for preparation, see Experimental.

The inhibitory effect on SAH-hydrolase was evaluated using the ratio of the initial rates of SAH-hydrolysis in the presence or absence of the inhibitor (v_i/v_0); under our experimental conditions this ratio is close to zero for potent inhibitors of SAH-hydrolase. In such instances other inhibition parameters were also determined. Since the reaction mixture for the hydrolytic assay contains an excess of adenosine amino-hydrolase to rapidly remove adenosine arising from the hydrolysis of SAH, we investigated in the most important cases whether the test compounds were not deaminated under the assay conditions.

Antiviral activity of the compounds was evaluated by inhibition of cytopathogenic effect of vesicular stomatitis virus (VSV) and vaccinia virus (VV) in cell culture. Compounds effecting a 50% inhibition at a minimum inhibitory concentration (MIC_{50}) higher than 400 μg/ml (*i.e.* about $1{\cdot}5 \cdot 10^{-3}$ mol l^{-1}) were considered inactive.

TABLE III
Compounds modified at the carboxyl groups

Compound	Configuration	Substituent	v_i/v_0	MIC_{50}, μg/ml[a]	
				VSV	VV
		Eritadenines			
20a	2*R*,3*R*	Methyl ester[3]	0·49	15	30
20b	2*R*,3*R*	Ethyl ester[3]	0·50	15	30
20c	2*R*,3*R*	n-Octyl ester	0·48	—	—
20d	2*S*,3*S*	Methyl ester[3]	0·62	100	70
21a	2*R*,3*R*	Amide[9]	0·70	200	NA
21b	2*R*,3*R*	N-Ethylamide[9]	0·90	NA	NA
21c	2*R*,3*R*	N,N-Dimethylamide[9]	1·00	—	—
22	2*S*,3*S*	Methyl ester[b,16]	1·00	—	—
		Other 9-substituted adenines			
23d	2*S*	$-CH_2CH(OH)COOCH_3$[3]	0·65	1·5	7·0
23a	2*R*	$-CH_2CH(OH)COOCH_3$[3]	0·83	1·5	7·0
23b	2*R*	$-CH_2CH(OH)COOC_2H_5$[3]	0·88	—	—
24	*RS*	$-CH(CH_3)CH(OH)COOCH_3$[14]	0·89	NA	300
25	—	$-CH_2COOCH_3$	0·94	NA	NA
26	—	$-CH{=}CHCH_2COOCH_3$[14]	0·80	—	—
27	—	$-CH_2CH{=}CHCOOCH_3$[14]	1·00	—	—
28	*RS*	$-CH_2CH(OH)CONH_2$	1·00	NA	NA

[a] NA not active ($MIC_{50} > 400$ μg/ml); [b] 2,3-O-isopropylidene-L-eritadenine methyl ester.

The high enzyme inhibitory activity of some compounds required very high chemical and isomeric purity of the test samples. Thus, traces of an impurity of $IC_{50} \sim 10^{-8}$ mol l^{-1} can completely depreciate measurements with inhibitor concentrations of 10^{-6} or 10^{-7} mol l^{-1}. Purity of compounds was verified by HPLC and their structure and isomeric homogeneity was guaranteed by stereospecific syntheses (*cf.* our previous papers: see Tables I–III); some new compounds are described in this communication.

EXPERIMENTAL

Unless stated otherwise, solutions were taken down at 40°C/2 kPa and compounds were dried at 13 Pa over phosphorus pentoxide. Melting points were determined on a Kofler block and are uncorrected.

Materials and enzymes. [^{14}C]-S-Adenosyl-L-homocysteine was prepared by enzymatic synthesis from L-homocysteine and [^{14}C]adenosine of specific activity 16 GBq mmol^{-1} (purchased from Institute for Research, Production and Application of Radioisotopes, Prague). Rat liver SAH-hydrolase was isolated according to ref.[4], L-1210 murine leukemia cell SAH-hydrolase was isolated as described in ref.[5]; both enzymes were purified to homogeneity by affinity chromatography[11] and dissolved in 20% aqueous glycerol. Rat liver SAH-hydrolase was stabilized by addition of serum albumin (50 μg ml^{-1}). Adenosine aminohydrolase (calf intestine) was from Boehringer (Mannheim, FRG).

The inhibitory effect in the direction of hydrolysis (v_i/v_0) was determined at an SAH concentration of $4 \cdot 10^{-6}$ mol l^{-1} and an inhibitor concentration of 10^{-6} or 10^{-7} mol l^{-1} (sodium, lithium or ammonium salt) in a standard reaction mixture[4]. Determination of IC_{50} and $t_{1/2}$ of the irreversible inhibitors was carried out as described[2].

Time dependence of v_i/v_0. The inhibitory effect of compound 23*a* was evaluated after pre-incubation (0, 10, 30 and 60 min at 37°C) of its solution in 0·1 mol l^{-1} phosphate buffer pH 7·4; aliquots corresponding to 0·25 μmol of the inhibitor were transferred at the given intervals to a reaction mixture of standard composition[4], containing L-1210 murine leukemia cell SAH-hydrolase (0·136 μg/ml), adenosine aminohydrolase and [^{14}C]-SAH. Incubation was for 5 and 10 min at 37°C. Determination of v_i was carried out according to ref.[2].

Substrate activity towards adenosine aminohydrolase was determined as described previously[1]. The antiviral effect was followed by inhibition of cytopathogenic effect of vesicular stomatitis virus (VSV) and vaccinia virus (VV) in cell cultures, as described in the preceding communication[1]. The antiviral activity is expressed as MIC_{50}, *i.e.* minimal inhibitory concentration of the compound which gave 50% reduction of the virus cytopathogenic effect.

2-Amino-D-eritadenine (*Ib*)

A mixture of 2,6-diaminopurine (20 mmol), sodium hydride (20 mmol) and dimethylformamide (80 ml) was heated to 80°C for 30 min under stirring and exclusion of moisture. 2,3-O-Cyclohexylidene-D-erythronolactone[19] (4·5 g; 22·7 mmol) was added, the mixture was stirred at 100°C for 14 h and taken down at 50°C/13 Pa. The residue was refluxed with 80% formic acid (150 ml) for 6 h, and the acid was evaporated. The residue was codistilled with water (4 × 50 ml) and deionized on a column of Dowex 50X8 (H^+-form; 200 ml). After washing the column to disappearance of UV absorption and conductivity, the product was eluted with 2·5% aqueous ammonia.

The product fraction was taken down and applied in aqueous solution (pH 9) on a column of Dowex 1X2 (acetate; 200 ml). The column was then eluted with a linear gradient of formic acid (0—1 mol l^{-1}; 2 litres each). The product fractions were combined, taken down *in vacuo*, formic acid was removed by codistillation with water and the residue was chromatographed on a column (80 × 4 cm) of microcrystalline cellulose (Macherey and Nagel) in 2-propanol–conc. aqueous ammonia–water (7 : 1 : 2); rate 20 ml/h. Fractions, containing the compound *Ib* (monitored by paper chromatography in the same solvent system; R_F 0·39), were combined, taken down *in vacuo*, the residue was dissolved in water (10 ml) and applied on a column of Dowex 50X8 (Li^+-form; 25 ml). Elution with water gave the lithium salt of *Ib* which after evaporation and precipitation with ether from methanol was isolated in a 34% yield (related to 2,6-diaminopurine). UV Spectrum (pH 2): λ_{max} 253 nm, 291 nm; ε_{291} = 10 000. Electrophoretical mobility (E_{Up}) related to uridine 3′-phosphate at pH 7·5 (20 V/cm): 0·33.

2′,3′-O-Isopropylidene-L-eritadenine (*4*)

was prepared by hydrolysis of the methyl ester *22* (ref.[16]) with 1% aqueous sodium hydroxide at room temperature overnight, neutralization of excess sodium hydroxide with Dowex 50X8 (H^+-form), filtration and evaporation of the filtrate *in vacuo*. The chromatographically pure sodium salt of the compound *4* was obtained by precipitation with ether from methanolic solution; yield 95%, R_F 0·65.

9-Dicarboxymethyladenine (*8*)

was prepared as the sodium salt by hydrolysis of the dimethyl ester[15] under conditions described for compound *4*. The chromatographically pure compound *8* was obtained in 90% yield; R_F 0·48; E_{Up} 0·68.

(2*R*,3*R*)-5-(Adenin-9-yl)-2,3-dihydroxypentanoic Acid (*5*)

1-(Adenin-9-yl)-1,2-dideoxy-3,4-O-isopropylidene-D-*erythro*pentitol[20] (1·7 mmol), followed by ruthenium oxychloride solution (corresponding to 10 mg Ru), was added to a solution of sodium periodate (3·5 mmol) in 70% aqueous acetone (80 ml). After stirring at room temperature overnight, the precipitate was filtered and washed with acetone (100 ml) and the filtrate was taken down. The residue was dissolved in 0·25 mol l^{-1} sulfuric acid, set aside overnight, neutralized with barium hydroxide, filtered and taken down *in vacuo*. The residue was deionized on Dowex 50 (see compound *1b*) and the product was chromatographed on a column of Dowex 1X2 (acetate; 100 ml) with a linear gradient (1 litre each) of 0—0·5 mol l^{-1} formic acid. Further purification on a column of cellulose (as described for *1b*) gave chromatographically pure lithium salt of *5* in 38% yield. R_F 0·29, E_{Up} 0·55. Purity (determined spectrophotometrically) higher than 95%.

(*RS*)-3-(8-Bromoadenin-9-yl)-2-hydroxypropanoic Acid (*12d*)

Sodium (*RS*)-3-(adenin-9-yl)-2-hydroxypropanoate[14] (*12a*; 4 mmol) was added with stirring to a solution of bromine (0·3 ml) in water (70 ml). The mixture was stirred overnight in a stoppered flask and taken down. The residue was codistilled with water (2 × 25 ml), dissolved in water (25 ml) and the solution was applied on a column of Dowex 50X8 (H^+-form; 200 ml). After washing with water to disappearance of UV absorption and conductivity, the ion-exchange resin was suspended in water (300 ml). Aqueous ammonia (2·5%) was added dropwise under stirring so as to keep the pH value under 9·0 until this value remained constant. After stirring for further 20 min the mixture was filtered, the resin was washed with water (300 ml) and the

combined filtrates were taken down *in vacuo*. The residue was chromatographed on a column of Dowex 1X2 (acetate; 100 ml) with a linear gradient of formic acid (0—0·5 mol l^{-1}; 2 litres each; 3 ml/min, fractions 10 min). The UV-absorbing material was analyzed by HPLC and the product-containing fractions were combined, taken down *in vacuo* and formic acid was removed by codistillation with water (3 × 25 ml). Crystallization from water gave 450 mg (37%) of compound *12d*, R_F 0·46 (*12a*: 0·39), E_{Up} 0·48. For $C_8H_8BrN_5O_3$ (302·2) calculated: 31·80% C, 2·67% H, 26·46% Br, 23·18% N; found: 31·46% C, 2·65% H, 26·51% Br, 23·03% N. UV Spectrum (pH 2): λ_{max} 267 nm, ε_{max} 19 000.

4-(Adenin-9-yl)-2-hydroxybutanoic Acid (*17*)

A solution of sodium periodate (2·3 g; 10·8 mmol) in water (20 ml) was added at 0°C to a stirred suspension of 9-(3,4-dihydroxybutyl)adenine[15] (3·2 g; 10 mmol) in water (50 ml). After stirring (ice cooling) for 3 h the mixture was applied on a column of Dowex 1X2 (acetate, 100 ml). The column was washed with water until the conductivity dropped and then eluted with 0·05 mol . . l^{-1} acetic acid. The UV-absorbing eluate was concentrated *in vacuo* to about 70 ml, the residue was made up with water to 100 ml, cooled to 5°C and sodium cyanide (3·5 g) was added. The mixture was rapidly neutralized with acetic acid and stirred in a stoppered flask overnight at room temperature. After addition of concentrated hydrochloric acid (15 ml), the mixture was boiled for 3 h and taken down *in vacuo*. The residue was coevaporated with water (3 × × 50 ml), dissolved in water (50 ml) and applied on a column of Dowex 50X8 (H^+-form; 400 ml) which was then washed with water to drop of conductivity. The product was eluted with 2·5% aqueous ammonia, the UV-absorbing ammonia eluate was taken down *in vacuo*, the residue dissolved in water (20 ml, adjusted to pH 9 with ammonia) and applied onto a column of Dowex 1X2 (acetate; 100 ml). After washing with water to disappearance of UV absorption, elution with a linear gradient of formic acid (0—1 mol l^{-1}, 1 litre each) gave the product (eluted at 0·3—0·5 mol l^{-1}). The product fractions were taken down and the residue was codistilled with water (4 × 25 ml) and crystallized from 70% ethanol–ether, affording 1·05 g (45%) of compound *17*, m.p. 261—263°C. For $C_9H_{11}N_5O_3$ (237·2) calculated: 45·56% C, 4·67% H, 29·53% N; found: 45·74% C, 4·73% H, 29·42% N. Mass spectrum: M^+ 237, UV spectrum (pH 2): λ_{max} 261 nm, ε_{max} 14 500. R_F 0·37 (S1), E_{Up} 0·57. HPLC (Sepharon SIX C18, 0·05 mol l^{-1} triethylammonium borate pH 8·4 with 5% methanol): k = 5·60 (*17*), 3·2 (*12a*), 4·35 (*15*) ($k = t_R/t_0 - 1$, t_R retention time, t_0 hold-up time).

9-(*RS*)-(3-O-Carboxymethoxy-2-hydroxypropyl)adenine (*18*)

Sodium hydride (20 mmol) was added to a solution of 9-(*RS*)-(2,3-dihydroxypropyl)adenine[17] (5 mmol) in dimethylformamide (25 ml) under exclusion of moisture. After stirring for 30 min, sodium chloroacetate (6 mmol) was added, the mixture was stirred at 100°C for 8 h and the solvent evaporated at 50°C/13 Pa. The further work-up procedure was the same as described for compound *12d*. The product was eluted with 0·05—0·07 mol l^{-1} formic acid and after evaporation of the eluent and codistillation with water it was crystallized from water; yield 20% of monohydrate of *18*; R_F 0·43, E_{Up} 0·50. For $C_{10}H_{15}N_5O_5$ (285·3) calculated: 42·10% C, 5·30% H, 24·55% N; found: 42·35% C, 5·30% H, 24·72% N.

(2*S*,3*S*)-9-(4-Carboxymethoxy-2,3-dihydroxybutyl)adenine (*19*)

The title compound was prepared from (2*S*,3*S*)-9-(2,3-O-isopropylidene-2,3,4-trihydroxybutyl)-adenine[18] (5 mmol) and sodium chloroacetate (6 mmol) as described for compound *18*. After evaporation of the solvent *in vacuo*, the residue was warmed to 37°C with 0·25 mol l^{-1} sulfuric

acid (40 ml) for 8 h. This solution was directly deionized and processed as described for *12d*. Crystallization from 90% ethanol gave 47% of *19*, m.p. 205–207°C, R_F 0·37, E_{Up} 0·50. For $C_{11}H_{15}N_5O_5$ (297·3) calculated: 44·44% C, 5·09% H, 23·56% N; found: 44·72% C, 5·00% H, 23·34% N.

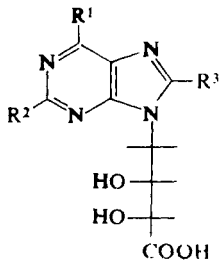
RESULTS AND DISCUSSION

Inhibition of S-*Adenosyl*-L-*homocysteine Hydrolase*

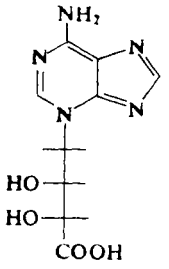
The investigations on SAH-hydrolase inhibition were based upon our experience with neutral adenosine analogues[1], and D-eritadenine and its isomers[3]. We evaluated the effects of the heterocyclic base, substituents in the alkyl side chain and modification of the carboxyl group. Table I summarizes the results obtained with D-eritadenine derivatives, modified in the adenine base. In the evaluation of these (and other) compounds, we consider as active compounds only those with v_i/v_0 close to zero. Examples of such active compounds are *1a*, *1b* and *2*. Introduction of a methylthio group into position 2 of adenine (*1c*), alkylation of the 6-amino group (*1e,f*) or substitution with bromine at position 8 (*1d*) results in a partial or complete loss of enzyme-inhibiting activity. However, if we compare the activity of these compounds with that of 9-(2,3-dihydroxypropyl)adenine, which can be regarded as the parent compound of derivatives *1*, it is evident that introduction of carboxyl group substantially enhances the inhibitory effect on SAH-hydrolase. It is thus not surprising that in the compound *1* series we detect inhibitory activity even for those base-modified compounds that prove inactive in the neutral form. However the inhibitory effects of compounds *1d*, *1e*, *1f* and *1g* are weak ($v_i/v_0 \sim 0{\cdot}50$), as compared with compound *1a*. More surprising is the potent SAH-hydrolase inhibition found for the N^3-isomer of D-eritadenine (*2*).

As in the series of the neutral analogues, most potent SAH-hydrolase inhibitor among derivatives *1* was the derivative of adenine itself. Therefore, further investigations were performed exclusively with derivatives of adenine, substituted with an ω-carboxyalkyl moiety (Table II). For an inhibitory effect on SAH-hydrolase the mere presence of a carboxyl function in the molecule is not sufficient, since compounds *7–9* are not inhibitors. Also, introduction of a methoxy or amino group into the side chain of the alkanoic acid (compounds *10,11*) is incompatible with activity. On the other hand, introduction of a single hydroxyl leads to a marked inhibitory activity, as attested by the enantiomers as well as the racemate of compounds *12a*, *i.e.* 3-(adenin-9-yl)-2-hydroxypropanoic acids. The presence of an additional alkyl group in the side chain (compounds *13*, *14*) lowers the activity. This effect may be explained in terms of hydrophobicity of the alkyl group. An analogous observation made in the series of the 2,3-dihydroxyalkyl derivatives[1] was interpreted by hindered interaction with the adenine binding site of the enzyme. The

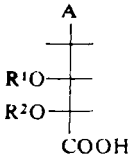
activity of a compound is determined by the position of the adenine moiety as well as the hydroxy and carboxy groups: *e.g.* the α-hydroxy acids *12a*, *13* and *17* are more potent inhibitors than the corresponding β-hydroxy acids *15* and *16*. The inhibitory effect is completely lost for the O-carboxymethyl derivative *18* where



	R^1	R^2	R^3		R^1	R^2	R^3
1a	NH_2	H	H	*1e*	NHC_2H_5	H	H
1b	NH_2	NH_2	H	*1f*	$N(CH_3)_2$	H	H
1c	NH_2	SCH_3	H	*1g*	OH	H	H
1d	NH_2	H	Br	*1h*	SCH_3	H	H

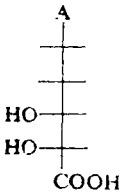


2



3, R^1 = H, R^2 = CH_3

4, R^1, R^2 = $(CH_3)_2C$ –



5

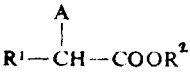
A—CH_2[CH(OH)]$_2$COOH

6

A—CH=CH—CH_2COOR

9, R = H

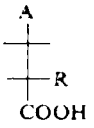
26, R = CH_3



7, R^1 = R^2 = H

8, R^1 = COOH, R^2 = H

25, R^1 = H, R^2 = CH_3

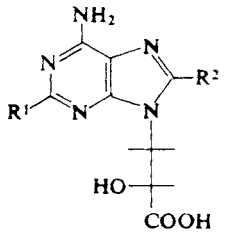


10, R = OCH_3

11, R = NH_2

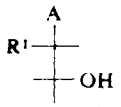
A = adenin-9-yl residue

the carboxyl function is still more distant from the adenine and hydroxy groups. Yet, this type of carboxy derivatives can inhibit SAH-hydrolase: *e.g.* the homologous compound *19* is definitely more active than the best inhibitor of the neutral series of compounds.



12a, $R^1 = R^2 = H$ *12c*, $R^1 = NH_2$ $R^2 = H$

12b, $R^1 = CH_3$, $R^2 = H$ *12d*, $R^1 = H$, $R^2 = Br$



13, $R^1 = CH_3$, $R^2 = OH$

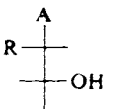
14, $R^1 = n\text{-}C_6H_{13}$, $R^2 = H$

23a, $R^1 = H$, $R^2 = OCH_3$

23b, $R^1 = H$, $R^2 = OC_2H_5$

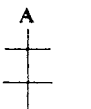
23c, $R^1 = H$, $R^2 = NH_2$

24, $R^1 = CH_3$, $R^2 = OCH_3$

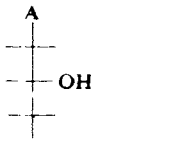


15, $R = H$

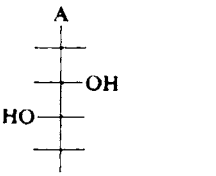
16, $R = CH_3$



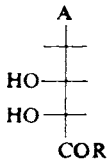
17



18



19



20a, $R = OCH_3$

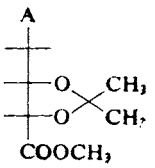
20b, $R = OC_2H_5$

20c, $R = n\text{-}C_8H_{17}$

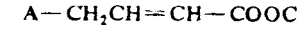
21a, $R = NH_2$

21b, $R = NHC_2H_5$

21c, $R = N(CH_3)_2$



22



27

A = adenin-9-yl residue

The difference between the activity of compounds *18* and *19* is probably due to the presence of a vicinal diol group in the alkanoic acid chain of compound *19*. This

effect has also been observed with the eritadenines[3]. As noted for the neutral compounds[1], O-methylation of one of the hydroxyl functions of the side chain results in a decrease of the inhibitory activity (compound *3*) and replacement of both hydroxyls by a 1,3-dioxolane group results in a complete loss of inhibitory potency (compound *4*). Less critical than O-substitution is the distance of the α,β-dihydroxy acid group from the adenine moiety; this is evident from a comparison of D-eritadenine with its homologue *5*.

Less straightforward is the situation of the homologous trihydroxyalkanoic acids *6* (Table II): some diastereoisomers of these compounds show very low activity whereas others reach the activity level of the *threo*-isomer of *1a* (ref.[3]); the enantiomers of *ribo* configuration (*6a,b*) are virtually ineffective, whereas the *lyxo*-enantiomers (*6e,f*) are active. Of the remaining enantiomeric pairs (*arabo* and *xylo* configuration) only the (3*S*,4*R*)-isomer is active (compounds *6c,h*).

Since the preparation of the racemic 3-(adenin-9-yl)-2-hydroxypropanoic acid (*12a*) is easier than that of D-eritadenine (*1a*) (ref.[13]) and since *12a* has almost the same inhibitory effect as D-eritadenine, we further examined the effects of substitutions in the adenine ring of *12a* (Table I). The 2-amino (*12c*) and 2-methyl (*12b*) derivatives were active, whereas, as could be expected, the 8-bromo derivative *12d* showed lower activity. Thus, for both series of compounds (*1* and *12*), substitution in the adenine ring brought about similar effects.

The last group of compounds tested contained the derivatives with a modified carboxyl group. They should help to determine whether the potent inhibitory effect of the carboxylic acid derivatives on SAH-hydrolase is due to the free carboxyl group or the electronegative substituent in the 9-(dihydroxyalkyl)adenine moiety. For this purpose, we investigated derivatives of the most active carboxylic acids in both series, *i.e.* D-eritadenine and 3-(adenin-9-yl)-2-hydroxypropanoic acid (Table III). Whereas amides (*21*, *28*) had no marked inhibitory effect on SAH-hydrolase, alkyl esters of both the *1a* and *12a* acid types (*20*, *23*) proved active. Their activity was lower than that of carboxylic acids *1a* and *12a*, but equal or higher than that of 9-(*S*)-(2,3-dihydroxypropyl)adenine or 9-(2-hydroxyethyl)adenine (parent compounds of these esters) (ref.[1]). However, this inhibitory effect cannot be ascribed directly to the esters *20*, *23*, because both are readily hydrolyzed, even under physiological conditions, *e.g.* the half-life of hydrolysis of the methyl ester *23a* at pH 7·4 is 22 h (ref.[21]) and hydrolysis to the acids *1a* and *12a* must occur during the assay. Since the arising acids are extremely active (Table II), their activity must be counteracted by the esters. Such interaction is evident from the time-dependence of v_i/v_0 for the methyl ester *23a*, preincubated under the assay conditions in the absence of the enzyme (Fig. 1). The v_i/v_0 value, extrapolated to zero time, is very near to one, thus showing inactivity for the starting ester (Fig. 1).

For the neutral adenosine analogues it was possible to specify rather accurately he requirements necessary for the inhibitor structure and the absolute configuration

of hydroxyl group in the side chain[1]. This is more difficult for the ω-carboxyalkyl derivatives. Whereas both enantiomers of the α-hydroxy acid *12a* are approximately equally active, the (3*S*)-isomer of the β-hydroxy acid *15* is obviously more effective than its (*R*)-enantiomer. In the eritadenine series, the (2*R*,3*R*)-*erythro*-isomer *1a* is markedly more active than the *threo*-derivatives[2]. If the inhibitory activity of a compound toward SAH-hydrolase is governed by its conformational adaptability, enabling to replace adenosine at its binding site of the enzyme, then in neutral analogues the intramolecular interaction of the vicinal diol group and orientation of the side chain with respect to adenine will be decisive. Since the chain is bound to the base by an sp_3-hybridized carbon atom it has a high degree of freedom in the conformational adaptation. However, in α-hydroxy and α,β-dihydroxy acids the interaction of the carboxylate anion with the protonated $N_{(1)}$ atom of the adenine ring should be of prime importance in determining the conformation of the molecule. Although the side chain is also bound by an sp_3-hybridized atom to the base, the conformational freedom is considerably restricted. In the α-hydroxy or β-hydroxy acids the isolated hydroxyl in the side chain can adopt a suitable position relative to the enzyme approximately equally easily in both enantiomers; consequently not much difference in activity can be expected. On the other hand, in the α,β-dihydroxy acids (eritadenines) the intramolecular interaction of the vicinal diol group represents an additional factor in determining conformation; its role may be significantly different for the individual geometric isomers. An optimal situation obviously exists in the *erythro*-isomer with absolute configuration 2*R*,3*R* (ref.[3]). Extension of the chain bearing the *erythro*-vicinal diol (compound *5*) does not significantly disturb the adaptability of the molecule. On the contrary, in the homologous trihydroxyalkanoic acids *6* the interactions of the three neighbouring hydroxyl groups with each other can de-

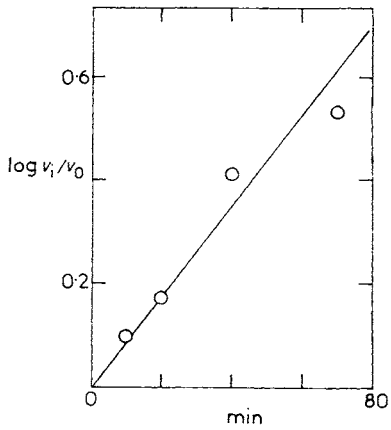


FIG. 1

Dependence of the inhibition of SAH-hydrolase from L-1210 cells on the time of non-enzymatic hydrolysis of compound *23a*. $t = T_0 + t_A$, t_0 preincubation time, t_A time of enzyme assay

stabilize the suitable conformation. Such a situation did occur with the trihydroxybutyl and tetrahydroxypentyl adenine derivatives[1].

It must be taken into account that the presence of the carboxyl in the molecule enhances the affinity toward SAH-hydrolase to such an extent that it prevails over the negative effect of substituents on the heterocyclic base (Table I). The high affinity of some compounds toward the enzyme is not due to the presence of any acid function, but specifically due to the carboxyl group. Phosphate esters, analogous to compounds *18* and *19* are completely inactive (data not shown). Moreover, the inhibition caused by the above derivatives is irreversible. Thus the interaction of ω-carboxyalkyl derivatives of adenine with the enzyme differs qualitatively from the interaction of neutral derivatives. If the decisive factor in the affinity of these carboxy derivatives for SAH-hydrolase is the binding of the carboxyl group to the active center (or its vicinity) of SAH-hydrolase, it is obvious that this carboxyl cannot participate in the intramolecular stabilization of the adenosine analogue (*vide supra.*) According to the current ideas about activation of the sugar component of adenosine prior to hydrolysis of SAH by SAH-hydrolase[22], the sugar hydroxyl groups would necessarily participate in the interaction with the enzyme. Since both the functional groups in the open-chain analogues are linked together by sp_3-hybridized carbon atoms, the hydroxyl groups in the side chain can eventually adopt orientation suitable for a cooperative interaction with the enzyme. Such conformational change would be independent of the absolute configuration of the α- and β-monohydroxy acids. For the eritadenines the vicinal diol is affected by intramolecular stabilization. Therefore, the affinity of such a molecule for the active site of SAH-hydrolase may depend on both relative and absolute configuration of the individual centers.

TABLE IV

Inhibition of SAH-hydrolase from L-1210 murine leukemia cells by selected compounds (hydrolytic reaction)

Formula	Configuration	Acid	v_i/v_0[a]
1a	2*R*,3*R*	4-(Adenin-9-yl)-2,3-dihydroxybutanoic (D-eritadenine)	0·21
6c	2*S*,3*S*,4*R*	5-(Adenin-9-yl)-2,3,4-trihydroxypentanoic	0·30
6e	2*S*,3*R*,4*R*		0·21
6h	2*R*,3*S*,4*R*		0·14
12a	*RS*	3-(Adenin-9-yl)-2-hydroxypropanoic	0
13	*RS*	3-(Adenin-9-yl)-2-hydroxybutanoic	0·31
15	3*S*	4-(Adenin-9-yl)-3-hydroxybutanoic	0·13

[a] $v_0 = 0{\cdot}84 \cdot 10^{-6}$ mol l^{-1} min^{-1}.

D-Eritadenine (*1a*) and its analogues (*12a*) not only inactivate the rat liver SAH-hydrolase but also SAH-hydrolase from L-1210 murine leukemia cells (Table IV), tobacco cell cultures[6] and insect ovaria[7].

Table V summarizes kinetic data estimated for several selected derivatives. ω-Carboxyalkyl derivatives of adenine, substituted in the base or in the side chain, act generally as irreversible enzyme inactivators with inactivation half-life times which do not markedly vary from one to another. Neither do these half-life times differ very much from the values for D-eritadenine and its geometric isomers[3]. Derivatives with modified carboxyl group (amides, esters) are either reversible competitive inhibitors (Table VI) or do not inhibit the enzyme at all. They behave thus as the neutral analogues[1], although they are less active than (*S*)-DHPA. It should be pointed out that the real K_i values of esters *20* and *23* should be lower than those indicated in Table VI, because of their partial hydrolysis to the respective free acids *1a* and *12a* during the enzymatic test.

Antiviral Effect

Within the group of substituted ω-carboxyalkyladenines the effect against vesicular stomatitis virus and vaccinia virus is limited only to the strongest SAH-hydrolase

TABLE V
Inactivation of rat liver SAH-hydrolase

Formula	Configuration	Compound	Synthesis		Hydrolysis
			IC_{50} mol l^{-1}	$t_{1/2}$, min	IC_{50} mol l^{-1}
1a	2*R*,3*R*	D-Eritadenine	$7{\cdot}5 \cdot 10^{-8}$	1·5	$7{\cdot}0 \cdot 10^{-9}$
1b	2*R*,3*R*	2-Amino-D-eritadenine	$9{\cdot}0 \cdot 10^{-7}$	1·5	$8{\cdot}0 \cdot 10^{-8}$
2	2*R*,3*R*	N^3-D-Eritadenine	$2{\cdot}0 \cdot 10^{-6}$	3·4	$1{\cdot}7 \cdot 10^{-7}$
5	2*R*,3*R*	5-(Adenin-9-yl)-2,3-dihydroxypentanoic acid	$1{\cdot}8 \cdot 10^{-6}$	2·0	$1{\cdot}1 \cdot 10^{-7}$
6a	2*S*,3*R*,4*R*	5-(Adenin-9-yl)-2,3,4-trihydroxypentanoic acid	$4{\cdot}0 \cdot 10^{-6}$	1·8	$1{\cdot}8 \cdot 10^{-7}$
12a	2*R*	3-(Adenin-9-yl)-2-hydroxypropanoic acid	$8{\cdot}9 \cdot 10^{-7}$	1·6	$5{\cdot}0 \cdot 10^{-8}$
12a	2*S*		$4{\cdot}2 \cdot 10^{-7}$	1·8	$2{\cdot}0 \cdot 10^{-8}$
12a	*RS*		$4{\cdot}7 \cdot 10^{-7}$	1·5	$3{\cdot}4 \cdot 10^{-8}$

inhibitors: in addition to D-eritadenine (*1a*) whose antiviral effect has been published previously[3], only the enantiomers (and the racemate) of 3-(adenin-9-yl)-2-hydroxypropanoic acid (*12a*) were found to be active. Their antiviral activity was demonstrated not only by the usual MIC_{50} determination (Tables I–III) but also by monitoring virus yield reduction in either human skin fibroblasts or primary rabbit kidney cells infected with vesicular stomatitis virus (Table VII). Under the given conditions, the (*S*)-enantiomer of compound *12a* decreased the virus titer by about 100 times. Also, the antiviral spectrum of the *12a* compounds was identical to that

TABLE VI

Inhibition constants (K_i) of reversible inhibitors of rat liver SAH-hydrolase (hydrolytic reaction)

Formula	Configuration	Compound	K_i, μmol l^{-1}	K_i/K_m[a]
-	2*S*	9-(2,3-Dihydroxypropyl)adenine	0·46	0·056
20a	2*S*,3*S*	L-Eritadenine methyl ester	4·50	0·54
20b	2*R*,3*R*	D-Eritadenine ethyl ester	0·65	0·078
21a	2*R*,3*R*	D-Eritadenineamide	1·50	0·18
23a	2*S*	3-(Adenin-9-yl)-2-hydroxypropanoic acid methyl ester	4·60	0·55

[a] $K_m^{SAH} = 8{\cdot}33 \cdot 10^{-6}$ mol l^{-1}.

TABLE VII

Effect of SAH-hydrolase inhibitors (100 μg/ml) on the growth of vesicular stomatitis virus in primary rabbit kidney (PRK) and human skin fibroblast (HSF) cells

Compound	Virus yield (log_{10} PFU/ml) at[a]					
	8 h		24 h		48 h	
	PRK	HSF	PRK	HSF	PRK	HSF
None	4·0	3·3	4·7	5·8	4·3	5·8
12a (2*S*)	2·2	1·8	2·3	3·9	3·2	3·9
12a (2*R*)	2·6	1·3	3·5	4·8	3·4	4·6
20a (2*S*,3*S*)	2·8	2·6	3·3	5·4	2·9	4·9
20b (2*R*,3*R*)	2·3	1·3	3·5	4·4	3·4	3·9

[a] PFU plaque forming units.

of (*S*)-DHPA and D-eritadenine (Table VIII). They were active against vesicular stomatitis virus, reovirus 1, parainfluenza type 3 virus and vaccinia virus but no effect was observed with coxsackie B-4, polio 1, sindbis and herpes simplex virus type 1. Rabies virus is also sensitive toward compounds of this type[25]: in cell cultures a 90% inhibition is achieved with D-eritadenine (*1a*) and the (*R*)-enantiomer of compound *12a* at a concentration of 5 μg/ml (about $2 . 10^{-5}$ mol l^{-1}); however, in mice infected with rabies virus neither peroral nor intravenous administration of *12a* proved therapeutically effective[26].

Those compounds that are active as antiviral agents show an inactivating effect on SAH-hydrolase (hydrolytic reaction) with IC_{50} values, ranging from $0{\cdot}3 . 10^{-8}$ to $5 . 10^{-8}$ mol ml l^{-1} (Table V). Weaker inhibitors (IC_{50} higher than $5 . 10^{-8}$ mol . $. l^{-1}$) have no antiviral activity under the given conditions. However, the extent of the antiviral activity does not correspond to the exquisitely high inhibitory potency of compounds *1a* or *12a* toward SAH-hydrolase; the reason for this discrepancy is undoubtedly the difficulty of the polar compounds to penetrate into the cells[3]. This process should be facilitated for derivatives with a modified carboxyl group. Actually, esters of both D-eritadenine (*20a*) and L-eritadenine (*20b*) show a higher antiviral activity than the parent compounds, and this antiviral spectrum is identical (Tables III and VIII). Even more marked is the antiviral activity of the 3-(adenin-

TABLE VIII

Antiviral activity (MIC_{50}, μg/ml) of selected compounds against[a] different viruses

Virus	Cell line	(*S*)-DHPA	*1a*[b]	(*S*)-*12a*	(*R*)-*12a*	(*S,S*)-*20a*	(*R,R*)-*20b*
Vesicular stomatitis	PRK	4	30	15	100	100	15
Vesicular stomatitis	HeLa	20	—	150	200	40	40
Reo-1	Vero	40	100	400	150	NA	>300
Parainfluenza-3	Vero	40	150	100	100	100	300
Sindbis	Vero	NA	NA	NA	NA	300	150
Coxsackie B-4	Vero	400	—	NA	NA	NA	NA
Coxsackie B-4	HeLa	NA	NA	NA	NA	400	NA
Polio-1	HeLa	NA	NA	NA	NA	200	NA
Vaccinia	PRK	20	70	40	200	70	30
Herpes simplex type 1 (strain KOS)	PRK	20-NA	NA	NA	NA	NA	NA

[a] NA not active (MIC_{50} > 400 μg/ml); [b] *cf.*[3].

-9-yl)-2-hydroxypropanoates: both the (*R*)- and (*S*)-enantiomers of *23a* have a strong inhibitory effect on vesicular stomatitis and vaccinia virus (Table III). We assume that the alkyl esters *20* and *23* penetrate more easily into the cells than their acidic precursors, and that once inside the cells they are hydrolyzed to give the parent acids *1a* and *12a*. Detailed information on the antiviral activity of these compounds (*23*), which *per se* are not inhibitors, or only weak inhibitors, of SAH-hydrolase, and of the effect of the alcohol moiety on the antiviral activity[27] of the esters will be described elsewhere[21].

The amides of *1a* and *12a* (compounds *21* and *28*) also have neutral character. Unlike the esters, these compounds are chemically stable. Among these amides, D-eritadenine amide (*21a*) exerts a weak activity against VSV and, concomitantly, has a weak inhibitory effect on SAH-hydrolase.

The increased ability of carboxylic acid esters to enter the cells, followed by the subsequent release of the active acid inside the cell contribute to the antiviral activity of the compounds. It may also have some undesirable consequences, *e.g.* a pronounced disturbance of transmethylation reactions. This effect may be potentiated by the catabolic stability and limited efflux of the acidic compounds into the medium. Therefore, the esters *20* and *23* may be more cytotoxic than the neutral derivatives of the (*S*)-DHPA type. This prediction has been borned out by a study of the antimitotic effect of these compounds on CER (chicken embryo-related) cells: the ethyl ester of D-eritadenine (*20b*) completely terminated cell mitosis after 24 hours' cultivation at a concentration of ≤ 25 μg/ml, whereas D-eritadenine (*1a*), compound (*S*)-*12a* and (*S*)-DHPA did so only at a concentration of 100 μg/ml[25]. Nevertheless, the ratio of the antimitotic concentration to antiviral concentration is favorable for all compounds. However, therapeutic usefulness of these compounds *in vivo*, their therapeutic index and pharmacokinetic behavior remain to be established.

All the compounds that were studied here are resistant toward adenosine aminohydrolase, are catabolically stable and do not serve as substrates of SAH-hydrolase in the SAH synthesis. In this respect, they differ from the regular nucleoside analogues[23], which in the organism can be transformed to SAH analogues and thus complicate the analysis of the subsequent biological processes. Nucleosides can also undergo phosphorylation followed by transformations leading to decomposition of the compound or synthesis of antimetabolites targeted at other enzymes. Therefore, (*S*)-DHPA, D-eritadenine and the easily accessible 3-(adenin-9-yl)-2-hydroxypropanoic acids and their esters are not only potential antiviral compounds but very useful tools for the study of the biological consequences of an inhibition of transmethylation reactions and other processes connected with the equilibrium state between S-adenosyl-L-methionine and S-adenosyl-L-homocysteine[24].

We are indebted to Dr K. Jošt for the kind gift of L-*homocysteine and to Dr A. Merta for* SAH-*-hydrolase from* L-1210 *murine leukemia cells. The excellent technical assistance of Mrs B. Miklová and Mrs B. Nováková is gratefully acknowledged.*

REFERENCES

1. Holý A., Votruba I., De Clercq E.: This Journal *50*, 245 (1985).
2. Votruba I., Holý A.: This Journal *47*, 167 (1982).
3. Holý A., Votruba I., De Clercq E.: This Journal *47*, 1392 (1982).
4. Votruba I., Holý A.: This Journal *45*, 3039 (1980).
5. Merta A., Votruba I., Veselý J., Holý A.: This Journal *48*, 2701 (1983).
6. Šebestová L., Votruba I., Holý A.: This Journal *49*, 1543 (1984).
7. Votruba I., Holý A., Sláma K.: Insect Biochem., in press.
8. Schanche J. S., Schanche T., Ueland P. M., Holý A., Votruba I.: Mol. Pharmacol. *26*, 553 (1984).
9. Okumura K., Matsumoto K., Fukamiru M., Yasuo H., Taguchi Y., Sugihara Y., Inoue I., Seto M., Sato Y., Takamura N., Kanno T., Kawazu M., Mizoguchi T., Saito S., Takashima K., Takayama S.: J. Med. Chem. *17*, 846 (1974).
10. Holý A.: Nucleic Acids Res. Symp. Ser. *11*, 199 (1982).
11. Holý A., Votruba I., Rosenberg I.: This Journal *48*, 2549 (1983).
12. Kawazu M., Kanno T., Yamamura S., Mizoguchi T., Saito S.: J. Org. Chem. *38*, 2887 (1973).
13. Holý A.: This Journal *47*, 2969 (1982).
14. Holý A.: This Journal *49*, 2148 (1984).
15. Holý A.: This Journal *43*, 3444 (1978).
16. Holý A.: This Journal *47*, 173 (1982).
17. Holý A.: This Journal *40*, 187 (1975).
18. Holý A.: This Journal *44*, 593 (1979).
19. Okumura K., Oina T., Yamada Y., Tomie M., Adachi T., Nagura T., Kawazu M., Mizoguchi T., Inoue I.: J. Org. Chem. *36*, 1573 (1971).
20. Holý A.: This Journal *47*, 2786 (1982)
21. De Clercq E., Holý A.: J. Med. Chem., in press.
22. Palmer J. L., Abeles R. H.: J. Biol. Chem. *254*, 1217 (1979).
23. Zimmerman T. P., Wolberg G., Duncan G. S., Elion G. B.: Biochem. *19*, 2252 (1980).
24. Daubner S. C., Krapf E., Matthews R. K. in the book: *Biochemistry of* S-*Adenosylmethionine and Related Compounds* (E. Usdin, R. T. Borchardt, C. R. Creveling, Eds), p. 617. Macmillan Press, London 1982.
25. Bussereau F., Chermann J. C., De Clercq E., Hannoun C.: Ann. Virol. *134E*, 127 (1983).
26. Sodja I.: Unpublished results.
27. Holý A., De Clercq E.: Czech. Appl. 7380—73.

Translated by M. Tichý.