SYNTHESIS AND ANTIVIRAL ACTIVITY OF STEREOISOMERIC ERITADENINES*

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D-Eritadenine (*Ia*) and L-eritadenine (*IIa*) were prepared from 5-(adenin-9-yl)-5-deoxyaldofuranoses or enantiomeric 2,3-disubstituted erythronolactones (*VIIIb, c, XIV*). Oxidation of methyl 2,3-O-isopropylidene-D-ribofuranoside (*IX*) with periodate in the presence of ruthenium, followed by acid hydrolysis and reduction with sodium borohydride, afforded L-ribonolactone (*XI*). Its 2,3-O-isopropylidene derivative was subjected to alkaline hydrolysis, followed by oxidation with periodate, reduction with sodium borohydride and reaction with cyclohexanone to give 2,3-O-cyclohexylidene-L-erythronolactone (*XIV*). Condensation of [U-^{14}C]-adenine with *VIIIb*, followed by acid hydrolysis, afforded [U-^{14}C-adenine]-D-eritadenine. The *threo*-eritadenines *III* and *IV* were prepared by oxidation of 1-(adenin-9-yl)-1-deoxy-2,3-O-isopropylidenethreitols *XVI* and *XVII* with sodium periodate in the presence of ruthenium, followed by acid hydrolysis. Reaction of 9-(2,2-diethoxyethyl)adenine (*XIX*) with malonic acid gave 4-(adenin-9-yl)-3-butenoic acid (*XXI*); its methyl ester *XXII*, prepared by treatment with methanol, was isomerized with triethylamine to give methyl 4-(adenin-9-yl)-2-butenoate (*XXIII*). Hydroxylation of *XXIII* with osmium tetroxide afforded the racemic mixture of D- and L-*threo*-eritadenine (*III* + *IV*). Eritadenines *Ia* and *IIa* were active against vaccinia, measles and vesicular stomatitis virus. Eritadenine *Ia* was also effective against reo- and parainfluenza virus. In general, the antiviral activity of the eritadenines decreased in the order D-*erythro* (*Ia*) > > L-*erythro* (*IIa*) ≫ D- and L-*threo* (*III, IV*).

In the scope of our studies of the structure–function relationship of aliphatic adenosine analogues as S-adenosyl-L-homocysteine hydrolase inhibitors we have found the natural D-eritadenine ((2*S*,3*S*)-4-(adenin-9-yl)-2,3-dihydroxybutanoic acid, *Ia*) to be an extraordinarily potent inhibitor of this enzyme[1]. We have now evaluated the influence of structural changes in the 9-(ω-carboxyalkyl)adenine series. This paper concerns the preparation and antiviral properties of the four stereoisomeric 4-(adenin-9-yl)-2,3-dihydroxybutanoic acids *Ia*–*IV*.

Since the natural D-eritadenine (*Ia*) – the active component isolated from the edible Japanese mushroom *Lentinus edodes shiitake*[2] – exhibits a marked hypocholesterolemic effect[3], its synthesis and properties have been studied intensively,

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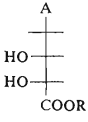
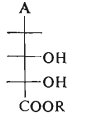
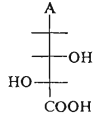
mainly by Japanese authors (for a review see ref.[4]). Stereospecific syntheses of both *erythro*-derivatives *Ia* and *IIa* start from 5-(adenin-9-yl)-5-deoxy-D-ribofuranose (*Vb*), -L-arabinofuranose (*VIb*) or its D-enantiomer. Oxidation of these compounds in an alkaline medium affords, among other compounds, the compounds *Ia* or *IIa*. However, this procedure does not yield pure products: the compounds must be purified by transformation into the alkyl esters (ref.[5,6]), hydrolysis and rechromatography of the recovered compound *Ia* or *IIa* on a strongly basic anion exchanger. Using HPLC analysis one can detect degradation products of sugar derivatives; this holds also for N^3-eritadenine (*VII*) which arises from the isomer of compounds *V* and *VI* and which is difficult to remove. Pure compounds *Ia* and *IIa* can be prepared in this way only with considerably low yield.



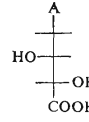
Ia, R = H
Ib, R = C_2H_5



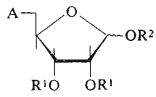
IIa, R = H
IIb, R = CH_3



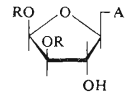
III



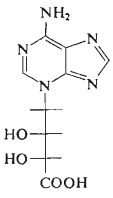
IV



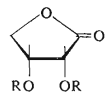
Va, $R^1, R^1 = (CH_3)_2C$; $R^2 = CH_3$
Vb, $R^1 = R^2 = H$



VIa, R,R = $(CH_3)_2C$
VIb, R = H



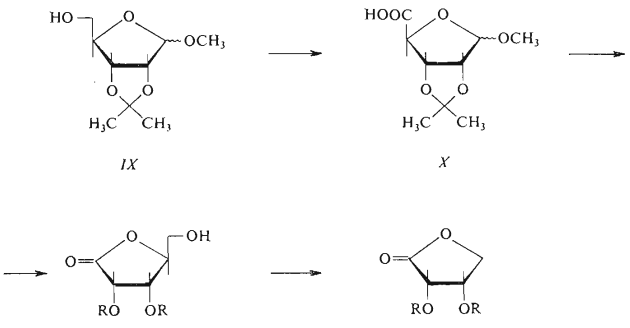
VII



VIIIa, R = H
VIIIb, R = $-(CH_2)_6-$
VIIIc, R = $(CH_3)_2C$

In formulae *I*–*VIII*: A = adenin-9-yl residue

An alternative method of preparation of the *erythro*-isomers *Ia* and *IIa* consists of condensation of sodium salt of adenine with 2,3-O-substituted erythronolactone[6]; although this synthesis requires a multiple-step preparation of the starting material, purification of the products is easier. We prepared both the enantiomeric eritadenines *Ia* and *IIa* by a modification of this method. The starting L-erythronolactone derivative was obtained by a novel procedure from methyl 2,3-O-isopropylidene-D-ribofuranoside (*IX*) (Scheme 1). This compound was oxidized with sodium periodate in 70% acetone in the presence of ruthenium ions to give 1-O-methyl-2,3-O-isopropylidene-D-riburonic acid (*X*) which on acid hydrolysis and reduction of the aldehyde function with sodium borohydride afforded L-ribonolactone (*XI*) and its 2,3-O-isopropylidene derivative *XII*. Compound *XII* was transformed into L-erythronolactone (*XIII*) and its 2,3-O-cyclohexylidene derivative *XIV* by saponification, degradation with periodate and reduction with sodium borohydride, using the modified methods, described for the D-enantiomer[6,7] (Scheme 1). Compound *XIV* was



IX *X*

XI, R = H
XII, R,R = $(CH_3)_2C$

XIII, R = H
XIV, R = $-(CH_2)_6-$

SCHEME 1

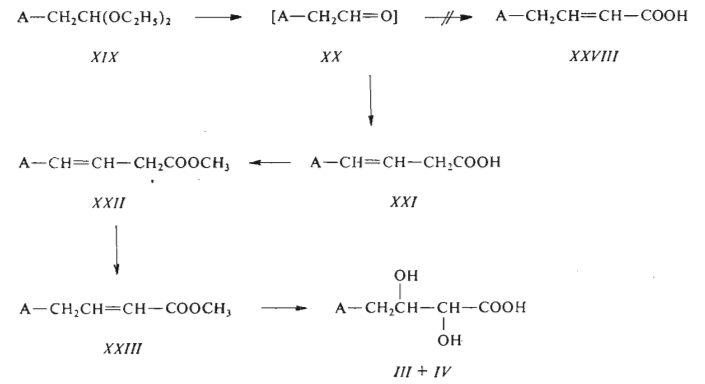
condensed in dimethylformamide with sodium salt of adenine under similar conditions as described for the D-enantiomer *VIIIb*. The chromatographically (HPLC) pure product was obtained only after the above mentioned esterification to *IIb* (ref.[8]), saponification and chromatography on strongly basic anion exchange resin in acetic acid or formic acid. When the reaction of *VIIIc* or *XIV* was carried out in dimethyl sulfoxide, it proved advantageous to add compound *VIIIc* to a mixture of adenine and potassium carbonate in dimethyl sulfoxide at elevated temperature. Under these conditions both the decomposition of compound *VIIIc* and formation of iso-

meric side-products could be prevented. After acid hydrolysis of the protecting group, pure D-eritadenine (*Ia*) was obtained by chromatography on anion exchange resin.

We have also used this modification in the preparation of D-eritadenine, labelled with radionuclide ^{14}C in the adenine ring. In the presence of dimethyl sulfoxide and excess of the lactone *VIIIb* we obtained a significant conversion of the labelled adenine. The isomeric compound *VII* was separated by combination of paper chromatography and electrophoresis. The desired [U-^{14}C-adenine]-D-eritadenine (*Ia*) was obtained in high radiochemical purity and high total yield. The only preparation of the labelled compound *Ia* that has been described so far[9] consisted in a closure of the imidazole ring of the acyclic intermediate by treatment with ^{14}C-formic acid; this method was therefore limited to synthesis of [8-^{14}C]-D-eritadenine of low specific activity. On the other hand, our procedure allows to prepare compound of high specific activity, and is limited only by the maximum obtainable specific activity of adenine.

The known[10] *threo*-(2*S*,3*R*)-isomer *III* was obtained by oxidation of 5-adenin-9-yl-5-deoxy-D-xylofuranose (*XV*); the starting material is not readily available and the method suffers from the same drawbacks as the analogous preparation of the *erythro*-isomers from compounds *V* and *VI*. The enantiomeric (2*R*,3*S*)-derivative *IV* was prepared by a seven-step procedure consisting in the synthesis of the adenine ring from (2*R*,3*S*)-4-amino-2,3-dihydroxybutanoic acid[10]. Our stereospecific synthesis of compounds *III* and *IV* was realized by oxidation of enantiomeric 1-(adenin-9-yl)-1-deoxy-2,3-O-isopropylidenethreitols *XVI* and *XVII* with sodium periodate in the presence of ruthenium. The starting compounds were easily accessible[8] from tartaric acid and D-mannitol, respectively. After removal of the dioxolane protecting group by acid hydrolysis, the pure reaction products *III* and *IV* were obtained in high yield by chromatography on an anion exchange resin.

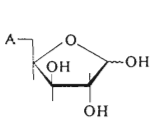
Alternatively, the racemic eritadenines *Ia* + *IIa* or *III* + *IV* could also be obtained by hydroxylation of the corresponding 4-adenin-9-yl-2-butenoic acid (*XVIII*). This compound should result from the condensation of adenin-9-ylacetaldehyde (*XX*) with malonic acid. Compound *XX* which was generated by mild hydrolysis of the acetal *XIX* (*cf.*[11]) was unstable and underwent autocondensation even in neutral medium. Nevertheless, condensation of compound *XX* with malonic acid in pyridine, employed usually for such reactions, afforded as the sole reaction product the isomeric 4-(adenin-9-yl)-3-butenoic acid (*XXI*) instead of the desired derivative *XVIII*. The same compound was formed in high yield directly from the acetal *XIX* and malonic acid in aqueous solution (Scheme 2). The structure of compound *XXI* was confirmed by elemental analysis, UV-spectrum (characteristic maximum at 236 nm and shoulder at 260 nm in acid medium), and its reaction with potassium permanganate or chlorate and osmium tetroxide in neutral or acidic solutions: the compound was degraded to adenine as the only product (the 3,4-dihydroxy derivative being the likely intermediate). Obviously, thermodynamic control of the reaction, combined with the



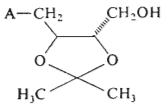
In formulae *XVIII*–*XXIII*: A = adenin-9-yl residue

SCHEME 2

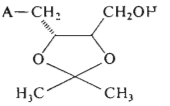
extraordinary insolubility of the acid *XXI* in water, explains why compound *XXI* was formed instead of its isomer *XVII*.



XV



XVI



XVII

A—CH_2COOR

XXVa, R = H
XXVb, R = C_2H_5
XXVc, R = CH_3

XXVIa, R = H
XXVIb, R = C_2H_5

XXVII

XXIV

In formulae *XV*–*XXVII*: A = adenin-9-yl residue

Acid-catalyzed esterification of the compound *XXI* with methanol afforded the ester *XXII* (with UV-spectrum corresponding to the starting acid) which on treat-

ment with triethylamine in anhydrous dioxane slowly isomerized into the ester *XXIII*, whose UV-spectrum (λ_{max} 262 nm in acid medium) corresponded to an isolated adenine chromophore. Hydroxylation of the ester *XXIII* with osmium tetroxide in the presence of sodium chlorate, followed by alkaline hydrolysis, afforded eritadenine which, according to HPLC analysis, was a racemic mixture of the *threo*-isomer *III* + *IV* (Scheme 2). This result indirectly confirmed the structure of compound *XXIII* as well as its configurational (*trans*) homogeneity. However, due to the difficult isomerization of *XXII* → *XXIII*, this procedure for the preparation of eritadenines is not very practical.

All the eritadenines were obtained in purity higher than 99% as found by HPLC analysis, paper chromatography and electrophoresis.

Compounds *Ia*, *Ib*, *IIa*, *IIb*, *III*, *IV* and *XXI*–*XXIII* were tested for antibacterial activity against *Escherichia coli* B. Under standard conditions (synthetic medium with glucose) neither of the afore mentioned compounds exhibited a significant effect at a concentration of 100 μg/ml.

Recently, we have reported that there is a correlation between the antiviral activity and inhibition of S-adenosyl-L-homocysteine (SAH) hydrolase for a series of neutral open-chain analogues of adenosine[12,13]. This finding suggests that the inhibition

TABLE I
Comparative antiviral activity of eritadenines against different viruses

Virus	Cell culture	Minimum inhibitory concentration[a], μg/ml				
		Ia	*IIa*	*III*	*IV*	*XXIV*
Vesicular stomatitis	PRK	30	70	400	300	10
Vesicular stomatitis	HSF	40	150	400	400	10
Vaccinia	PRK	70	200	400	400	70
Vaccinia	HSF	100	200	400	400	40
Herpes simplex 1 (KOS)	PRK	>400	>400	—	—	40–>400[b]
Newcastle disease	HSF	>400	—	—	—	>400
Coxsackie B4	HeLa	>400	—	—	—	>400
Polio I	HeLa	>400	—	—	—	>400
Sindbis	Vero	>400	—	—	—	>400
Measles	Vero	10–70[c]	40	40	200	10–40[c]
Parainfluenza 3	Vero	150	>400	>400	>400	50
Reo 1	Vero	100	>400	>400	>400	50

[a] Required to reduce virus-induced cytopathogenicity by 50%; [b] range of values obtained with different strains of herpes simplex 1 virus strains; [c] range of values obtained in different assays.

of SAH hydrolase may pertain to the mode of action of the aliphatic adenosine analogues. A similar explanation for the antiviral activity of some other analogues of adenosine and SAH has been proposed earlier[14-16]. Since eritadenines (*Ia*, *IIa*, *III*, *IV*) are extraordinarily potent inhibitors of SAH hydrolase[1], these compounds were examined for their antiviral properties. The experiments were performed in cell cultures inoculated with various viruses, according to procedures that have been described earlier[17].

Table I comprises data on the antiviral activity of eritadenines and 9-(*S*)-(2,3-dihydroxypropyl)adenine ((*S*)-DHPA, *XXIV*). In analogy with the inactivation of SAH-hydrolase[1], the antiviral activity of the eritadenines decreased in the order of D-*erythro*(2*S*,3*S*) (*Ia* > L-*erythro* (2*R*,3*R*) (*II*) ⩾ *threo*(2*R*,3*S*) (*IV*) ~ *threo*(2*S*,3*R*) (*III*). D-Eritadenine(*Ia*), the most active congener of the present series, resembled (*S*)-DHPA both by its distinct antiviral activity against vesicular stomatitis virus, parainfluenza virus, reo virus, measles virus and vaccinia virus, and lack of activity against Polio I, Coxsackie, Sindbis and Newcastle disease virus, on the one hand, and by its negligible cytotoxicity at the highest concentration tested (400 μg/ml), on the other hand. The antiviral activity of L-eritadenine (*IIa*) was lower than that of its D-enantiomer *Ia* (*i.e. IIa* was ineffective against parainfluenza and reo virus), whereas the *threo*-isomers *III* and *IV* could be regarded as inactive for all viruses assayed, except for compound *III versus* measles virus.

These findings were confirmed by experiments where inhibition of virus yield rather than inhibition of viral cytopathogenicity was monitored as a parameter of antiviral activity. As could be clearly established with the virus titres obtained at 24 h after

TABLE II

Effect of eritadenines on multiplication of vesicular stomatitis virus (VSV) in primary rabbit kidney (PRK) cell cultures

Compound[a]	Virus yield ($\log_{10}$ PFU/ml) at			
	1 h	8 h	24 h	48 h
Control	1·8	3·4	5·1	4·9
Ia	1·3	2·8	4·2	4·2
IIa	1·3	3·5	4·7	4·4
III	1·6	3·4	5·2	4·8
IV	1·3	3·5	5·1	5·0
XXIV	1·3	2·3	3·5	3·6

[a] Concentration 100 μg/ml.

virus inoculation, the order of (decreasing) antiviral activity was *XXIV* > D-*erythro* (*Ia*) > L-*erythro* (*IIa*) > D-*threo* (*IV*) ~ L-*threo* (*III*) (Table II).

The antiviral activity of the eritadenines *Ia* and *IIa* strongly depended on the nature of the host cell line used. For example, a more marked antiviral effect was obtained in diploid fibroblast cell cultures (PRK, HSF) than in heteroploid tumor cell lines (HeLa, L-929). This, again, corresponded to the behaviour of (*S*)-DHPA (Table III).

In contrast to the neutral open-chain analogues which are reversible inhibitors of SAH hydrolase[13], eritadenines inactivate the enzyme irreversibly[1]. Consequently, it would seem difficult to establish a quantitative correlation between the antiviral and enzyme-inhibitory effects for this group of compounds. The *erythro*-isomers, and in particular compound *Ia*, are almost as active but not more active than (*S*)-DHPA. Yet, their activity as SAH hydrolase inhibitors exceeds that of (*S*)-DHPA by 3–4 orders of magnitude[1]. Thus, SAH hydrolase inhibition may not be the only mechanism by which eritadenines and (*S*)-DHPA exert their antiviral action. Other mechanisms include an inhibition of protein kinase[18]. Furthermore, the zwitterionic character of eritadenine (or, rather, their anionic character at physiological pH) would probably diminish their penetration into the host cells, thereby decreasing their antiviral potency. A proper comparison of the biological activities or (*S*)-DHPA and eritadenines should be based upon the actual concentrations achieved within the cells.

TABLE III

Comparative antiviral activity of eritadenines in different cell cultures inoculated with vesicular stomatitis virus

Cell culture	Minimum inhibitory concentration[a] (μg/ml)		
	Ia	*IIa*	*XXIV*
PRK	4	30	4
HSF (VGS)	30	100	10
HSF (T-21)	30	70	10
E_1SM	40	100	10
L-929	100	300	20
BS-C-1	150	400	40
Feline lung	25	35	10
HeLa	400	—	400

[a] Required to reduce virus-induced cytopathogenicity by 50%.

EXPERIMENTAL

Unless stated otherwise, the solutions were taken down at 40°C/2 kPa and the compounds were dried over phosphorus pentoxide at 13 Pa. Melting points were determined on a Kofler block and are uncorrected. Paper chromatography was carried out in a descending arrangement on a paper Whatman No 1 or No 3 MM in the systems S1 2-propanol–concentrated aqueous ammonia–water (7 : 1 : 2), S2 1-butanol–acetic acid–water (10 : 1 : 3). Thin layer chromatography on silica gel was carried out on Silufol UV 235 sheets (Kavalier, Czechoslovakia) in the systems S3 benzene–ethyl acetate (7 : 3), S4 chloroform, S5 chloroform–methanol (4 : 1). Paper electrophoresis was performed on a paper Whatman No 3 MM at 20 V/cm in the buffers E1 0·1M triethylammonium hydrogen carbonate (pH 7·5), E2 0·01M formic acid. HPLC analyses were carried out on a Separon SI C18 column (250 × 4 mm, 2 ml/min) in the system H1 0·1M ammonium hydrogen phosphate and 0·002M 11-aminoundecanoic acid (pH 3·95) (ref.[19]). Elution times (min): *Ia*, *IIa* 10·4, *III*, *IV* 13·0, *VII* 3·7, *XXVa* 12·0, *XXVIa* 13·3. UV spectra were taken on a Specord UV-VIS instrument (Carl Zeiss, Jena, G.D.R.) in aqueous solutions.

Antiviral Assays

Inhibition of virus-induced cytopathogenicity (Tables I and III) was evaluated in PRK (primary rabbit kidney) cells, HSF (human skin fibroblasts) disomic for chromosome-21 (VGS) or trisomic for chromosome-21 (T-21), human embryonic skin muscle (E_1SM) fibroblasts, mouse L-929 fibroblasts, Vero cells (derived from African green monkey kidney), BS-G-1 cells (also derived from African green monkey kidney), feline lung fibroblasts or HeLa cells (the latter five being continuous cell lines). When grown to confluency in Sterilin microtiter trays, the cell cultures were inoculated with 100 $CCID_{50}$ of either VSV (vesicular stomatitis virus) or any of the other viruses indicated in Table I, one $CCID_{50}$ being the virus dose required fo infect 50% of the cell cultures. After 1 h of virus adsorption, residual virus was removed and the cell cultures were incubated with maintenance medium (Eagle's minimal essential medium supplemented with 3% calf serum) containing varying concentrations of the test compounds (400, 200, 100, ... μg/ml). Virus-induced cytopathogenicity was recorded as soon as it reached completion in the control virus-infected cell cultures. The antiviral activity is expressed as the minimum inhibitory concentration of compound required to reduce viral cytopathogenicity by 50%.

Inhibition of virus multiplication (Table II) was assessed in PRK cells grown to confluency in 50 mm Falcon plastic petri dishes. The cells were inoculated with $10^{4.5}$ $CCID_{50}$ of VSV per petri dish, and, after 1 h of virus adsorption, residual virus was removed and replaced by maintenance medium containing 100 μg/ml of the test compounds. The cells were incubated for 1, 8, 24 or 48 h, and after one cycle of freeze-thawing the virus content of the cell homogenates was determined by plaque-formation in mouse L-929 fibroblasts. The virus titre is expressed in PFU (plaque forming units) per ml.

5-(Adenin-9-yl)-5-deoxy-1,2-O-isopropylidene-L-arabinofuranose (*VIa*) (*cf.* ref.[20])

A mixture of adenine (40·5 g; 0·3 mol), potassium carbonate (41·4 g; 0·3 mol), 5-O-*p*-toluenesulfonyl-1,2-O-isopropylidene-L-arabinofuranose[20] (97·8 g; 0·3 mol) and dimethylformamide (350 ml) was stirred at 140°C for 15 h under exclusion of moisture, taken down *in vacuo* and codistilled with toluene (2 × 100 ml). The residue was extracted with boiling methanol (2 l), filtered, the solid washed with methanol (100 ml) and the filtrate concentrated *in vacuo* to 1 l. Crystallization in a refrigerator afforded 45·2 g (49%) of the compound *VIa*, identical with the product prepared according to ref.[20]. The D-enantiomer of *VIa* was prepared analogously in 52% yield.

2,3-O-Isopropylidene-L-ribonolactone (*XII*)

To a solution of D-ribose (50 g; 0·33 mol) in methanol (1 l) was added concentrated sulfuric acid (5 ml) at 0°C and the mixture was set aside at 4°C overnight. Calcium carbonate (100 g) was added, the suspension was stirred until it was neutral and was filtered through Celite. The filtrate was taken down *in vacuo* and the residue taken up in a mixture of acetone (600 ml) and triethyl orthoformate (80 ml). A solution of 6M-HCl in dimethylformamide (2 ml) was added, the mixture was set aside overnight, made alkaline with triethylamine and taken down *in vacuo*. The residue was mixed with water (300 ml) and extracted with ether (5 × 100 ml), the extract dried over magnesium sulfate and taken down *in vacuo*. Distillation of the residue afforded 51·1 g (76%) of the product *IX*, b.p. 96—102°C/13 Pa; R_F 0·43 (S3). (Reported[21] b.p. 88—89°C/78 Pa, R_F 0·50).

A solution of the compound *IX* (40·8 g; 0·2 mol) in 70% acetone was added to a stirred solution of sodium periodate (85·6 g; 0·4 mol) in 70% acetone (1 l), followed immediately by a solution of ruthenium oxychloride (1 ml, 20 mg of Ru). The mixture was stirred without cooling until the reaction was complete (thin layer chromatography in S3; R_F of *X* 0·28). The precipitate was filtered and washed with acetone (200 ml). Acetone was removed *in vacuo* from the filtrate and the remaining solution was treated with barium acetate dihydrate (13·7 g; 50 mmol) in water (50 ml). The precipitate was filtered through Celite and the filtrate applied on a column of Dowex 50 X 8 (H^+-form; 700 ml). The column was washed with water until the eluate conductivity dropped (2 l). The eluate was taken down *in vacuo*, the residue codistilled with water (2 × 100 ml), dissolved in water (200 ml) and set aside at room temperature overnight. The mixture was neutralized with sodium hydroxide to pH 7·2—7·4 and evaporated *in vacuo*. The residue was dissolved in water (200 ml), cooled with ice to 0°C and a solution of sodium borohydride (7·6 g; 0·2 mol) in water (100 ml) was added dropwise with stirring so as the temperature did not exceed 20°C. After stirring for 2 h in an ice-bath the mixture was neutralized by adding Dowex 50 X 8 (H^+-form). The resin was removed by filtration, washed with water (100 ml), and the filtrate was applied on a column (700 ml) of the same type of Dowex. After elution with water (2 l) the eluate was taken down *in vacuo*, the residue codistilled with dioxane (2 × 100 ml) and taken up in acetone (200 ml). The solution was filtered, the solid washed with acetone (50 ml) and the filtrate again taken down *in vacuo*. The residue was dried by successive codistillation with dioxane and toluene and finally over phosphorus pentoxide overnight. The obtained oil was stirred for 20 h with a mixture of acetone (400 ml), anhydrous copper sulfate (100 g) and concentrated sulfuric acid (2 ml), filtered, the solid washed with acetone (100 ml) and the filtrate stirred with silver carbonate (40 g) for 1 h until neutral. After filtration and washing the solid with acetone (200 ml), the filtrate was taken down *in vacuo*. The residue was extracted with ether (200 ml), the extract dried over magnesium sulfate and again taken down. Crystallization from ether afforded 18·6 g (49·5% from *IX*) of compound *XII*, m.p. 68—69°C, $[\alpha]_D^{20}$ +77·0° (*c* 0·5, chloroform).

2,3-O-Cyclohexylidene-L-erythronolactone (*XIV*)

A solution of the compound *XII* (22·6 g; 0·12 mol) and sodium hydroxide (4·8 g; 0·12 mol) in water (200 ml) was set aside overnight and neutralized (pH 7·0—7·1) by addition of Dowex 50 X 8 (H^+). The resin was filtered, the filtrate made up to 250 ml, mixed with acetone (700 ml) and the mixture cooled with ice. A solution of sodium periodate (26·1 g; 0·122 mol) in water (50 ml) was added with stirring which was continued for 2 h (ice bath). The mixture was filtered and the solid washed with acetone (200 ml). Acetone was removed *in vacuo* and the ice-cooled aqueous residue was treated with sodium borohydride (2·5 g; portionwise) so as the temperature did not exceed 10°C. The mixture was stirred for 1 h at 0°C, acidified with Dowex 50 X 8 (H^+-form), filtered, the resin washed with water (100 ml) and the filtrate passed through a column

(500 ml) of the same ion exchange resin. The column was washed with water (1 l), the filtrate taken down *in vacuo*, the residue taken up in acetone (200 ml), filtered, the solid washed with acetone, the filtrate taken down *in vacuo* again and the residue dried over phosphorus pentoxide *in vacuo* overnight. The obtained compound *XIII* (15·6 g; R_F 0·20 in S4) was refluxed with a mixture of benzene (150 ml), cyclohexanone (13·2 g) and *p*-toluenesulfonic acid monohydrate (0·7 g) with simultaneous azeotropic removal of water. After 4 h the mixture was cooled down, diluted with benzene (100 ml), washed twice with saturated sodium hydrogen carbonate solution and twice with water (50 ml portions), dried over magnesium sulfate, filtered, the filtrate taken down *in vacuo* and the residue mixed with light petroleum (100 ml) to induce crystallization. The separated product was collected on filter, washed with light petroleum and crystallized from cyclohexane, affording 7·4 g (28% from *XII*) of compound *XIV*, m.p. 76–77°C. For $C_{10}H_{14}O_4$ (198·2) calculated: 60·59% C, 7·12% H; found: 61·02% C, 6·75% H. $[\alpha]_D^{20}$ +108·2° (*c* 1·0, chloroform); R_F 0·40 (S4).

2,3-O-Cyclohexylidene-D-erythronolactone (*VIIIb*)

Prepared similarly to compound *XIV* from D-ribonolactone according to ref.[6]; m.p. 78°C (cyclohexane), $[\alpha]_D^{20}$ −107·6° (*c* 1·0, chloroform), R_F 0·40 (S4).

2,3-O-Isopropylidene-D-erythronolactone (*VIIIc*)

Prepared in 52% yield according to ref.[7] similarly as described for compound *XII*; m.p. 68°C (reported[7] m.p. 68–68·5°C; $[\alpha]_D^{20}$ −77·3° (*c* 0·5; chloroform).

D-Eritadenine (*Ia*)

A) *From* Va: A solution of the compound *Va* (6·4 g; 20 mmol) in 0·2M sulfuric acid (100 ml) was heated to 75°C for 2 h, neutralized with saturated barium hydroxide solution, heated to 80°C, filtered through Celite, washed with water and the filtrate made up to 1 l. Sodium hydroxide (2·4 g; 60 mmol) was dissolved in this solution and the mixture was stirred under oxygen (100 kPa overpressure) for 48 h. The mixture was neutralized with Dowex 50 X 8 (H^+-form), filtered, the resin washed with water and the filtrate concentrated *in vacuo* to 300 ml. After adjusting to pH 9 with ammonia, the solution was applied on a column of Dowex 1 X 2 (100–200 mesh, acetate form; 500 ml) which was then washed with water until the UV absorption disappeared. The column was then eluted with dilute acetic acid (0–1M, linear gradient, 2 l each) and the UV-absorbing fractions were analyzed by HPLC (system H1). The pertinent fractions were combined, taken down *in vacuo*, the residues codistilled with water (3 × 20 ml) and crystallized from water. Fraction, obtained by elution with 0·6–0·7M acetic acid, afforded 50 mg of the N^3-isomer *VII*, not melting below 260°C, $[\alpha]_D^{20}$ +43·2° (*c* 0·5, 1M-HCl). UV spectrum (pH 2): λ_{max} 275 nm (ε_{max} 16 500) (pH 12): λ_{max} 275 nm (ε_{max} 12 000). For $C_9H_{11}N_5O_4$ (253·2) calculated: 42·68% C, 4·38% H, 27·66% N; found: 41·70% C, 4·56% H, 27·22% N. R_F 0·35 (S1), 0·40 (S2), E_{Up} = 0·45 (E1). Fraction 0·8–1M afforded 2·45 g (48%) of compound *Ia*, not melting below 260°C, $[\alpha]_D^{20}$ +15·6°(*c* = 0·5, 1M-HCl). UV-spectrum (pH 2,12): λ_{max} 261 nm (ε_{max} 14 500). For $C_9H_{11}N_5O_4$ (253·2) calculated: 42·68% C, 4·38% H, 27·66% N; found: 42·52% C, 4·42% H, 26·99% N. R_F = 0·35 (S1), 0·50 (S2), E_{Up} = 0·45 (E1). Further elution with 1M acetic acid afforded 0·2 g of a mixture of compound *Ia*, *XXVa* and *XXVIa*.

B) *From* Va via *ester* Ib: The compound *Va* (20 mmol) was processed in the same way as described under *A*) and applied on a column of Dowex 1 X 2 (acetate form; 250 ml). After washing the column with water until the UV absorption disappeared, the Dowex was stirred with 2M

formic acid (1 l) for 1 h, filtered and washed with boiling water (1 l). The filtrate was taken down *in vacuo*, the residue codistilled with ethanol (3 × 50 ml) and dried *in vacuo* overnight. The residue was refluxed under stirring with a mixture of ethanol (150 ml) and concentrated sulfuric acid (1·5 ml) for 10 h, cooled with ice, neutralized with Amberlite IR 45 (pre-washed with ethanol), filtered, the solid washed with ethanol and the filtrate taken down *in vacuo*. The residue was chromatographed on a column of silica gel (according to Pitra, 30—50 mesh, 300 g) and the material eluted with an ethanol–chloroform mixture. The pertinent fractions (analyzed by thin-layer chromatography in S5) were combined and the residues crystallized from ethanol–light petroleum. Elution with chloroform–ethanol (95 : 5) afforded 0·10 g (2%) of the ester *XXVb*, R_F 0·43 (S5), $[\alpha]_D^{20}$ 0° (*c* 0·5, methanol), m.p. 234—235°C. For $C_9H_{11}N_5O_2$ (221·2) calculated: 48·86% C, 5·01% H, 31·66% N; found: 48·28% C, 4·97% H, 31·79% N. UV-Spectrum (pH 2,12): λ_{max} 260 nm (ε_{max} 12 500). Elution with chloroform–ethanol (92·5 : 7·5) afforded 0·42 g (8%) of the ester *XXVIb*, R_F 0·32 (S5), $[\alpha]_D^{20}$ +3·5° (*c* 0·5, methanol), m.p. 167—169°C. UV-Spectrum (pH 2,12): λ_{max} 260 nm (ε_{max} 13 000). For $C_{10}H_{13}N_5O_3$ (251·2) calculated: 47·80% C, 5·21% H, 27·88% N; found: 48·25% C, 5·34% H, 27·15% N. Elution with chloroform–ethanol (9 : 1) gave 2·2 g (39%) of compound *Ib*, m.p. 92—93°C; R_F 0·20 (S5), $[\alpha]_D^{20}$ +24·3° (*c* 0·5, methanol). UV-Spectrum (pH 2,12): λ_{max} 260 nm (ε_{max} 14 500). For $C_{11}H_{15}N_5O_4$ (281·3) calculated: 46·97% C, 5·38% H, 24·90% N; found: 47·18% C, 5·23% H, 24·64% N. A solution of the compound *Ib* (1·0 g; 3·6 mmol) in 0·05M sodium hydroxide (100 ml) was set aside overnight, neutralized with Dowex 50 X 8 (H^+-form), filtered, the resin washed with water and the filtrate concentrated *in vacuo* to about 20 ml. This solution was applied on a column of Dowex 1 X 2 (acetate form; 100 ml) and eluted with dilute formic acid (linear gradient 0—1M, 2 l each). The eluate from 0·1—0·2M acid afforded the pure (HPLC) product which after evaporation and codistillation with water was crystallized from water, affording 0·86 g (95·5%) of compound *Ia*, identical (in systems E1, H1, S1 and S2) with the product prepared according to the procedure *A*); $[\alpha]_D^{20}$ +15·4° (*c* 0·5, 1M-HCl).

C) *From compound* VIIIb (cf. ref.[6]). Sodium hydride (0·3 g; 12·5 mmol) was added to a suspension of adenine (1·7 g; 12·5 mmol) in dimethylformamide (50 ml) and the mixture was stirred at 100°C for 3 h. Compound *VIIIb* (2·4 h; 12 mmol) was then added, the mixture stirred at 140°C for 12 h under exclusion of moisture and taken down *in vacuo*. The residue was heated with 0·25M sulfuric acid (100 ml) to 60°C for 6 h, neutralized with a saturated barium hydroxide solution, heated to 80°C and filtered through Celite which was then washed with water (200 ml). The filtrate was taken down *in vacuo* and the product purified as described under *A*). Crystallization from water afforded 0·80 g (26%) of *Ia*, identical (UV-spectrum, E1, S1, S2 and H1) with the product, obtained according to procedure *A*); $[\alpha]_D^{20}$ +15·2° (*c* 0·5, 1M-HCl).

D) *From compound* VIIIc. A solution of the compound *VIIIc* (14·8 g; 94 mmol) in dimethyl sulfoxide (50 ml) was added dropwise at 100°C during 1 h to a stirred suspension of adenine (13·5 g; 0·1 mol) and potassium carbonate (0·1 mol) in dimethyl sulfoxide (100 ml) and the mixture was stirred under exclusion of moisture at 120—130°C for 14 h. After evaporation at 70°C/13 Pa the residue was codistilled with dimethylformamide (2 × 50 ml), dissolved in water (250 ml), the solution adjusted to pH 1·2 with concentrated sulfuric acid and heated to 70°C for 6 h. The mixture was neutralized with barium hydroxide to pH 3·3, filtered through celite which was then washed with water (100 ml) and the filtrate applied on a column of Dowex 50 X 8 (300 ml; H^+-form). The column was washed with water until the eluate did not exhibit UV absorption and then with 2·5% aqueous ammonia. The UV-absorbing eluate was taken down and worked up by chromatography on Dowex 1 X 2, as described under *A*). Fractions, containing pure *Ia* (HPLC in H1), were combined, taken down and the residue codistilled with water and crystallized from the same solvent, affording 7·2 g (30%) of pure (E1, S1, S2 and H1) compound *Ia*, identical with the product obtained according to *A*); $[\alpha]_D^{20}$ +14·8° (*c* 0·5, 1M-HCl).

L-Eritadenine (*IIa*)

A) *From compound* VIa. A solution of compound *VIa* (13·1 g; 42·7 mmol) in 0·25M sulfuric acid (150 ml) was kept at 37°C for 20 h, neutralized with 40% sodium hydroxide*, made up to 1 l with water, mixed with sodium hydroxide (6·8 g; 0·17 mol) and stirred under oxygen (100 kPa overpressure) for 24 h. The mixture was then neutralized with Dowex 50 X 8 (H^+-form) and the suspension applied on a column of the same ion exchange resin (500 ml). The column was washed with water till the eluate did not absorb in UV, and then with 2·5% ammonia. The UV-absorbing eluate was taken down, codistilled with ethanol (3 × 50 ml) and dried at 13 Pa. The residue was refluxed under stirring with methanol (500 ml) and concentrated sulfuric acid (5 ml) for 4 h. After cooling, the mixture was worked up in the same manner as the compound *Ia* (procedure *B*) and the products were crystallized from ethyl acetate–light petroleum, affording the following three products: Compound *XXVc* (0·43 g, 5%), R_F 0·50 (S5), m.p. 237°C, $[\alpha]_D^{20} = 0°$ (*c* 0·5, methanol). Mass spectrum: M^+ 207. For $C_8H_9N_5O_2$ (207·2) calculated: 46·37% C, 4·38% H, 33·81% N; found: 46·58% C, 4·57% H, 33·69% N. Compound *XXVII* (0·87 g; 9%), R_F 0·42 (S5), m.p. 194–196°C. Mass-spectrum: M^+ 237; $[\alpha]_D^{20}$ −3·4° (*c* 0·5, methanol). For $C_9H_{11}N_5O_3$ (237·2) calculated: 45·56% C, 4·67% H, 29·53% N; found: 45·45% C, 4·64% H, 29·76% N. Compound *IIb* (4·56 g; 40%), R_F 0·24 (S5), m.p. 217–219°C, $[\alpha]_D^{20}$ −23·0° (*c* 0·5, dimethylformamide). Mass spectrum: M^+ 267. For $C_{10}H_{13}N_5O_4$ (267·2) calculated: 44·34% C, 4·90% H, 26·21% N; found: 44·81% C, 4·63% C, 4·63% H, 26·32% N.

A solution of compound *IIb* (2·7 g; 10 mmol) in 0·1M sodium hydroxide (100 ml) was set aside at room temperature overnight and neutralized (pH 8·5) with Dowex 50X8 (H^+-form). This solution was chromatographed on a column of Dowex 1 X 2 (100 ml) as described for *Ia*, procedure *B*). Crystallization from water afforded 2·3 g (90%) of compound *IIa* which did not melt below 260°C; $[\alpha]_D^{20}$ −14·8° (*c* 0·5, 1M-HCl), R_F 0·35 (S1), 0·50 (S2), E_{Up} 0·45 (E1). For $C_9H_{11}N_5O_4$ (253·2) calculated: 42·68% C, 4·38% H, 27·66% N; found: 42·40% C, 4·21% H, 28·04% N.

B) *From compound* XIV. The reaction was carried out in the same manner as described for the compound *Ia* (procedure *C*). Thus, 20 mmol of *XIV* and 25 mmol of adenine afforded after crystallization from water 1·7 g (34%) of *IIa* which was homogeneous and identical (S1, S2, E1, and H1) with the product prepared under *A*); $[\alpha]_D^{20}$ −14·7° (*c* 0·5; 1M-HCl).

[U-^{14}C-Adenine]-D-eritadenine

A mixture of [U-^{14}C]-adenine (10 MBq; 814 MBq/mmol, 12·25 μmol), compound *VIIIb* (20 mg) potassium carbonate (5 mg) and dimethyl sulfoxide (250 μl) was heated in a thick-walled test tube (ground-joint stopper) to 140°C for 7 h. The mixture was subjected to electrophoresis on a sheet (16 cm width) of paper Whatman No 3 MM in E1 (90 min, 20 V/cm). The mobile UV-absorbing band of the product (E_{Up} 0·45–0·50) was eluted with water (3–5 ml) and the eluate taken down *in vacuo*. The residue was warmed with formic acid (2 ml) to 37°C for 48 h, the solution taken down, the residue codistilled *in vacuo* with water (3 × 5 ml) and chromatographed on a strip (2 cm width) of paper Whatman No 3 MM in the system S1. The spot corresponding to *Ia* was eluted with water (2–3 ml) and the eluate taken down and chromatographed in the system S2. The spot of eritadenine (*Ia*) (R_F 0·50) and compound *VII* was eluted with water (2 ml): composition 78% *Ia* and 22% *VII*. The eluate of *Ia* was again chromatographed in the system S1, eluted with water and the eluate freeze-dried; yield 65% (based on adenine), radiochemical purity (S1, S2, E1, E2) higher than 99%.

* Neutralization with barium hydroxide (see *Ia*) was not suitable because the compound *VIb* is poorly soluble in water.

(2*S*, 3*R*)-*threo*-4-(Adenin-9-yl)-2,3-dihydroxybutanoic Acid (*III*)

A mixture of compound *XVI* (ref.[8]; 8·4 g; 30 mmol), sodium periodate (17·1 g; 80 mmol) and 70% aqueous acetone (800 ml) was stirred until it became homogeneous. A ruthenium oxychloride solution (3 ml; 30 mg ruthenium) was added and the mixture was stirred at room temperature overnight. Methanol (10 ml) was added, after 30 min the mixture was filtered, the solid washed with acetone (100 ml) and the filtrate taken down *in vacuo*. The residue was warmed to 37°C with 0·25M sulfuric acid (100 ml) for 20 h and treated as described for compound *Ia* under *A*). Crystallization from water afforded 4·4 g (58%) of compound *III*, not melting below 260°C, $[\alpha]_D^{20}$ −68·7° (*c* 0·5; 1M-HCl); R_F 0·37 (S1), E_{Up} 0·45 (E1). UV spectrum (pH 2,12): λ_{max} 261 nm (ε_{max} 14 200). For $C_9H_{11}N_5O_4$ (253·2) calculated: 42·68% C, 4·38% H, 27·66% N; found: 42·59% C, 4·40% H, 27·65% N.

(2*R*,3*S*)-threo-(4-Adenin-9-yl)-2,3-dihydroxybutanoic Acid (*IV*)

A mixture of compound *XVII* (ref.[8]; 1·4 g; 5 mmol), sodium periodate (4·0 g; 18·7 mmol), 70% acetone (250 ml) and ruthenium oxychloride solution (1 ml; 10 mg ruthenium) was stirred overnight at room temperature and worked up similarly as described for *III*. Crystallization from water gave 0·89 g (70%) of compound *IV* which did not melt below 260°C; $[\alpha]_D^{20}$ +68·6° (*c* 0·5; 1M-HCl), identical with *III* (S1, E1 and H1). For $C_9H_{11}N_5O_4$ (253·2) calculated: 42·68% C, 4·38% H, 27·66% N; found: 42·73% C, 4·48% H, 27·99% N.

9-(2,2-Diethoxyethyl)adenine (*XIX*) (modified according to ref.[11])

A mixture of adenine (135 g; 1 mol), potassium carbonate (150 g; 1·09 mol) and dimethylformamide (2·2 l) was heated to 120°C and bromoacetaldehyde diethylacetal (236 g; 1·2 mol) was added dropwise with stirring in the course of 90 min. The mixture was then stirred at 140°C for 14 h, filtered while hot, the solid washed with dimethylformamide (400 ml), the filtrate concentrated to 800 ml at 60°C/2 kPa and allowed to crystallize in a refrigerator. The product was collected on filter, washed with ethanol (200 ml), ether (200 ml) and dried *in vacuo*, affording 167·2 g (66%) of compound *XIX*, m.p. 218—219°C (reported[11] m.p. 212°C). For $C_{11}H_{17}N_5O_2$ (251·3) calculated: 52·57% C, 6·82% H, 27·87% N; found: 52·72% C, 6·74% H, 27·22% N. R_F 0·70 (S5).

4-(Adenin-9-yl)-3-butanoic Acid (*XXI*)

A mixture of compound *XIX* (15·1 g; 60 mmol), malonic acid (25·2 g; 0·24 mol) and water (500 ml) was stirred under reflux for 11 h, cooled with ice, filtered, the solid washed with water, ethanol and ether and dried *in vacuo*; yield 10·2 g (71·7%) of compound *XXI*, not melting below 260°C. For the monohydrate $C_9H_{11}N_5O_3$ (237·2) calculated: 45·56% C, 4·67% H, 29·53% N; found: 46·18% C, 4·31% H, 29·46% N. R_F 0·47 (E1), E_{Up} 0·55 (E1). UV-Spectrum (pH 2): λ_{max} 234 nm (ε_{234} 19 000), λ_{sh} 260 nm; (pH 12): λ_{max} 260 nm (ε_{260} 12 500), λ_{sh} 232 nm.

Methyl 4-(Adenin-9-yl)-3-butenoate (*XXII*)

A mixture of compound *XXI* (3·5 g; 14·7 mmol), concentrated sulfuric acid (1·3 ml) and methanol (130 ml) was refluxed with stirring for 2 h, poured into a suspension of Amberlite IR 45 (50 ml) in methanol (100 ml), stirred until neutral and filtered. The resin was washed with methanol (300 ml), the filtrate taken down and the residue crystallized from methanol–ether, affording 3·2 g (93%) of compound *XXII*, m.p. 185—186°C. R_F 0·48 (S5). For $C_{10}H_{11}N_5O_2$ (233·2) cal-

culated: 51·49% C, 4·75% H, 30·03% N; found: 51·39% C, 4·77% H, 29·95% N. Positive reaction with potassium permanganate. UV spectrum identical with that of compound *XXI*.

Methyl *trans*-4-(Adenin-9-yl)-2-butenoate (*XXIII*)

Compound *XXII* (1·3 g; 5·6 mmol) was dissolved in 33% solution of triethylamine in dioxane (120 ml) and the solution was set aside at room temperature for 48 h. After evaporation *in vacuo*, the residue was chromatographed on two layers of silica gel (35 × 15 × 0·3 cm), containing a fluorescent indicator, in chloroform–methanol (93 : 7). The bands of the products were separated, eluted with methanol (300 ml) and the eluates taken down. Crystallization of the residues from methanol–ether afforded 0·85 g (65·4%) of the recovered compound *XXII*, identical with the starting material according to chromatography in S5 and its UV spectrum. There was obtained further the compound *XXIII* (0·40 g; 31%), m.p. 211—212°C; R_F 0·63 (S5). UV spectrum (pH 2,12) λ_{max} 260 nm. For $C_{10}H_{11}N_5O_2$ (233·2) calculated: 51·49% C, 4·75% H, 30·03% N; found: 51·23% C, 4·69% H, 29·78% N.

Hydroxylation of Compound *XXIII*

Osmium tetroxide (8 mg) was added to a solution of compound *XXIII* (233 mg; 1 mmol) and sodium chlorate (426 mg; 4 mmol) in 50% methanol (20 ml) and the mixture was stirred at room temperature overnight (quantitative reaction according to chromatography in S5). The mixture was taken down *in vacuo*, the residue dissolved in 1% lithium hydroxide (20 ml), set aside at room temperature overnight, acidified (pH 3) with Dowex 50 X 8 (H^+-form), poured on a column of the same resin (100 ml) and the column washed with water until the UV absorption of the eluate dropped. Elution with 2·5% aqueous ammonia afforded a UV-absorbing eluate which was taken down and the residue chromatographed on a column of Dowex 1 X 2 (acetate, 100 ml) with dilute formic acid (linear gradient 0 — 1M, 2 l each). Fraction, obtained with 0·1—0·12M formic acid was taken down, the residue codistilled with water and crystallized from the same solvent, affording 215 mg (85%) of compound *III* + *IV*, identical with the authentic sample (E1, S1, S2). According to HPLC (H1), no compound *Ia* + *IIa* was found in the product or the mother liquor from its crystallization.

Hydroxylation of Compound *XXI*

A) *With osmium tetroxide*: The reaction was performed in the same manner as the hydroxylation of compound *XXIII*. After 10 min the reaction mixture contained only adenine (S1, E1, H1).

B) *With potassium permanganate*: A solution of compound *XXI* (1 mmol) in water (10 ml) was neutralized (pH 7·0) with sodium hydroxide and 0·1M potassium permanganate (20 ml) was added. After 15 min the mixture contained adenine (S1, S5, E1, H1) as the only UV-absorbing material.

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