PREPARATION OF ISOMERIC 3-AMINOPROPYLAMINO DERIVATIVES OF 9-(*RS*)-(2,3-DIHYDROXYPROPYL)ADENINE*

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Treatment of 9-(*RS*)-(2,3-dihydroxypropyl)adenine (*III*) with bromine in water afforded the 8-bromo derivative *IV* which on reaction with acetone was converted into the 1,3-dioxolane derivative *VI*. Reaction of compound *VI* with 1,3-diaminopropane, followed by acid hydrolysis, gave 9-(*RS*)-(2,3-dihydroxypropyl)-8-(3-aminopropylamino)adenine (*VIII*). Compound *IV* reacts with 1,3-diaminopropane under formation of a mixture of compound *VIII* and isomeric 9-(*RS*)-[3(2)-(3-aminopropylamino)-2(3)-hydroxypropyl]-8-hydroxyadenines (*IX*, *X*). 9-(*RS*)-(2,3-Dihydroxypropyl)-8-hydroxyadenine (*XVII*) was prepared by reaction of compound *VI* with sodium benzoxide in dimethylformamide and subsequent acid hydrolysis. Its tosylation, followed by reaction of the obtained 3'-O-*p*-toluenesulfonyl derivative *XVIII* with 1,3-diaminopropane, furnished also the compound *IX*. In an analogous way, 9-(*RS*)-[3-(3-aminopropylamino)-2-hydroxypropyl]-adenine (*XXI*) was prepared from the 3'-O-*p*-toluenesulfonyl derivative of compound *III* (*XX*).

Recently, a new efficient purification of S-adenosyl-L-homocysteine hydrolase has been published which utilises the strong binding of the enzyme to carriers, containing 8-(3-aminopropylamino)adenosine (*IIa*) linked to ω-carboxyhexyl groups in CH-Sepharose[1]. However, adenosine and some of its derivatives are known to be relatively weak inhibitors of this enzyme[2]. In one of our previous communications we described an unusually strong reversible inhibition effect of 9-(*S*)- or 9-(*RS*)-(2,3-dihydroxypropyl)adenine (*III*) on S-adenosyl-L-homocysteine hydrolase[3]. Contrary to adenosine, compound *III* is not a substrate of this enzyme. Since we have found that in the compound *III* any substitution at the N^6 position of adenine or modification of the hydroxy groups in the side chain removed completely the mentioned inhibitory effect[4], it appeared interesting to ascertain whether the 8-(3-aminopropylamino)derivative of compound *III*, as an analogue of the adenosine derivative *IIa*, could also be used for affinity chromatography of the mentioned enzyme.

The compound *IIa* has been synthesized[1] by reaction of moist 1,3-diaminopropane with 8-bromoadenosine (*Ia*). When preparing material for a comparison, we found that the published isolation procedure (gel filtration of the crude reaction mixture) was not satisfactory, obviously because the strongly basic components of the

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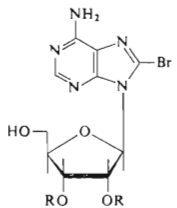
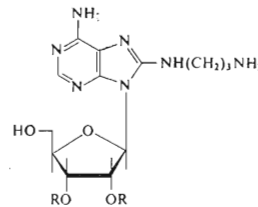
reaction mixture formed carbonates during the purification. For complete deionization of the product *IIa* and removal of the persistently contaminating traces of the diamine, chromatography on a cation-exchange resin proved to be advantageous. The product was eluted with ammonia with difficulty and its complete elution was achieved only by gradual elution with triethylamine in aqueous methanol. Still better was the use of 2′,3′-O-isopropylidene derivative *Ib* which on reaction with 1,3-diaminopropane afforded the well-isolable isopropylidene derivative *IIb*. After its purification, the product *IIa* was obtained by hydrolysis with sulfuric acid, neutralization with barium hydroxide and the above-mentioned chromatography on an ion-exchange resin.



Ia, R = H
Ib, R,R = $(CH_3)_2C$



IIa, R = H
IIb, R,R = $(CH_3)_2C$

The desired 9-(*RS*)-(2,3-dihydroxypropyl)-8-(3-aminopropylamino)adenine (*VIII*) was prepared starting from 9-(*RS*)-(2,3-dihydroxypropyl)-8-bromoadenine (*IV*). This starting material has been prepared previously[5] by bromination of triacetyl derivative of compound *III* with N-bromosuccinimide. However, direct bromination of compound *III* with bromine in water proved to be more satisfactory: after accurate neutralization of the crude hydrobromide of compound *IV*, the pure water-insoluble bromo derivative *IV* was obtained in a high yield. Reaction of *IV* with acetone gave the 2′,3′-O-isopropylidene derivative *VI* which can be prepared also by bromination of the isopropylidene derivative *V* (ref.[5,6]) with bromine in a phosphate buffer, analogously to the bromination of 2′,3′-O-isopropylidene-adenosine (*Ib*) (ref.[7]).

The reaction of compound *VI* with 1,3-diaminopropane in the presence of water proceeds smoothly giving the isopropylidene derivative *VII* which can be hydrolyzed with sulfuric acid. After removal of the excess acid with barium hydroxide, the compound *VIII* is isolated by chromatography on a medium acidic ion-exchange resin with a gradient of acetic acid. This procedure affords the pure diacetate salt of compound *VIII*; the free base can be prepared from the purified diacetate either by the above-mentioned chromatography on a strongly acid ion-exchange resin (elution

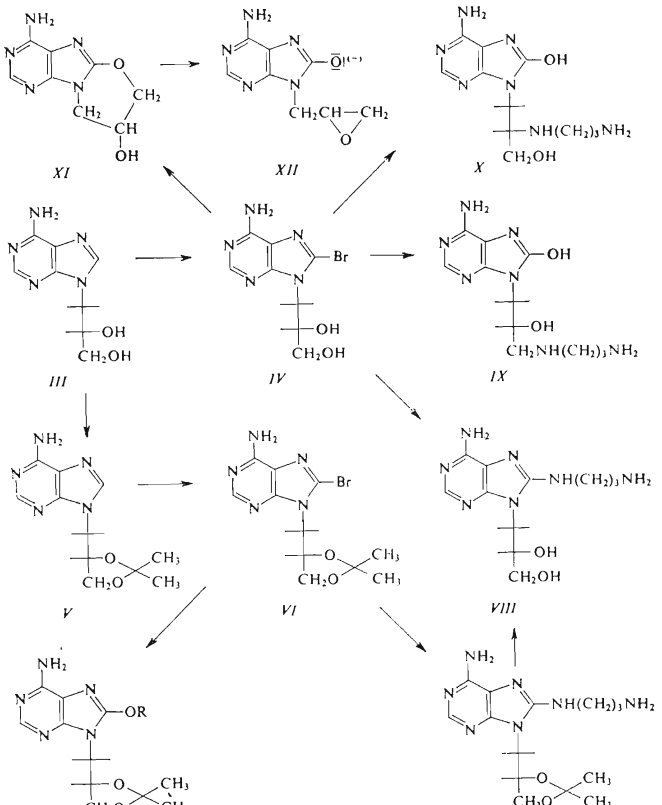
with triethylamine solutions) or chromatography on silica gel in an ammonia-containing system. The latter system is suitable for small-scale preparations but, because of high separation efficiency of silica gel in ammonia-containing systems, preparation of even gram quantities presents no difficulties. This procedure was used also in the preparation of the (*S*)-enantiomer of *VIII* from the (*S*)-enantiomer of compound *III*.

Under the mentioned conditions, the reaction of 9-(*RS*)-(2,3-dihydroxypropyl)-8-bromoadenine (*IV*) with 1,3-diaminopropane has a more complex course: although it is quantitative relative to the compound *IV*, it gives, in addition to the desired 8-(3-aminopropylamino) derivative *VIII* identical with the above-described product, two further ninhydrin-positive strongly basic compounds. Their UV spectra (characteristic double absorption maximum at 272 and 281 nm and its hypochromic shift to 286 nm in alkaline medium) indicate an 8-hydroxyadenine structure. Mass spectra of both compounds exhibit a molecular ion M^+ 281 ($C_{11}H_{19}N_7O_2$) and a peak due to 8-hydroxyadenine (M^+ 151). Accordingly, in these compounds one of the hydroxy groups in the side chain of the substituted 8-hydroxyadenine (*IX*, *X*) is replaced by 3-aminopropylamino group. Their structures can be assigned also from the mass spectra: the fragment 194 arises by loss of the $NH_2(CH_2)_3NCH_2$ group only in compound *IX* whereas compound *X* is characterized by the fragment 207 formed by loss of $NH_2(CH_2)_3NH$ moiety from the secondary carbon $C_{(2')}$. Both these fragmentation patterns markedly differ from that of the third isomeric compound *VIII*, exhibiting a typical 8-(3-aminopropylamino)adenosine fragment.

Structure of all the three isomers was confirmed also by the ^1H NMR spectra, particularly by the different chemical shifts of protons in positions 2' and 3' of the side-chain; compounds *IX* and *X* contain a hydroxy and/or secondary amino group whereas two hydroxy groups are bonded in compound *VIII* in these positions. Another characteristic feature is also the difference in the chemical shifts of protons at the NH—CH_2 carbon atom in compound *VIII* on the one hand and compounds *IX* and *X* on the other hand; this effect is due to a different character of the substituent on the amino group.

The anomalous reaction of compound *IV* with 1,3-diaminopropane results from formation of a cyclic intermediate of the type *XI* which, in reaction with a strong nucleophile, can either undergo a reaction at $C_{(8)}$ to give compound *VIII*, or afford the 2',3'-epoxide *XII*. The oxirane ring is then opened with the amine to give a mixture of compounds *IX* and *X*. In the case of the 2',3'-O-isopropylidene derivative *IV* the formation of a cyclic derivative is excluded and therefore the reaction with 1,3-diaminopropane affords compound *VII* as the only product. The derivative *VI* is stable in aqueous hydroxides, however, treatment with sodium hydroxide in 50% aqueous methanol leads to the 8-methoxy derivative *XIII* as the sole reaction product, obviously as a result of a higher nucleophilicity of the methoxide ion. The structure of compound *XIII* was confirmed by its mass and ultraviolet spectra.

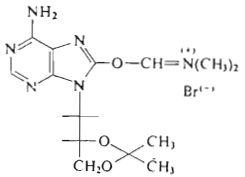
The assumed presence of the 2',3'-epoxide *XII* (rather than the isomeric 2',8-O-anhydro derivative) in the reaction of compound *IV* with 1,3-diaminopropane is sup-



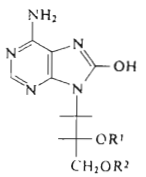
ported by the unusualy facile decomposition of the 8-bromo derivative *IV* even in weakly basic medium: the intermediate *XI* (identified by mass and UV spectra) is rapidly transformed into a mixture of products, apparently of oligomeric character. The product of ring-opening in compound *XI* by an attack at position 8, *i.e.* 9(*RS*)-(2,3-dihydroxypropyl)-8-hydroxyadenine (*XVII*) is formed only in a low yield. Because of instability of compound *IV* in alkaline media, maximum care must be exercised when isolating the 8-bromoadenine derivative *IV* by neutralization of its hydrobromide (*vide supra*). The extraordinarily facile formation of the 3',8-cyclic derivative *XI* is especially marked when compared with the drastic conditions[8], required for preparation of the 2',8- or 5',8-anhydro derivatives from 8-bromoadenosine and its derivatives.

To prove the structure of compound *IX* by an independent synthesis, we chose a reaction sequence starting from the 8-hydroxyadenine derivative *XVII*. The starting compound could not be prepared from the 8-bromo derivatives *IV* or *VI* by treatment with hydroxide; the originally employed procedure, *i.e.* nucleophilic substitution of 8-bromo derivative with sodium acetate, gave as the main product the 8-acetoxy derivative[5], whose deacetylation was difficult. In an attempt to prepare this compound by reaction of the 8-bromo derivative with sodium benzoxide[9] (analogously to the introduction of 8-hydroxy group in the purine nucleoside series), the isopropylidene derivative *VI* afforded only small amount of the 8-benzyloxy derivative *XIV*. This reaction, proceeding smoothly in hot dimethylformamide, gave directly the 8-hydroxy derivative *XV* as the principal product. Its acid hydrolysis furnished the well characterizable 9-(*RS*)-(2,3-dihydroxypropyl)-8-hydroxyadenine (*XVII*). The UV spectrum of compounds *XV* and *XVII* corresponds to those of the mentioned isomers *IX* and *X*. The direct formation of compound *XV* in the reaction may be explained by participation of dimethylformamide which under the reaction conditions can be O-alkylated with the adenine moiety to give an O-substituted salt of the type *XVI*. Its subsequent hydrolysis results in the 8-hydroxyadenine derivative *XV*. Analogous reactions occur frequently in the dimethylformamide chemistry (*e.g.* in the reaction with dimethyl sulfate[10]).

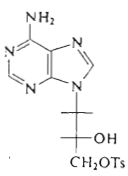
The free 8-hydroxy derivative *XVII* reacts with *p*-toluenesulfonyl chloride affording a mixture of 3'-mono(*XVIII*) and 2',3'-di-O-*p*-toluenesulfonyl (*XIX*) derivative. The compound *XVIII* reacted with 1,3-diaminopropane under the mentioned conditions to give the 3-(3-aminopropylamino)-2-hydroxypropyl derivative *IX* as the only product. The absence of the isomer *X* in the reaction mixture can be interpreted in two ways: either the nucleophilic substitution reaction is so fast that the 2',3'-epoxide is not formed, or the intermediate *XII* is specifically opened to afford compound *IX*. The latter alternative explains the formation of the isomer *X* from the bromo derivative *IV* only then, if the substitution by the amine in positions 2' and 3' of the side-chain proceeds by a concerted mechanism, involving structures analogous to compound *XI* without formation of covalent structures.



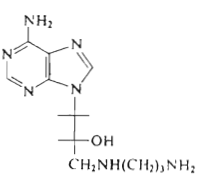
XVI



XVII, $R^1 = R^2 = H$
XVIII, $R^1 = H$, $R^2 = Ts$
XIX, $R^1 = R^2 = Ts$



XX



XXI

In formulae *XVIII*–*XX*, Ts = *p*-toluenesulfonyl residue.

Analogously, also the reaction of 3'-O-*p*-toluenesulfonyl derivative of compound *III* (*XX*; ref.[11]) with 1,3-diaminopropane proceeds in an unequivocal way, affording isomerically pure 3-(3-aminopropylamino)-2-hydroxypropyl derivative *XXI*.

The prepared 3-aminopropylamino derivatives *VIII*–*X* and *XXI* were used for preparation of carriers for affinity chromatography of S-adenosyl-L-homocysteine hydrolase. The results of this study will be described in another communication of this series[12].

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. Unless otherwise stated, the solutions were evaporated on a rotarory evaporator at 2 kPa and the compounds were dried over phosphorus pentoxide at 13 Pa. Paper chromatography was carried out on a Whatman No 1 paper in the system S1, 2-propanol–conc. aqueous ammonia–water (7 : 1 : 2), preparative chromatography on a column (80 × 4 cm) of microcrystalline cellulose (Macherey + Nagel) in the same system (20 ml/h); detection by continuous measurement of UV absorption on a Uvicord (Uppsala, Sweden) instrument. Chromatography on silica gel was performed on Silufol UV 254 plates (Kavalier, Czechoslovakia) in systems S1 (see above), S2, chloroform–ethanol (95 : 5), S3, chloroform–methanol (9 : 1), S4, chloroform–methanol (3 : 2). Preparative chromato-

graphy on silica gel was run on loose layers (40 × 16 × 0·3 cm) of silica gel (30—40 μ), containing fluorescent indicator (made by Service Laboratories of this Institute) or on a column of silica gel according to Pitra (150—200 g).

UV absorption spectra were measured in water on a Specord UV-VIS (Carl Zeiss, Jena, GDR), ^{1}H NMR spectra on a Varian XL-200 spectrometer in hexadeuteriodimethyl sulfoxide with tetramethylsiloxane as internal standard; chemical shifts are given in ppm, coupling constants in Hz.

8-(3-Aminopropyl)aminoadenosine (*IIa*)

a) *From* 8-*bromoadenosine* (Ia) (ref.[1]): 8-bromoadenosine (*Ia*; see ref.[7]; 1·73 g; 5 mmol) was added to a stirred mixture of 1,3-diaminopropane (10·5 ml; 126 mmol) and water (2 ml), pre-heated to 120°C. After stirring at this temperature for 4 h (soda lime protecting tube), the mixture was taken down at 40°C/13 Pa, the residue codistilled with dimethylformamide (2 × 25 ml) under the same conditions and dissolved in water (25 ml). The solution was mixed with Dowex 50X8 (H^+ form) to acid reaction and the suspension was applied on a column (100 ml) of the same ion exchange resin. After washing with water until conductivity and UV absorption of the eluate dropped, the elution was carried out with a 20% solution of triethylamine in 50% aqueous methanol. The UV-absorbing eluate was taken down *in vacuo* and the residue chromatographed on a column of cellulose in the system S1. Fractions, containing the compound of R_F 0·40 (S1) were combined, evaporated *in vacuo* and dried, affording 63% of the chromatographically homogeneous (R_F 0·40 in S1, 0·13 in S5) product *IIa* as a foam with positive ninhydrine reaction. Mass spectrum: M^+ 339 (calculated 339·4). UV spectrum (pH 2): λ_{max} 280 nm (ε_{280} 11 000); (pH 12): λ_{max} 280 nm (ε 12 300).

b) *From* 2,3-O-*isopropylidene*-8-*bromoadenosine* (Ib): The reaction was carried out in the same manner as described under *a*) with 14 mmol of 2,3-O-isopropylidene-8-bromoadenosine (*Ib*; see ref.[7]). After evaporation, the residue was dissolved in water (20 ml) and applied on a column of Dowex 1X2 (100 ml; OH^- form). The column was washed with water until the eluate no longer absorbed in the UV region and its conductivity dropped. The product was eluted with 20% aqueous methanol. The UV-absorbing fraction was taken down and dried at 13 Pa over phosphorus pentoxide, affording 6·1 g (100%) of chromatographically pure compound *IIb*; R_F 0·63 (S1). Its UV spectrum was identical with that of compound *IIa*. A solution of this product in 0·5M-H_2SO_4 (100 ml) was set aside at room temperature overnight, neutralized with saturated barium hydroxide solution to pH 7·00 and heated to 80°C for 1 h. The suspension was filtered through Celite, the filtrate concentrated *in vacuo* and purified on Dowex 50 as described under *a*) to give 72% of chromatographically pure (S1, S4) compound *IIa*, identical with an authentic sample.

9-(*RS*)-(2,3-Dihydroxypropyl)-8-bromoadenine (*IV*)

Compound *III* (20·9 g; 0·1 mol) was added to a solution of bromine (9·2 ml) in water (1 500 ml). The mixture was stirred at room temperature overnight and evaporated *in vacuo*. The residue was dissolved in water (300 ml) and the stirred solution neutralized with 2M-KOH to pH 7·00. After stirring for 10 min the product was collected on filter, washed with water (1 litre), acetone and ether and dried *in vacuo*. The obtained compound *IV* (23·3 g; 80·9%) did not melt below 240°C. R_F 0·63 (S1); *III*: R_F 0·54 (S1). UV spectrum (pH 2, 12): λ_{max} 267 (ε = 19 000). For $C_8H_{10}BrN_5O_2$ (288·2) calculated: 33·34% C, 3·50% H, 27·75% Br, 24·31% N; found: 33·46% C, 3·59% H, 27·48% Br, 24·17% N.

2',3'-O-Isopropylidene-9-(*RS*)-(2,3-dihydroxypropyl)-8-bromoadenine (*VI*)

a) A mixture of compound *IV* (20 g; 70 mmol), acetone (150 ml), dimethylformamide (50 ml), ethyl orthoformate (40 ml) and 6M-HCl in dimethylformamide (15 ml) was stirred at room temperature for 2 h, made alkaline with triethylamine and taken down *in vacuo*. The residue was co-distilled with toluene and crystallized from 80% aqueous ethanol, affording 19·8 g (86%) of compound *VI*, m.p. 195–196°C; R_F 0·40 (S3). For $C_{11}H_{14}BrN_5O_2$ (328·2) calculated: 40·25% C, 4·30% H, 24·36% Br, 21·34% N; found: 40·77% C, 4·20% H, 23·99% Br, 20·98% N.

The (*S*)-enantiomer of compound *VI* was prepared in an analogous way; m.p. 196°C; $[\alpha]_D^{20}$ −3·0° (*c* 0·5, dimethylformamide).

b) A solution of crystalline sodium hydrogen phosphate (40 g) in water (400 ml) was added to a solution of compound *V* (see ref.[6]; 7·47 g; 30 mmol) in dioxane (400 ml). Then, bromine (1·8 ml; 35·6 mmol) was added portionwise under stirring. Stirring was continued at room temperature for 4 h, the excess bromine was destroyed with saturated solution of sodium hydrogen sulfite and the mixture was extracted with chloroform (6 × 200 ml). The chloroform extract was dried over magnesium sulfate, evaporated *in vacuo* and the residue was dissolved in boiling ethanol (100 ml). The solution was filtered to remove small amount of impurities and light petroleum was added to the filtrate to persistent turbidity. Crystallization at 0°C afforded 7·0 g (71%) of the compound *VI*, identical with the product obtained according to the procedure *a*).

9-(*RS*)-(2,3-Dihydroxypropyl)-8-(3-aminopropylamino)adenine (*VIII*)

Compound *VI* (2·0 g; 6 mmol) was added to a stirred mixture of 1,3-diaminopropane (6 ml) and water (1·2 ml), preheated to 120°C. The mixture was stirred at 120°C for 5 h (soda lime protecting tube) and evaporated. After codistillation with dimethylformamide (2 × 25 ml) at 60°C/13 Pa and then with toluene (2 × 25 ml) under the same conditions, the residue (R_F 0·58 in S1; *VI*: R_F 0·75 in S1) was kept at 37°C with 0·25M-H_2SO_4 (25 ml) for 20 h. The mixture was diluted with water (100 ml) and filtered through Celite. The filtrate was taken down *in vacuo* and the residue applied to a column of Amberlite IRC 50 (H^+ form; 200 ml). The column was washed with water until the UV absorption and conductivity dropped and the product was eluted with a linear gradient of 0–1M acetic acid (à 2 litres); elution rate 3 ml/min, fractions 10 min. The UV-absorbing product fractions (0·2–0·4M acetic acid) were combined and taken down *in vacuo*, leaving the diacetate salt of compound *VIII* as an amorphous foam; yield 1·50 g (62%). For $C_{11}H_{19}N_7O_2.2\,CH_3CO_2H$ (401·4) calculated: 44·87% C, 6·78% H, 24·43% N; found: 44·51% C, 7·11% H, 25·05% N.

This product was chromatographed on a column of cellulose in the system S1. The product fractions were combined, taken down *in vacuo* and the residue was codistilled with ethanol (3 × 20 ml). Crystallization from ethanol (with addition of ether to turbidity) afforded 85% (based on the diacetate salt of *VIII*) of compound *VIII*, m.p. 182–183°C. For $C_{11}H_{19}N_7O_2$ (281·3) calculated: 46·96% C, 6·81% H, 34·86% N; found: 46·81% C, 6·81% H, 34·39% N. R_F 0·40 (S1). Mass spectrum: 281 (M^+), 251 (M− CH_2NH_2), 250 (M−CH_2OH), 207 (M−$CH_2CH.(OH)CH_2OH$). ^{1}H NMR spectrum 1·72 (pent. 2 H, J = 6·6) $NHCH_2CH_2$; 2·69 (t, 2 H, J = 6·6) CH_2NH_2; 3·36 (2 × dd, 2 H, J_1 = 5·0, J_2 = 4·8, J_{gem} = 11·8) CH_2OH; 3·42 (t, 2 H, J = 6·2) $NHCH_2$; 3·83–4·09 (m, 3 H) $H_1 + H_2$·; 6·47 (br, 2 H), NH_2 (adenine), 7·92 (s, 1 H) H_2 (adenine). UV spectrum (pH 2): λ_{max} 280 nm (ε_{max} 13 300); (pH 7 and 12): λ_{max} 280 nm (ε_{max} 15 600).

Reaction of Compound *IV* with 1,3-Diaminopropane

Compound *IV* (7·2 g; 25 mmol) was added to a stirred mixture of 1,3-diaminopropane (25 ml) and water (5 ml), preheated to 120°C. The mixture was heated with stirring (soda lime protecting tube) to 120°C for 10 h, taken down and worked up by codistillation with dimethylformamide and toluene as described for the compound *VIII*. The residue was chromatographed on a column of Amberlite IRC 50 (H^+-form; 300 ml), as described for compound *VIII*. The acid eluate (0·4M) was evaporated, the residue codistilled with ethanol (4 × 50 ml) and chromatographed on a column of cellulose in the system S1. The UV-absorbing fractions were checked by thin-layer chromatography on silica gel in the system S1, the corresponding fractions were combined, taken down *in vacuo*, the residues codistilled with ethanol (2 × 25 ml), dissolved in methanol (5 ml) and precipitated with ether (200 ml). The precipitated products were filtered, washed with ether and dried *in vacuo*.

This procedure gave 1·9 g (27%) of the compound *VIII*, identical with the authentic material (UV and mass spectrum, thin-layer chromatography in S1).

Fraction of R_F 0·36 (S1) afforded 1·4 g of compound *IX*, *i.e.* 20%, based on *IV*; m.p. 144°C (decomposition). For $C_{11}H_{19}N_7O_2$ (281·3) calculated: 46·96% C, 6·81% H, 34·86% N; found: 46·83% C, 6·91% H, 33·88% N. Mass spectrum: 281 (M^+), 263 ($M-H_2O$), 237 ($M-CH_2CH_2.NH_2$), 233 ($263-CH_2NH_2$), 152 (BH). ^{1}H NMR spectrum: 1·51 (pent, 2 H, J = 6·6) NH—CH_2—CH_2; 2·48 (d, 2 H, J = 5·2) 3′-CH_2; 2·53 (t, 2 H, J = 6·9) CH_2NH_2; 2·66 (br t, 2 H, J = 6·5) CH_2NH—; 3·75 (d, 2 H, J = 6·1) 1′-CH_2; 3·97 (pent, 1 H, J = 6·0) 2′-CH; 6·77 (br, 2 H) NH_2 (adenine); 7·97 (s, 1 H) H_2 (adenine). UV spectrum (pH 2): λ_{max} 270, 281 nm (ε = 10 300); (pH 7): λ_{max} 273 nm (ε 12 900); (pH 12): λ_{max} 283 nm (ε_{max} 14 800).

Fraction of R_F 0·27 (S1) gave 1·8 g (25·6%) of compound *X*, m.p. 138°C. For $C_{11}H_{19}N_7O_2$ (281·3) calculated: 46·96% C, 6·81% H, 34·86% N; found: 46·38% C, 6·94% H, 34·77% N. Mass spectrum: 281 (M^+), 251 ($M-CH_2NH_2$), 250 ($M-CH_2OH$), 207 ($M-NH_2CH_2CH_2CH_2NH_2$), 51 (B). ^{1}H NMR spectrum: 1·46 (pent, 3 H, J = 6·6) NH—CH_2CH_2; 2·61 (t, 4 H, J = 6·6) $NHCH_2$ + NH_2CH_2; 2·87 (pent, 1 H, J = 5·6) 2′-CH; 3·28 + 3·38 (2 × dd, 2 H, J = 5·3, J_{gem} = 11·2) 3′-CH_2; 3·74 (d, 2 H, J = 6·2) 1′-CH_2; 6·69 (br, 2 H) NH_2 (adenine), 8·01 (s, 1 H) H_2 (adenine). UV spectrum (pH 2, 7 and 12) identical with that of compound *IX*.

2′,3′-O-Isopropylidene-9-(*RS*)-(2,3-dihydroxypropyl)-8-hydroxyadenine (*XV*)

Benzyl alcohol (10·8 g; 0·1 mol) was added to a stirred suspension of sodium hydride (2·4 g; 0·1 mol) in dimethylformamide (150 ml) under exclusion of moisture. After stirring at 40°C for 30 min, compound *VI* (13·2 g; 40 mmol) was added. The mixture was stirred at 100°C for 9 h under exclusion of moisture, cooled, treated with acetic acid (3·6 ml; 60 mmol) and taken down at 40°C/13 Pa. The residue was dissolved in chloroform (300 ml) and the solution extracted with water (10 × 50 ml). The aqueous extract was taken down *in vacuo* and the residue codistilled with ethanol (2 × 100 ml) and extracted with boiling chloroform (3 × 200 ml). The chloroform extract was taken down *in vacuo* and the residue crystallized from ethanol (ether added), affording 6·9 g (65%) of compound *XV*, m.p. 251—252°C; R_F 0·25 (S2); *VI*: 0·60 (S2). UV spectrum identical with that of *IX* and *X*. For $C_{11}H_{15}N_5O_3$ (265·3) calculated: 49·79% C, 5·72% H, 26·40% N; found: 49·59% C, 5·45% H, 25·82% N.

The chloroform solution after extraction of compound *XV* with water (*vide supra*) was chromatographed on a column of silica gel affording another 1·35 g (12·7%) of compound *XV*. Further fraction on crystallization from ethanol (ether added) gave 1·05 g (7·4%) of compound *XIV*, m.p. 161—162°C; R_F 0·57 (S2). For $C_{18}H_{21}N_5O_3$ (355·4) calculated: 60·82% C, 5·96% H, 19·71% N; found: 60·98% C, 5·96% H, 19·64% N. UV spectrum (pH 2): λ_{max} 272 nm (ε_{272} 9 200), 288 nm (ε_{288} 8 900); (pH 7 and 12): λ_{max} 276 nm (ε_{max} 11 300).

9-(*RS*)-(2,3-Dihydroxypropyl)-8-hydroxyadenine (*XVII*)

Concentrated sulfuric acid (5 ml) was added dropwise to a stirred suspension of compound *XV* (7·9 g; 30 mmol) in water (100 ml) and the stirring was continued until the mixture became homogeneous. After standing at room temperature for 2 days, the mixture was diluted with water (100 ml), neutralized to pH 7·00 with saturated barium hydroxide solution, heated to the boil and filtered while hot through Celite which was then washed with boiling water (500 ml). The filtrate was taken down *in vacuo*, the residue dissolved in boiling 80% aqueous ethanol and ether was added until the solution became turbid. Standing in a refrigerator afforded crystals of compound *XVII* (5·54 g; 82%), m.p. 229–230°C. R_F 0·48 (S1). For $C_8H_{11}N_5O_3$ (225·2) calculated: 42·66% C, 4·92% H, 31·10% N; found: 42·71% C, 4·81% H, 31·08% N. UV spectrum (pH 3): λ_{max} 270, 282 nm ($\varepsilon_{270,282}$ 11 000); (pH 7): λ_{max} 271 nm (ε_{max} 13 600); (pH 12): λ_{max} 281 nm (ε_{max} 15 800).

Reaction of Compound *VI* with Sodium Hydroxide in Aqueous Methanol

A 1-M sodium methoxide solution (5 ml) in methanol was added to a suspension of compound *VI* (500 mg; 1·52 mmol) in 50% aqueous methanol (30 ml). The mixture was refluxed for 3 h, cooled, neutralized with Dowex 50X8 (H^+ form), filtered and the resin washed with methanol. The filtrate was taken down *in vacuo* and the residue chromatographed on a plate of silica gel in the system S2 (R_F 0·30 in S2, *VI*: R_F 0·37), the product being eluted with methanol (300 ml). After evaporation *in vacuo*, the residue was crystallized from ethyl acetate (light petroleum added), affording 300 mg (70·7%) of compound *XIII*, m.p. 125–126°C. For $C_{12}H_{17}N_5O_3$ (279·3) calculated: 51·60% C, 6·14% H, 25·08% N; found: 51·91% C, 6·30% H, 25·37% N. Mass spectrum: 279 (M^+), 264 (M–CH_3), 221 ($C_9H_{11}N_5O_2$, M–isopropylidene), 165 ($C_6H_7N_5O$, BH). UV spectrum (methanol): λ_{max} 261 nm (ε_{max} 12 000).

When the reaction was carried out under the same conditions in aqueous sodium hydroxide at 100°C, no change was observed (S1, S2) after 3 h.

Reaction of Compound *IV* with Sodium Hydroxide

A stirred solution of compound *IV* (0·50 g; 1·74 mmol) in 0·2M-NaOH (20 ml) was refluxed for 8 h (till the starting compound disappeared on thin-layer chromatography in S1). The mixture was neutralized with Dowex 50X8 (H^+ form), filtered, the resin washed with water and the filtrate evaporated. Chromatography of the residue on cellulose in the system S1 afforded 150 mg of compound *XI*, R_F 0·30 (S1). Mass spectrum: 207 (M^+, $C_8H_9N_5O_2$), 155 ($C_6H_9N_3O_2$), 112, 138 ($C_6H_8N_3O$). UV spectrum (pH 2, 7, 12): λ_{max} 267 nm. Compound *III* (50 mg) was obtained as a side product; it was identified by comparison (S1, UV and mass spectra) with the authentic sample.

Reaction of Compound *XVII* with *p*-Toluenesulfonyl Chloride

4-Dimethylaminopyridine (0·1 g), followed by *p*-toluenesulfonyl chloride (5·0 g; 26·2 mmol), was added under stirring to a suspension of compound *XVII* (4·3 g; 20 mmol) in pyridine (50 ml). After the exothermic reaction had subsided, the solution was set aside for 2 days at room temperature. Water (300 ml) was added and the mixture was extracted with chloroform (6 × 50 ml). The extract was evaporated *in vacuo* and the residue codistilled with ethanol (2 × 50 ml) *in vacuo*. Chromatography on a column of silica gel in the system S2 and subsequent crystallization from ethyl acetate (with addition of light petroleum) afforded 4·25 g (52·5%) of compound *XVIII*, m.p. 259–260°C, R_F 0·23 (S3). For $C_{15}H_{17}N_5O_5S$ (379·4) calculated: 47·48% C, 4·52% H, 18·46% N, 8·45% S; found: 46·91% C, 4·70% C, 18·56% N, 8·36% S.

The second product obtained was compound *XIX* (1·0 g; 9·4%), m.p. 134—136°C, R_F 0·30 (S3). For $C_{22}H_{23}N_5O_7S_2$ (533·6) calculated: 49·52% C, 4·34% H, 13·13% N, 12·02% S; found: 49·46% C, 4·31% H, 13·08% N, 11·60% S.

9-(*RS*)-[3-(3-Aminopropylamino)-2-hydroxypropyl]-8-hydroxyadenine (*IX*) from Compound *XVIII*

A mixture of compound *XVIII* (1·52 g; 4 mmol), water (2 ml) and 1,3-diaminopropane was stirred at 100°C for 3 h under soda lime protecting tube, taken down and codistilled with dimethylformamide (2 × 25 ml) at 60°C/13 Pa. The residue was deionized on a column of Amberlite IRC 50 (H^+ form; see the preparation of compound *VIII*) by gradient elution with acetic acid. The obtained diacetate salt of compound *IX* was chromatographed on a column of cellulose in the system S1. Precipitation of the product from methanol with ether (see preparation of compound *VIII*) afforded 0·70 g (62·3%) of compound *IX* which, according to mass, UV and ^{1}H NMR spectra and thin-layer chromatography (S1) was identical with the product obtained from compound *IV* by the above-described procedure.

9(*RS*)-[3-(3-Aminopropylamino)-2-hydroxypropyl]adenine (*XXI*)

A mixture of compound *XX* (ref.[11]) (0·90 g; 2·5 mmol), water (0·8 ml) and 1,3-diaminopropane (4 ml) was heated to 100°C for 6 h (soda lime protecting tube) and worked up as described for the preparation of compound *IX* from compound *XVIII*. Precipitation of the product, previously purified by chromatography on cellulose in the system S1, gave 0·45 g (64·4%) of the amorphous compound *XXI*; R_F 0·34 (S1). For $C_{11}H_{19}N_7O$ (265·3) calculated: 49·79% C, 7·22% H, 36·96% N; found: 49·77% C, 7·03% H, 36·90% N. UV spectrum (pH 2, 7 and 12): λ_{max} 262 nm (ε_{max} 13 200). ^{1}H NMR spectrum: 1·48 (pent, 2 H, J = 6·8) NH—CH_2CH_2, 2·47 (d, 1 H, $J_{3',2'}$ = 5·8) 3'-CH_2; 2·52 (t, 2 H) CH_2NH_2; 2·59 (br t, J = 6·5) NH—CH_2; 3·93 (m, 1 H) 2'-CH; 4·05 (dd, 1 H, J = 7·5, J_{gem} = 13·5) 1'-CH_2; 4·24 (dd, 1 H, J = 3·8, J_{gem} = 13·5) 1'-CH_2; 7·18 (br, 2 H) NH_2 (adenine); 8·04 (s, 1 H) H_2 (adenine), 8·14 (s, 1 H) H_8 (adenine). Mass spectrum: 266 (M^+H), 247 (M—H_2O), 235 (M—CH_2NH_2), 221 (M—$CH_2CH_2NH_2$), 192 (M—$NH_2CH_2CH_2CH_2$.NH_2), 148 (B —CH_2), 136 (BH_2), 135 (BH).

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