9-(AMINOALKYL)-8-HYDROXYADENINES: PREPARATION, MECHANISM OF FORMATION AND USE IN AFFINITY CHROMATOGRAPHY OF S-ADENOSYL-L-HOMOCYSTEINE HYDROLASE*

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Bromine reacts with 9-(2-hydroxyethyl)- (*Va*), 9-(3-hydroxypropyl)- (*Vb*), 9-(2-hydroxypropyl)- (*Vc*), 9-(2,3-dihydroxypropyl)- (*Vd*), 9-(1,3-dihydroxy-2-propyl)- (*Ve*), 9-threo-(2,3-dihydroxybutyl)- (*Vf*) and 9-threo-(2,3,4-trihydroxybutyl)adenine (*Vg*) to give 8-bromoadenine derivatives (*VI*). Reaction of compounds *VI* with ammonia results in intramolecular cyclization to five- and six-membered cyclic ethers which are regiospecifically opened to afford the respective 2-aminoalkyl-8-hydroxyadenines (*VIIa, b*) and 3-aminoalkyl-8-hydroxyadenines (*VIIc—VIIf*). Binding of compounds *VII* to CH-Sepharose 4B led to the polymeric material *XIII* capable of binding S-adenosyl-L-homocysteine hydrolase. Compounds *XIII* derived from the amino derivatives *VIIa, b, d* are of affinity support character and liberate the enzyme only on elution with dilute adenosine solution.

In one of our previous communication of this series[1] we described the preparation of 9-[3-(3-aminopropylamino)-2-hydroxypropyl]-8-hydroxyadenine (*II*) and its position isomer by reaction of 1,3-diaminopropane with 9-(2,3-dihydroxypropyl)-8-bromoadenine (*I*)**. The facile course of this reaction is due to formation of a cyclic intermediate with participation of the substituent on $C_{(8)}$ of the adenine ring. Nucleophilic opening of this ring with an aliphatic amine gives rise exclusively to an 8-hydroxyadenine derivative the substituted amino group being bonded to the side-chain. This course contrasts with the opening of analogous adenine 2',8- or 5',8-anhydronucleosides leading to the corresponding 8-aminoadenine derivatives[2]. The reaction is interesting not only for its mechanism but also for its products which are very useful as affinity ligands[3], utilized in a new highly effective isolation of S-adenosyl-L-homocysteine hydrolase (SAH-hydrolase) from various biological materials[4-6].

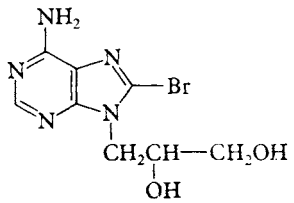
* Part XVI in the series Studies on S-Adenosyl-L-homocysteine Hydrolase; Part XV: This Journal *50*, 1514 (1985).

** All the acyclic adenosine analogs mentioned in this work are racemates; the prefix (*RS*) is omitted.

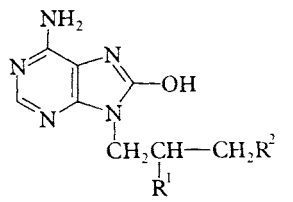
The present work investigates the reaction of hydroxy derivatives of 9-alkyl--8-bromoadenines with ammonia instead of 1,3-diaminopropane with the aim to study the general character of the mentioned transformation, the effect of the side--chain character on its course and the properties of the synthesized compounds as inhibitors and affinity ligands for isolation of SAH-hydrolases.

Starting compounds for our studies were the so-called open-chain adenosine analogs, *i.e.* hydroxy derivatives of 9-alkyladenines with a two- to four-carbon chain (*V*), obtained by previously described procedures. Only 9-(3-hydroxypropyl)adenine (*Vb*) was prepared by a new method, starting from 1,3-propanediol (*IIIa*): The monobenzoyl derivative *IIIb* (prepared by reaction of propane-1,3-diol with benzoyl cyanide[7]) was first converted into the *p*-toluenesulfonyl derivative *IIIc*. Its condensation with sodium salt of adenine, followed by methanolysis, afforded 9-(3-hydroxypropyl)adenine (*Vb*), identical with the previously described[8] compound. The preparation[9] of the isomeric 9-(2-hydroxypropyl)adenine (*VI*) was slightly modified in that the 2-benzoyl derivative *IVb* instead of the unprotected *IVa* was used as synthone in the reaction with adenine. This modification excludes the concurrent elimination and the work-up of the reaction mixture is much simpler. Methanolysis of the reaction intermediate gave compound *Vc*, identical with an authentic material.

The adenine derivatives *V* were converted into the corresponding 8-bromoadenine compounds *VI* by reaction with bromine water without protecting the hydroxyl



I



II, R^1, R^2 = OH, $NH(CH_2)_3NH_2$

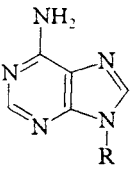
$R^1OCH_2CH_2CH_2OR^2$

IIIa, $R^1 = R^2 = H$
IIIb, $R^1 = COC_6H_5$, $R^2 = H$
IIIc, $R^1 = COC_6H_5$, $R^2 = SO_2C_6H_4CH_3(4\text{-})$

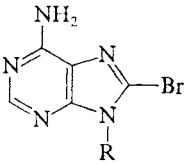
$CH_2OSO_2C_6H_4CH_3(4\text{-})$

IVa, R = H
IVb, $R = C_6H_5CO$

groups. The arising hydrogen bromide converted the compounds *VI* into the hydrobromides; these were accurately neutralized with concentrated solution of alkaline hydroxide. The neutralization must be carried out very carefully since the reaction products are very sensitive to alkaline medium. In some instances, the bromo derivative *VI* was sparingly soluble in water and was easily isolated, in others, the product was obtained after desalting and chromatography on silica gel. The thus-prepared 8-bromo derivatives *VI* had the expected analytical values and their UV spectra did not differ from those of the 9-substituted 8-bromoadenines (λ_{max} 265 nm in acidic medium).



V



VI

In formulae *V*, *VI*

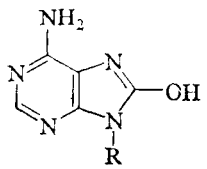
a, R = CH_2CH_2OH
b, R = $CH_2CH_2CH_2OH$
c, R = $CH_2CH(OH)CH_3$
d, R = $CH_2CH(OH)CH_2OH$
e, R = $CH(CH_2OH)_2$
f, R = $CH_2CH(OH)CH(OH)CH_3$ (*threo*)
g, R = $CH_2CH(OH)CH(OH)CH_2OH$ (*threo*)

The bromo derivatives *VI* reacted with concentrated aqueous ammonia on heating to 100–120°C in an autoclave and the starting compounds disappeared completely within few hours. Because of basic character of the formed amino derivatives, the reaction gave their hydrobromides, usually well soluble in water and alcohols (the only exception being the 2-hydroxypropyl derivative *VIc* whose hydrobromide was sparingly soluble in water and was isolated directly in the pure state). As shown by previous studies on 3-aminopropylamino derivatives[1], deionization of these salts is difficult and therefore the free amino derivatives *VII* were isolated by chromatography on cellulose in an ammonia-containing system. This procedure led to chromatographically homogeneous products whose elemental composition corresponded to 8-hydroxyadenine derivatives with a side-chain containing one amino group instead of the original hydroxyl.

All these compounds exhibit characteristic ultraviolet spectra: the absorption maximum of neutral compounds at 270 nm is shifted on alkalization by 12 nm to longer wavelengths with a marked hyperchromic effect whereas in an acidic medium the

spectrum exhibits a characteristic double maximum at 270 and 282 nm, with a clear hypsochromic effect. Such behaviour is typical for compounds of the type *II* with a substituted amino group in the side-chain as well as for 9-substituted 8-hydroxyadenines[1].

The structure of the 8-hydroxyadenine derivatives *VI* was determined by mass spectrometry (*vide infra*). From the data obtained we can make the following conclusions: *a*) Derivatives with an isolated primary or secondary hydroxyl in the side-chain (*VIa* – *VIc*) afford in the studied reaction the corresponding 9-aminoalkyl-8-hydroxyadenines (*VIIa* – *VIIc*). The 1,3-propanediol derivative with two isolated hydroxyl groups gives solely the monoamine *VIIe*. *b*) Compounds whose side-chain contains a *cis*-diol system, either with one primary or both secondary groups (*VId*, *VIf*), give isomerically homogeneous 3-amino-2-hydroxyalkyl derivatives (*VIId*, *VIIf*). Similarly as in the reaction with 1,3-diaminopropane[1], reaction of compounds *VI* with ammonia proceeds via the cyclic ether ("anhydro derivative") with participation of the substituent at the 8-position of the adenine ring.



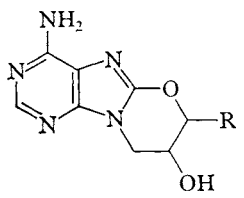
VII

a, R = $CH_2CH_2NH_2$
b, R = $CH_2CH_2CH_2NH_2$
c, R = $CH_2CH(CH_3)NH_2$
d, R = $CH_2CH(OH)CH_2NH_2$
e, R = $CH(CH_2OH)CH_2NH_2$
f, R = $CH_2CH(OH)CH(CH_3)NH_2$
g, R = $CH_2CH(OH)CH(CH_2OH)NH_2$

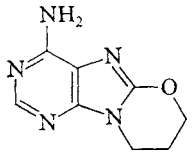
The transformation of 2-hydroxyethyl (*VIa*), 2-hydroxypropyl (*VIc*) or 1,3-dihydroxypropyl (*VIe*) derivative, leading to 9-(2-aminoalkyl)-8-hydroxyadenines *VIIa*, *c*, *e*, requires formation of an oxazoline intermediate (or a transition state of analogous geometry); however, no covalent compound of this type has been detected. On the other hand, 2,3-dihydroxy compounds *VId*, *f*, *g* form preferentially the six-membered oxazine ring of the type *VIII* as follows from the specific formation of the corresponding products. This intermediate is preferred over that with seven-membered ring which should arise if the compound *VIg* gave a 4-aminobutyl derivative. The oxazine structure was proved directly: in the reaction of 9-(3-hydroxy-

propyl)-8-bromoadenine (*VIb*) with ammonia the corresponding intermediate *IX* (besides the aminopropyl derivative *VIIb*) was isolated and characterized. Another derivative of this type, the compound *VIIIa*, was found in minor amount in the preparation of the amine *VIId* from the 2,3-dihydroxypropyl derivative *VId*. This mixture also contained 9-(2,3-dihydroxypropyl)-8-methoxyadenine (*Xa*), arising from the oxazine *VIIIa* during isolation.

The five- and six-membered cyclic ethers can thus arise readily but the six-membered oxazine derivatives decompose less easily on the nucleophilic attack by ammonia. If, however, the oxazine ring carries a hydroxyl in position 3 (*VIII*), it is more sensitive to a nucleophilic attack than the unsubstituted heterocyclic system in compound *IX*.

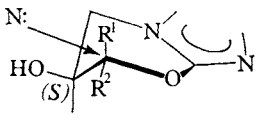


VIIIa, R = H
VIIIb, R = CH_2
VIIIc, R = CH_2OH

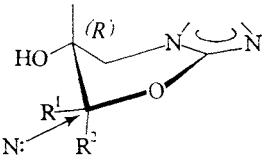


IX

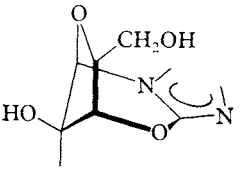
From the stereochemistry of the formed intermediates it is obvious that the five--membered oxazoline ring, fused with the adenine (imidazole) ring in positions 8 and 9, is practically planar and highly strained. If its formation is forced (by the structure of compound *VI*), it is easily opened under the reaction conditions. On the contrary, an oxazine ring, fused to the adenine system, is more stable and can exist



A1



A2

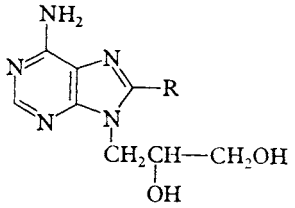


B

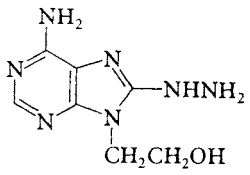
in two energetically similar conformations A1 and A2. Both enantiomers of *VIIIa* formed from the racemic compound *VIa* (3*S* and 3*R*) can thus assume a conformation with equatorial hydroxyl. The conformation of the oxazine ring in A1 is almost identical with that of 3',8-anhydro-9-(β-D-xylofuranosyl)-8-hydroxyadenine (**B**).

As seen from the stereochemistry of the intermediates (A1 and A2), there is no steric hindrance to a nucleophilic attack at either center in these systems.The arising 8-hydroxyadenine anion is sufficiently mesomerically stabilized by the assistance of the $N_{(7)}$ atom.

Reactions of adenosine 2',8-anhydronucleosides with ammonia or amines[2,12] proceed exclusively under formation of 8-aminoadenine nucleosides with nucleophilic attack at the substituent at $C_{(8)}$ of the imidazole ring, *i.e.* with configurational inversion in position 2'. The same course has *e.g.* the reaction with a hydrogen halide in alcohol[2]; in aprotic solvents, however, the nucleophilic attack takes place from the direction of the $C_{(2)}$ atom[2,13]. Although the chemistry of 3',8-anhydronucleosides of this type, analogous to the cyclic ethers *VIII* and *IX*, is not sufficiently investigated[14], we may assume that the reaction with amines will also lead to 8-aminoadenine derivatives by fission at the $C_{(8)}$ atom. There is thus a fundamental difference in mechanism of reaction of both types of compounds (nucleosides and their open-chain analogues) that cannot be evoked by a different electron density at $C_{(8)}$: The oxazine or oxazoline ring in the open-chain analogs is opened exclusively by a nucleophilic attack in position 2. The reaction of compounds *VIa* gave a very small amount of 9-(2,3-dihydroxypropyl)-8-aminoadenine (*Xb*) (whose structure was proved by UV and mass spectra and by comparison with an authentic material[10]), however, we cannot exclude that this compound is a minor product of direct nucleophilic substitution not involving the cyclic intermediate[11].



Xa, R = OCH_3
Xb, R = NH_2
Xc, R = OH



XI

Whereas compounds *VI* react with ammonia regiospecifically (forming exclusively 3-aminoalkyl derivatives), the reaction with the more nucleophilic 1,3-diaminopropane affords about equal amounts of the 2-amino and 3-amino derivatives[1]. The intramolecular cyclization – or formation of an analogous transition state – is apparently thermodynamically controlled in the former case and kinetically controlled in the latter.

To check the general character of these reactions, we studied also the reaction of 8-bromoadenine hydroxyalkyl derivatives *VI* with hydrazine, using 9-(2-hydroxyethyl)-8-bromoadenine (*VIa*) as the model compound. It appeared that on treatment

with 30% aqueous hydrazine hydrate under conditions comparable with those used in the reaction with ammonia the reaction has another course, affording 9-(2-hydroxyethyl)-8-hydrazinoadenine (*XI*). Ultraviolet spectrum of this product (λ_{max} 270 nm in acidic and 267 nm in alkaline medium with hyperchromic effect) differs unequivocally from that of 8-hydroxyadenine (*VII*); its ^{1}H NMR spectrum proves the presence of methylene groups as well as hydroxyl in the side-chain. The spectrum exhibits also typical signals due to C—NH_2 and N—NH_2 groups and the NH group of the hydrazine moiety. The only singlet of the proton at $C_{(2)}$ confirms substitution in position $C_{(8)}$ but the NMR spectrum gives no information on the mutual position of the amino and hydrazino groups. In the mass spectrum the most intensive peak is due to the molecular ion (*m/z* 209, $C_7H_{11}N_7O$) which loses the NH, NH_2, NH_3 and N_2H_2 species. Another abundant peak corresponds to the (base + H) ion (*m/z* 165, $C_5H_7N_7$) from which the NH, NH_2 and NH_3 fragments are cleaved off. The arising ions lose finally also HCN. The mass spectrum also confirms the presence of an amino and hydrazino group bonded to the heterocyclic ring. The final structural proof was obtained only after removal of the hydrazino group by action of silver oxide[15]. This reaction gave a single product whose mass spectrum (*m/z* 179) and ^{1}H NMR spectrum (2 singlets of protons on the heterocyclic ring, 1 amino group and a hydroxyethyl group) proved the expected removal of the hydrazine functionality. Its UV spectrum corresponded to a 9-substituted adenine (λ_{max} 260 nm) and the chromatographical behaviour (HPLC) to the authentic 9-(2-hydroxyethyl)adenine (*Va*). The hydrazinolysis product can therefore have only the structure *XI* and it is probably formed *via* a cyclic oxazoline derivative which is then opened by hydrazine attack in position C(8). The same reaction path has been observed for the reaction of hydrazine with 2',8-anhydronucleosides of adenine[12]. Thus, this way cannot lead to derivatives analogous to compounds *VII* with the hydrazino group in the side-chain.

Mass spectra of 9-(aminoalkyl)-8-hydroxyadenines (*VII*) are given in Table I. All the studied compounds of this series give molecular peaks of relatively small intensity (rel. intensity 1–4%), the only exception being the 3-aminopropyl derivative *VIIb* (37%). Further fragmentation depends on the structure of the aminoalkyl chain (presence of hydroxy groups). The β-fission of the C—C bond in an alkyl chain vicinal to the amino or hydroxyl group predominates[16] and the arising ions form one of the most intensive peaks from which the position of the OH and NH_2 groups on the alkyl chain can be determined by high resolution technique. Compounds with a terminal CH_2NH_2 group on the alkyl chain (*VIIa*, *VIIb*, *VIId* and *VIIe*) lose this group; except *VIId* in which this cleavage predominates due to the hydroxyl on the neighbouring carbon atom all the compounds lose also a fragment of *m/z* 29. According to high resolution measurements, this fragment is probably a NH_2CH group, arising by β-cleavage with hydrogen transfer[17]. In the case of compound *VIIe* the formed ion eliminates water and then CO (metastable transitions). Elimination of ammonia was observed only with compounds *VIIb* and *VIIf*.

TABLE I

Mass spectra of 9-(aminoalkyl)-8-hydroxyadenines (*VII*)

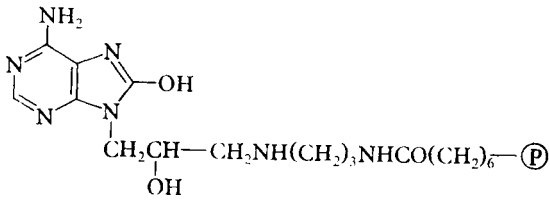
Ion + compound	*m/z* and (relative intensity)						
	VIIa	*VIIb*	*VIIc*[a]	*VIId*	*VIIe*[b]	*VIIf*	*VIIg*
M + H	—	—	—	225 (1·3)	225 (2·2)	239 (0·9)	—
M	194 (4·4)	208 (37·4)	208 (0·7)	—	—	238 (1·2)	254 (2·8)
M − NH_3	—	191 (15·4)	—	—	—	221 (6·0)	—
M − $CHNH_2$	165 (79·8)	179 (26·9)	—	—	195 (17·6)	—	—
M − CH_2NH_2	164 (29·1)	178 (32·4)	—	194 (100)	194 (7·6)	—	—
BCH_2CHOH	—	—	—	—[c]	—	194 (41·4)	194 (16·8)
B + CH_3	—[d]	165 (100)	165 (100)	165 (11·5)	165 (9·0)	165 (8·6)	165 (14·3)
B + CH_2	—[c]	164 (17·0)	164 (14·7)	164 (9·1)	164 (5·1)	164 (14·4)	164 (13·8)
B + 2 H	152 (100)	152 (53·8)	152 (7·8)	152 (69·7)	152 (65·8)	152 (100)	152 (47·7)
B + H	151 (10·3)	151 (72·0)	151 (2·6)	151 (48·5)	151 (19·3)	151 (41·4)	151 (100)
Adenine + H	136 (88·2)	136 (54·9)	136 (41·1)	136 (13·2)	136 (20·7)	136 (13·3)	136 (49·3)

[a] 193/2·1 (M − CH_3); [b] 177/100 (195 − H_2O), 149/82·9 (177 − CO); [c] identical with M − CH_2NH_2; [d] identical with M − $CHNH_2$.

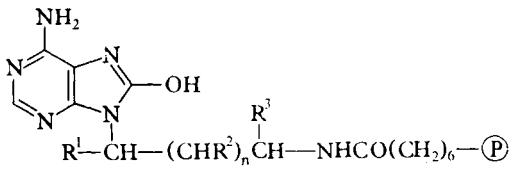
Characteristic for the spectra of compounds *VII* is the (base + 2 H) peak which corresponds to the 8-hydroxyadenine nucleus (*m/z* 152) and is usually accompanied by the (base + H) peak (*m/z* 151). The fragmentation of 8-hydroxyadenine manifests itself only in the low mass region by gradual elimination of HCN (ref.[18]). The peaks are of low intensity since the heteroaromatic 8-hydroxyadenine system is stable.

Behaviour of compounds VII *towards* SAH-*hydrolase.*

Polymeric materials of the Sepharose type with bound compounds *II* exhibit a high affinity towards rat liver SAH-hydrolase[3]. These carriers contain bonds of the type *XII* in which the aminopropyl group acts as a spacer. Since materials of the type *VII* are more accessible than the aminopropylamino derivatives *II* we investigated the polymeric carriers *XIII*, derived from the former compounds (*VII*). They were pre-



XII



XIII

a, $R^1 = R^3 = H$, $n = 0$
b, $R^1 = R^2 = R^3 = H$, $n = 1$
c, $R^1 = H$, $R^3 = CH_3$, $n = 0$
d, $R^1 = R^3 = H$, $R^2 = OH$, $n = 1$
e, $R^1 = CH_2OH$, $R^3 = H$, $n = 0$
f, $R^1 = H$, $R^2 = OH$, $R^3 = CH_3$, $n = 1$
g, $R^1 = H$, $R^2 = OH$, $R^3 = CH_2OH$, $n = 1$

Ⓟ ... Sepharose 4B matrix

pared by reaction of compounds *VII* (usually 2–3 equivalents of the carrier carboxylate capacity) with a ω-carboxyhexyl derivative of a dextrane gel (*e.g.* CH-Sepharose 4B) in water in the presence of a soluble carbodiimide. After removal of excess compound and reagent, the binding of compounds *VII* to the carriers was followed directly by UV-spectral measurement of the product dispersed in 50% aqueous

glycerol. The affinity of the carriers *XIII* was evaluated using a partially purified rat liver SAH-hydrolase preparation[19]. We followed the binding of the enzyme to, and its liberation from, the carrier in elution with solutions of increasing ionic strength (in addition to the activity of the liberated enzyme we determined also the amount of the liberated contaminating proteins) as well as in final elution with dilute adenosine solution. The results of the evaluation are given in Table II. Although under the experimental conditions used SAH-hydrolase was bound quantitatively to all the studied preparations, the binding was not strong enough for carriers containing ligands with amino group on the secondary carbon atom (*VIIc*, *VIIf*, *VIIg*). On the contrary, all ligands bound *via* amino group located on the primary carbon atom (*VIIa*, *b*, *d*, *e*) are highly active towards the enzyme. It is immaterial whether the alkyl chain is composed of two or three carbon atoms (*VIIa*, *b*, *e*) or contains a hydroxyl (*VIIb*, *VIId*). The affinity character of the enzyme binding to these carriers is thus obviously due solely to the 8-hydroxyadenine moiety bound to the polymeric chain *via* the alkyl group in position 9. It is further apparent that the hexyl group of the polymer represents a sufficiently long spacer so that introduction of an additional three--carbon chain in compounds of the type *II* is not necessary.

The reason for this behaviour of compounds *VII* is not known with certainty. As follows from the values in Table III, neither the ligands *VII* nor 9-(2,3-dihydroxypropyl)-8-hydroxyadenine[20] (*Xc*) show any inhibitory activity towards SAH--hydrolase. Also compounds *II* do not inhibit the enzyme *in vitro*[3]. Therefore, the affinity towards SAH-hydrolase may be exhibited also by carriers prepared from

TABLE II

Affinity of modified CH-Sepharose 4B towards rat liver SAH-hydrolase

Compound	Ligand		SAH-Hydrolase yield, %	
	type	content[a]	nonspecific desorption	specific elution[b]
XIIIa	*VIIa*	8·0	2·5	32
XIIIb	*VIIb*	12·0	0	39
XIIIc	*VIIc*	8·0	15	29
XIIId	*VIId*	10·0	0	40
XIIIe	*VIIe*	7·0	0	33
XIIIf	*VIIf*	7·0	[c]	n.d.[d]
XIIIg	*VIIg*	10·0	[c]	n.d.

[a] μmol *VII* bound per ml gel; [b] 0·025 mol l^{-1} adenosine in 0·75 mol l^{-1} potassium chloride; [c] very strong; [d] n.d. not determined.

other types of polymers and ligands, *e.g.* from 9-(6-aminohexyl)-8-hydroxyadenine and cyanogen bromide-activated Sepharose 4B. From the practical viewpoint, the ligands *VII* (preferentially *VIIa* and *VIId*) are more advantageous because of good accessibility of the starting compounds *Va* and *Vd* and their facile conversion to the aminoalkyl derivatives *VIIa* and *VIId*. Since the polymeric carrier *XIIId* appears to be more effective, it can be recommended as an easily accessible and most effective affinity material for isolation of SAH-hydrolases from crude homogenates. Its practical use has been also confirmed *e.g.* by isolation of SAH-hydrolase from crude homogenate of ovaries of the bugs *Pyrrhocoris apterus* L. (ref.[6]).

EXPERIMENTAL

Unless otherwise stated, the solvents were evaporated at 40°C/2 kPa and the compounds dried at 13 Pa over phosphorus pentoxide. Melting points were determined on a Kofler block and are uncorrected. Paper chromatography was performed on paper Whatman No 1 in 2-propanol–conc. aqueous ammonia–water (7 : 1 : 2) (S1), thin-layer chromatography on Silufol UV 254 plates in the following systems: chloroform (S2), chloroform–methanol (4 : 1) (S3), chloroform–methanol (7 : 3) (S4), chloroform–methanol (3 : 2) (S5). The R_F values are given in Table IV. Preparative chromatography on silica gel was performed on columns of Silpearl (20—30 μ, 200 g) in chloroform. Chromatography on microcrystalline cellulose (Macherey and Nagel) was carried out on a 100 × 2·5 cm column in the system S1; elution rate 20 ml/h, detection at 254 nm on a Uvicord (LKB, Uppsala, Sweden) instrument. Ultraviolet absorption spectra were measured in aqueous solutions on a Specord UV-VIS (Carl Zeiss, Jena, G.D.R.) spectrophotometer. ^{1}H NMR Spectra were taken on a Varian 100 instrument in hexadeuterodimethyl sulfoxide with hexamethyldisiloxane as standard; chemical shifts are given in ppm, coupling constants in Hz. Mass spectra were obtained with an AEI MS 902 spectrometer (ion source temperature 120°C, electron energy 70 eV) using a direct inlet system. The elemental composition was determined at the resolution 10 000.

TABLE III
in vitro Inhibition of rat liver SAH-hydrolase

Compound	v_i/v_0[a]	Compound	v_i/v_0[a]
Vd	0·63	*VIIe*	0·92
VIIa	0·95	*VIIf*	0·92
VIIb	1·00	*VIIg*	0·86
VIIc	0·92	*Xc*	0·92
VIId	0·73		

[a] Initial rate of inhibited (v_i) and noninhibited (v_0) hydrolysis of S-adenosyl-L-homocysteine; conditions *cf.* Experimental. K_M SAH = $8{\cdot}33 \cdot 10^{-6}$ mol, $v_0 = 0{\cdot}84 \cdot 10^6$ mol l^{-1} min^{-1}.

Starting compounds. The following compounds were prepared according to the described procedures: *Vb* (ref.[8]), *Vc* (ref.[9]), *Vd* (ref.[8]), *Vf* (ref.[21]), *Vg* (ref.[22]), *VId* (ref.[1]). All compounds were pure according to the published properties.

9-(2-Hydroxyethyl)adenine (*Va*)

A stirred mixture of adenine (14 g; 0·1 mol), ethylene carbonate (10 g; 0·18 mol) and dimethylformamide (400 ml) was taken to the boil (calcium chloride protecting tube) and solid sodium hydroxide (0·15 g) was added. The mixture was refluxed under stirring for 2 h (bath temperature 150°C) and evaporated at 60°C/2 kPa. The residue was taken up in boiling ethanol (800 ml) and the extract was filtered through Celite and set aside overnight in a refrigerator. The crystallized product was collected on filter, washed with ethanol and ether and dried. The mother liquor was taken down *in vacuo*, the residue was crystallized from ethanol and processed as above; total yield of compound *Va*, m.p. 241–242°C, was 13·2 g (71%) (reported[23] m.p. 238–239°C). For $C_7H_9N_5O$ (179·2) calculated: 46·91% C, 5·06% H, 39·09% N; found: 47·06% C, 5·07% H, 39·04% N.

9-(3-Hydroxypropyl)adenine (*Vb*)

A solution of benzoyl cyanide (28·8 g; 0·22 mol) in acetonitrile (50 ml) was added dropwise during 2 h to an ice-cooled stirred solution of 1,3-propanediol (15·2 g; 0·2 mol) and triethylamine (5 ml) in acetonitrile (200 ml) under exclusion of moisture. The mixture was stirred and cooled

TABLE IV
Values of R_F

Compound	R_F		Compound	R_F (S1)
	S1	S3		
Va	0·60	0·40[a]	*VIIa*	0·38
Vb	0·73	0·32	*VIIb*	0·40
Vc	0·78	0·40	*VIIc*	0·53
Vd	0·57	—	*VIId*	0·35
Ve	0·60	0·27	*VIIe*	0·40
Vf	0·62	0·43[b]	*VIIf*	0·40
Vg	0·50	—	*VIIg*	0·35
VIa	0·72	—	*VIIIa*	0·27
VIb	0·85	0·63	*IX*	0·56
VIc	0·84	0·63	*Xa*	0·70
VId	0·65	—	*Xb*	0·43
VIe	0·68	0·57	*XI*	0·55[c]
VIf	0·72	0·68[b]	*XII*	0·60[a]
VIg	0·60	—		

[a] 0·40 in S5; [b] in S4; [c] 0·10 in S5.

with ice for 3 h and coevaporated with ethanol (10 ml). Chromatography of the residue on a column of silica gel in chloroform (*vide supra*) afforded 11·7 g (20·6%) of 1,3-dibenzoyloxypropane (R_F 0·44, crystallized from light petroleum) and 20·2 g (56%) of the monobenzoate *IIIb* (R_F 0·15 in S2). Compound *IIIb* (0·112 mol) in pyridine (50 ml) was added dropwise with stirring and ice-cooling during 1 h to a solution of *p*-toluenesulfonyl chloride (23 g; 0·12 mol) and 4-dimethylaminopyridine (0·2 g) in pyridine (100 ml). After stirring at 0°C for 3 h and at room temperature overnight, the mixture was diluted with ethyl acetate (500 ml), washed successively with two 100 ml portions of water, dilute (1 : 10) hydrochloric acid (to acid reaction), water, saturated sodium hydrogen carbonate solution (2×), water, and dried over magnesium sulfate. The solvents were evaporated and the product was crystallized from ether – light petroleum to give 22 g (58%) of compound *IIIc*, m.p. 78°C; R_F 0·40. For $C_{17}H_{18}O_5S$ (334·4) calculated: 61·06% C, 5·42% H, 9·59% S; found: 60·76% C, 5·24% H, 9·49% S.

Compound *IIIc* (10·4 g; 31 mmol) was added to a suspension of sodium salt of adenine (prepared by stirring of adenine (4·05 g; 30 mmol) and sodium hydride (0·72 g; 30 mmol) in dimethylformamide (100 ml) for 1 h at 100°C. The arising solution was heated to 100°C for 9 h under exclusion of moisture and taken down at 60°C/2 kPa. The residue was extracted several times with boiling chloroform (500 ml total), the extract filtered through Celite and the solvent evaporated *in vacuo*. Crystallization from methanol (150 ml) afforded 5·25 g (57%) of the O-benzoyl derivative of *Vb* (R_F 0·50 in S3). This product was taken to the boil with 0·05 mol l^{-1} methanolic sodium methoxide (150 ml) and set aside for 1 h. The mixture was neutralized by addition of dry Dowex 50 X 8 (H^+ form), made alkaline with triethylamine, filtered and the solid washed with methanol (300 ml). The filtrate was taken down *in vacuo*, the residue mixed with water (200 ml) and extracted with ether (3 × 100 ml). The aqueous layer was taken down and the residue was crystallized from ethanol (100 ml) with ether added to saturation. The product, crystallized in an refrigerator, was collected on filter, washed with ether and dried *in vacuo*; yield 2·7 g (79%) of compound *Vb*, m.p. 211 °C. For $C_8H_{11}N_5O$ (193·2) calculated: 49·73% C, 5·74% H, 36·25% N; found: 49·69% C, 5·63% H, 36·33% N. UV Spectrum (pH 2): λ_{max} 261 nm, ε_{max} 13 400. The product was identical (in the systems S1 and S3) with the material prepared according to ref.[8].

9-(*RS*)-(2-Hydroxypropyl)adenine (*Vc*)

Triethylamine (1 ml) was added at 0°C to a stirred solution of 1-O-*p*-toluenesulfonylpropane-1,2-diol (*IVa*, ref.[9]; 22 g; 96 mmol) in dichloromethane (300 ml), and a solution of benzoyl cyanide (13·1 g; 0·1 mol) in dichloromethane was added dropwise at 0°C over 20 min with stirring. After stirring at 0°C for 1 h, methanol (10 ml) was added, the mixture washed with water (100 ml), dried over magnesium sulfate and taken down *in vacuo*. The residue which crystallized on mixing with light petroleum (200 ml) at 0°C, was filtered, washed with light petroleum and dried *in vacuo*, affording 26·8 g (84%) of compound *IVb*, m.p. 90—91°C (ethyl acetate–light petroleum), R_F 0·44 in S2 (for *IVa* R_F 0·22 in S2). For $C_{17}H_{18}O_5S$ (334·4) calculated: 61·06% C, 5·42% H, 9·59% S; found: 61·20% C, 5·44% H, 9·51% S.

Compound *IVb* (25 g; 75 mmol) was added to a suspension of sodium salt of adenine (75 mmol) in dimethylformamide (300 ml), prepared as described in the preparation of *Vb*, and the mixture was heated to 100°C for 8 h under exclusion of moisture. After evaporation at 60°C/2 kPa, the residue was extracted with chloroform (500 ml total), the extract was filtered through Celite and taken down. The residue was purified by chromatography on silica gel in chloroform (*vide supra*), affording 13 g (58·5%) of 2-O-benzoyl derivative of *Vc* (R_F 0·50 in S3). This product was briefly boiled with 0·05 mol l^{-1} methanolic sodium methoxide (200 ml) and processed as described for compound *Vb*. Crystallization from ethanol (ether added to turbidity) afforded 7·5 g (85%) of compound *Vc*, m.p. 200°C (dec.), identical with an authentic specimen[9] in S1 and S3.

Preparation of 9-Alkyl-8-bromoadenines (*VI*)

Method A. Compound *V* (10 mmol) was added to a solution of bromine (1 ml; 19·6 mmol) in water (150 ml). After stirring at room temperature in a stoppered flask overnight, the mixture was taken down at 40°C/2 kPa. The residue was dissolved in water (100 ml), the stirred solution adjusted to pH 7·0 (±0·05) with 4 mol l^{-1} sodium or lithium hydroxide and the formed suspension cooled with ice. After 2 h the product was collected on filter, washed successively with ice-cold water (200 ml), acetone (100 ml) and ether (100 ml), and dried *in vacuo*. Yields and properties of the compounds *VI* prepared by this procedure are given in Table V.

Method B. The reaction was performed in the same manner as described under *A*). If the product had not precipitated (or had precipitated only partly) on neutralization, the mixture was applied on a column of Dowex 50 X 8 (H^+-form, 100 ml). The column was washed with water to drop of conductivity and UV-absorption of the eluate. The resin was suspended in water (200 ml) and dilute (1 : 20) aqueous ammonia was added to keep the pH value below 8·5 (monitored with a pH-meter) until a constant value of 7·5—8·0 (15 min) was achieved. The suspension was filtered, washed with water (200 ml) and the filtrate was taken down. The residue was dried by codistillation with ethanol (2 × 50 ml), dissolved in methanol (100 ml) and adsorbed on silica gel (50 g). After evaporation of the solvent *in vacuo*, the silica gel was suspended in chloroform, applied on a column of silica gel (*vide supra*) and the product was eluted with chloroform–methanol. Fractions, containing the pure compound *VI*, were combined, taken down *in vacuo* and the product was crystallized from ethanol (ether added to saturation). Yields and properties of the obtained compounds *VI* are given in Table V.

Preparation of 9-(Aminoalkyl)-8-hydroxyadenines (*VII*)

A suspension of compound *VI* (10 mmol) in concentrated aqueous ammonia (80 ml) was heated in a steel autoclave to 100—110°C for 8 h. After cooling, the clear pink solution was taken down *in vacuo*, the residue was dissolved in the system S1 (30 ml) and chromatographed on a column of cellulose in the same system (monitoring by thin-layer chromatography in S1). The pertinent fractions were combined, taken down *in vacuo*, the residue was codistilled with ethanol and crystallized from methanol (with ether added to turbidity). The filtered product *VII* was washed with ether and dried *in vacuo*. The mother liquor contained a further portion of *VII* in the form of its hydrogen carbonate which was re-precipitated from ether and dried over potassium hydroxide. Yields and properties of the obtained compounds *VII* are listed in Table VI.

Ammonolysis of Compound *VIc*

The reaction was carried out with 7·5 mmol of compound *VIc* according to the general procedure. After isolation by chromatography in the system S1, the residue was crystallized from 70% aqueous ethanol; yield 1·1 g (51%) of hydrobromide of compound *VIIc* which did not melt to 260°C. For $C_8H_{13}BrN_6O$ (289·2) calculated: 33·22% C, 4·53% H, 27·65% Br, 29·06% N;found: 33·26% C, 4·41% H, 27·20% Br, 29·20% N. Its UV spectrum and R_F (S1) were identical with those of the free base *VIIc*.

Evaporation of the mother liquor and crystallization of the residue from methanol–ether (see the general procedure) afforded further 0·55 g (35%) of compound *VIIc* (see Table VI).

Ammonolysis of Compound *VIb*

The reaction was performed with 10 mmol of compound *VIb* as described in the general procedure. Chromatography in the system S1 afforded two products which were crystallized from ethanol–

TABLE V

Preparation and properties of 9-alkyl-8-bromoadenines *VI*

Compound	Method (mmol)	Yield, % M.p., °C	UV spectrum[a] λ_{max}, nm ε_{max}	Formula (mol. mass)	M^{+b} *m/z*	Calculated/found % C	% H	% Br	% N
VIa	*A*	58	267	$C_7H_8BrN_5O$	258	32·57	3·12	30·97	27·14
	(30)	238–239	18 600	(258·1)		33·11	3·03	30·63	26·67
VIb	*B*	62	266	$C_8H_{10}BrN_5O$	272	35·30	3·70	29·38	25·74
	(15)	205–207	18 500	(272·2)		35·80	3·46	29·72	25·57
VIc	*B*	70	267	$C_8H_{10}BrN_5O$	272	35·30	3·70	29·38	25·74
	(40)	191–192	18 400	(272·2)		35·37	4·12	29·11	25·98
VId	*A*	92	267	$C_8H_{10}BrN_5O_2$	288	37·51	3·50	27·75	24·31
	(40)	260	18 500	(288·2)		37·29	3·48	27·82	24·60
VIe	*A*	50	266	$C_8H_{10}BrN_5O_2$	288	37·51	3·50	27·75	24·31
	(4)	235–236	18 800	(288·2)		38·02	3·20	28·00	24·73
VIf	*B*	75	266	$C_9H_{12}BrN_5O_2$	302	35·77	4·00	26·46	23·18
	(6)	224	18 300	(302·2)		35·89	3·83	27·13	22·76
VIg	*B*	95	267	$C_9H_{12}BrN_5O_3$	318	33·97	3·80	25·13	22·01
	(2)	260	18 200	(318·2)		34·15	3·98	25·02	21·83

[a] at pH 2; [b] mass-spectrum (mol. peak).

TABLE VI

Preparation and properties of 9-(aminoalkyl)-8-hydroxyadenines *VII*

Compound	Yield, % M.p. °C	UV-spectra, λ_{max} (ε_{max})			Formula (mol. mass)	M^{+a} *m/z*	Calculated/found		
		pH 2	pH 7	pH 12			% C	% H	% N
VIIa	57 251–252	269; 280 (11 200)	271 (13 000)	281 (14 200)	$C_7H_{10}N_6O$ (194·2)	194	43·29 43·02	5·19 5·14	43·28 42·90
VIIb	48 217–218	270; 282 (11 000)	271 (13 300)	280 (14 000)	$C_8H_{12}N_6O$ (208·2)	208	46·14 45·91	5·81 5·55	40·37 40·05
VIIc	86[b] 175–176	269; 281 (11 200)	271 (13 200)	280 (14 100)	$C_8H_{12}N_6O$ (208·2)	208	46·14 46·35	5·81 5·92	40·37 40·90
VIId	73 174	269; 280 (10 800)	272 (12 900)	281 (14 100)	$C_8H_{12}N_6O_2$ (224·2)	224	42·85 42·59	5·40 5·48	37·49 37·35
VIIe	56 145 (r.)	268; 281 (10 700)	271 (12 800)	281 (14 000)	$C_8H_{12}N_6O_2$ (224·2)	224	42·85 42·40	5·40 5·15	37·49 37·38
VIIf	54 168–170	269; (282) (11 000)	270 (13 200)	280 (14 100)	$C_9H_{14}N_6O_2$ (238·2)	238	45·37 45·62	5·92 6·12	35·28 35·41
VIIg	42 149	269; 284 (14 800)	270 (13 000)	281 (14 000)	$C_9H_{14}N_6O_3$ (254·3)	254	42·51 42·77	5·55 5·90	33·06 32·64

[a] Mass spectrum (mol. peak); [b] total yield (incl. hydrobromide).

-ether : compound *VIIb* (1·1 g; 53%, see Table VI) and compound *IX* (from the fraction of R_F 0·56 in S1), obtained as the hydrobromide (0·90 g; 33%) which did not melt up to 250°C. For $C_8H_{10}BrN_5O$ (272·2) calculated: 35·30% C, 3·70% H, 29·38% Br, 25·74% N; found: 35·80% C, 3·77% H, 28·86% Br, 26·30% N. Mass spectrum, *m/z* (%): 191 (100, M^+; $C_8H_9N_5O$), 163 (12·8, M − C_2H_4), 136 (48·9, 163 − HCN). UV Spectrum: λ_{max} 268 nm (pH 2 and 7).

Identification of Side-products of Reaction of Compound *VId* with Ammonia

A mixture of compound *VId* (11·6 g; 40 mmol) and concentrated aqueous ammonia (350 ml) was heated to 100°C for 12 h with stirring in an autoclave. After evaporation *in vacuo*, the residue was chromatographed in two parts on a column of cellulose in the system S1 and the product-containing fractions (R_F 0·30 in S1) were processed as described (see the general procedure); for yield and properties see Table VI. Fractions of R_F higher than 0·30 from both chromatographies were combined and applied to a column of Dowex 1X2 (OH^--form, 200 ml). The column was eluted with water (1 litre), 25% aqueous methanol (0·5 l) and 50% aqueous methanol (1 litre). On crystallization from water, the second eluate afforded 100 mg (1%) of compound *Xb*, not melting up to 260°C. For $C_8H_{12}N_6O_2$ (224·2) calculated: 42·85% C, 5·40% H, 37·49% N; found: 42·94% C, 5·32% H, 37·08% N. Mass spectrum, *m/z* (%): 224 (89·7; M), 207 (12·1; M − OH), 193 39·9; M − CH_2OH), 163 (62·6; B + CH_2), 151 (32·6; BH_2), 150 (100, BH), 123 (33·3; BH − HCN)*. UV Spectrum (λ_{max}): 270 nm (pH 2), 274 nm (pH 7, 12).

The third eluate gave on crystallization from water 20 mg (0·2%) of compound *Xa*, m.p. 199—200°C. For $C_9H_{13}N_5O_3$ (239·2) calculated: 45·18% C, 5·47% H, 29·28% N; found: 44·70% C, 5.22% H, 29·12% N. Mass spectrum, *m/e* (%): 239 (40·6; M), 222 (14·5; M − OH), 208 (58·3; M − CH_2OH), 179 (18·5; B + CH_3), 178 (34·5; B + CH_2), 166 (38·3; BH_2), 165 (100; BH), 164 (58·7; B), 150 (12·3), 136 (23·8), 135 (7·5), 123 (9·1), 108 (8·2). UV Spectrum (λ_{max}, nm): 265 (pH 2), 264 (pH 7, 12).

The further product obtained after crystallization from water was compound *VIIIa* (300 mg; 3·6%), not melting up to 260°C. For $C_8H_9N_5O_2$ (207·2) calculated: 46·37% C, 4·38% H, 33·81% N; found: 46·23% C, 4·30% H, 34·19% N. Mass spectrum, *m/z* (%): 207 (100; M, $C_8H_9N_5O_2$), 164 (28·9; B + CH_2, $C_6H_6N_5O$), 163 (10·2; M − CH_2CHOH), 151 (9·8; B, $C_5H_5N_5O$), 136 (43·5; 163 − HCN). UV Spectrum (pH 2, 7, 12): λ_{max} 266 nm.

Reaction of 9-(2-Hydroxyethyl)-8-bromoadenine (*VIa*) with Hydrazine Hydrate

A mixture of compound *VIa* (1·05 g; 4 mmol) and 30% aqueous hydrazine hydrate (30 ml) was heated to 110°C for 7 h in an autoclave. After cooling, the crystalline product was collected on filter, washed with water and recrystallized from water; yield 0·62 g (74%) of compound *XI*, not melting up to 260°C. For $C_7H_{11}N_7O$ (209·2) calculated: 40·18% C, 5·30% H, 46·87% N; found: 40·34% C, 5·45% H, 45·92% N. Mass spectrum, *m/z* (%): 209 (100; M; $C_7H_{11}N_7O$), 194 (13·1), 193 (7·2), 192 (4·5), 191 (5·1), 179 (12·0), 176 (5·6), 175 (7·7), 165 (74·1; BH, $C_5H_7N_7$), 164 (10·9), 163 (27·5), 150 (29·9; 165 − NH), 149 (51·5; 165 − NH_2), 148 (18·9), 136 (26·1), 135 (30·7; adenine), 134 (13·1), 123 (15·7), 122 (24·3), 121 (12·8). ^{1}H NMR Spectrum: 3·65 (br q, 2 H), 2′-CH_2; 4·01 (t, 2 H, J = 5·6), 1′-CH_2; 4·30 (br, 2 H) N—NH_2; 5·10 (br t, 1 H, J = 4·8) OH; 6·56 (br, 2 H) C—NH_2; 7·76 (s, 1 H) NH; 7·95 (s, 1 H) H_2. UV Spectrum: λ_{max} 269·5 nm (ε_{max} 17 200) (pH 2); 267 nm (13 600) (pH 12).

* B denotes heterocyclic base moiety.

Reaction of Compound *XI* with Silver Oxide

A stirred mixture of compound *XI* (0·41 g; 2 mmol), silver oxide (0·4 g) and water (25 ml) was refluxed for 90 min, filtered while hot through Celite which was then washed with boiling water (200 ml), and the filtrate was taken down *in vacuo*. Crystallization from ethanol (ether added to saturation) afforded 0·28 g (78%) of compound *Va*, m.p. 241–243°C. For $C_7H_9N_5O$ (179·2) calculated: 46·91% C, 5·06% H, 39·09% N; found: 47·15% C, 5·21% H, 38·84% N. Mass spectrum, *m/z* (%): 179 (27·5; M), 149 (38·7; B + CH_3), 148 (37·2; B + CH_2), 135 (100, BH), 108 (34·7; BH − HCN). ^{1}H NMR Spectrum: 3·70 (br q, 2 H) OCH_2; 4·20 (t, 2 H, J = 5·5) N—CH_2; 5·00 (br t, 1 H) OH; 7·14 (br, 2 H) NH_2; 8·07 + 8·13 (2 s, 2 × 1 H) H_2 + H_8. UV Spectrum: 261 nm (ε_{max} 13 900) (pH 2); 263 (14 500) (pH 7, 12).

Preparation of Polymer Carriers *XIII*

On a fritted glass filter, swollen CH-Sepharose 4B (200 ml) was prewashed successively with 0·1 mol l^{-1} sodium hydrogen carbonate (5 l) and water (4 l). For the reaction, this gel (10 ml) was suspended in water (30 ml). Compound *VII* (250–500 μmol) was dissolved in this suspension which was then adjusted to pH 5·0 with 2 mol l^{-1} hydrochloric acid under magnetic stirring. N-Cyclohexyl-N′-trimethylammoniumpropylcarbodiimide *p*-toluenesulfonate (600–1 200 μmol), or N-cyclohexyl-N′-methylmorpholiniumcarbodiimide hydrochloride (600–1 200 μmol), was added, the pH being rendered between 5–5·5 by addition of hydrochloric acid. After stirring for 30 min, the same portion of the carbodiimide as before was added and the procedure was repeated. When the pH value remained constant, the suspension was adjusted to pH 5·0, gently shaken at room temperature overnight and filtered. The remaining gel was washed with water (500 ml) and suspended in 0·5 mol l^{-1} 2-aminoethanol hydrochloride (20 ml), pH 5·0. The third, same, portion of carbodiimide was added and, after stirring at pH 5·0–5·5 for 30 min and gentle shaking for 3·5 h, the solid was collected on filter and washed with water (1 litre) and saturated potassium chloride solution. The carriers were stored in saturated potassium chloride solution containing 0·02% of sodium azide. The data on the prepared carriers are given in Table II.

Determination of Activity Towards SAH-Hydrolase

The enzyme solution was prepared from the partially purified rat liver SAH-hydrolase[19] in Sörensen 0·02 mol l^{-1} sodium potassium phosphate buffer pH 7·37 with 0·001 mol l^{-1} dithiothreitol (0·18 e.u./ml). The carrier *XIII* (0·6 ml) was added to 1 ml of this solution, the suspension was gently shaken for 20 min at 0°C and centrifuged. After removal of the supernatant, the carrier was subsequently shaken for 20 min with the following solutions (à 1 ml): 0·01 mol l^{-1} sodium potassium phosphate buffer (*vide supra*), 0·2 mol l^{-1} sodium potassium phosphate buffer (*vide supra*), 1 mol l^{-1} potassium chloride, 1·5 mol l^{-1} potassium chloride, 2 mol l^{-1} potassium chloride, 0·025 mol l^{-1} adenosine in 0·75 mol l^{-1} potassium chloride (all the solutions contained 0·001 mol l^{-1} dithiothreitol). The enzymatic activity of all supernatants was determined by synthesis of S-adenosyl-L-homocysteine (*vide infra*). Evaluation of the carriers according to this procedure is given in Table II.

Determination of S-Adenosyl-L-homocysteine Hydrolase

The tested supernatant (50 μl) was added to a solution (200 μl) containing 0·1 mol l^{-1} sodium potassium phosphate pH 7·37 (*vide supra*), 0·003 mol l^{-1} dithiothreitol, $3{\cdot}75 \cdot 10^{-3}$ mol l^{-1} L-homocysteine and $2{\cdot}5 \cdot 10^{-5}$ mol l^{-1} adenosine. The mixture was incubated for 10 min at 37°C and a sample (10 μl) was applied on a 3·3 × 150 mm column of Separon SIX C18 (5 μ);

elution (0·4 ml/min) with 0·01 mol l^{-1} potassium dihydrogen phosphate, pH 2·8, containing 10% of methanol, detection at 254 nm. The adenosine and S-adenosyl-L-homocysteine peaks were integrated and from their ratio the enzymatic activity was determined (1 e.u. converts 1 μmol of the substrate in 1 min).

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REFERENCES

1. Holý A.: This Journal *48*, 1910 (1983).
2. Ikehara M., Ogiso Y.: Tetrahedron *28*, 3695 (1972).
3. Votruba I., Holý A., Rosenberg I.: This Journal *48*, 2459 (1983).
4. Merta A., Votruba I., Veselý J., Holý A.: This Journal *48*, 2701 (1983).
5. Šebestová L., Votruba I., Holý A.: This Journal *49*, 1543 (1984).
6. Votruba I., Holý A., Rosenberg I., Sláma K.: Insect Biochem. *15*, 631 (1985).
7. Holý A., Souček M.: Tetrahedron Lett. *1971*, 185.
8. Holý A.: This Journal *43*, 3444 (1978).
9. Holý A.: This Journal *40*, 187 (1975).
10. Holý A.: This Journal *43*, 3103 (1978).
11. Long R. A., Robins R. K., Townsend L. B.: J. Org. Chem. *32*, 2751 (1967).
12. Chattopadhyaya J. B., Reese C. B.: J. Chem. Soc., Chem. Commun. *1977*, 414.
13. Ikehara M., Maruyama T., Miki H., Takatsuka Y.: Chem. Pharm. Bull. *25*, 754 (1977).
14. Ikehara M., Nakenahara Y., Yamada S.: Chem. Pharm. Bull. *19*, 538 (1971).
15. Holý A.: This Journal *44*, 2846 (1979).
16. Grohlke R. S., McLafferty F. W.: Anal. Chem. *34*, 1281 (1962).
17. Beynon J. H., Saunders R. A., Williams A. E.: *The Mass Spectra of Organic Molecules*, p. 266. Elsevier, Amsterdam 1968.
18. Shaw S. J., Desiderio D. M., Tsuboyama K., McCloskey J. A.: J. Amer. Chem. Soc. *92*, 2510 (1970).
19. Votruba I., Holý A.: This Journal *45*, 3039 (1980).
20. Holý A., Votruba I., DeClercq E.: This Journal *50*, 245 (1985).
21. Holý A.: This Journal *47*, 173 (1982).
22. Holý A.: This Journal *44*, 593 (1979).
23. Kondo K., Iwasaki H., Ueda N., Takemoto K., Imoto M.: Makromol. Chem. *120*, 21 (1968).

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