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Notes

Potential Inhibitors of *S*-Adenosylmethionine-Dependent Methyltransferases. 7. Role of the Ribosyl Moiety in Enzymatic Binding of *S*-Adenosyl-L-homocysteine and *S*-Adenosyl-L-methionine

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A series of 2′,3′-acyclic analogues of *S*-adenosyl-L-homocysteine were synthesized and evaluated as inhibitors of *S*-adenosyl-L-methionine-dependent methyltransferases. The 2′,3′-acyclic analogues were prepared by periodate oxidation of the corresponding ribonucleosides, followed by reduction of the intermediate dialdehydes with sodium borohydride. These 2′,3′-acyclic ribonucleosides were inactive as inhibitors of histamine *N*-methyltransferase, catechol *O*-methyltransferase, phenylethanolamine *N*-methyltransferase, and hydroxyindole *O*-methyltransferase. These results suggest that the rigidity of the ribosyl ring of *S*-adenosyl-L-homocysteine is crucial to its enzymatic binding.

A general feature of *S*-adenosylmethionine (L-SAM)[2] dependent methyltransferases is the inhibition produced by the product, *S*-adenosyl-L-homocysteine (L-SAH).[3] In an effort to develop inhibitors of methyltransferases, several laboratories have reported the syntheses and the in vitro and in vivo biological activities of base or amino acid modified analogues of SAH.[3-7] Various sugar-modified analogues of SAH have also been synthesized and their inhibitory activities toward methyltransferases examined.[4,8-11] For example, the ribosyl group of SAH has been replaced by 2′-deoxyribosyl,[10] 3′-deoxyribosyl,[10] arabinofuranosyl,[10] 2′,3′-dihydroxycyclopentyl,[9,11] and cyclopentyl[8] groups. Coward and Sweet[8] have also reported the synthesis of a series of five carbon acyclic SAH analogues in which the 1′,4′-oxygen bridge of the ribosyl moiety was removed.

In an effort to further characterize the role of the ribosyl group of SAH in enzymatic binding, we have synthesized an acyclic analogue of SAH in which the 1′,4′-oxygen bridge has been retained but the 2′,3′-carbon bond cleaved, e.g., 2′-[*O*-[(*R*)-hydroxymethyl(adenin-9-yl)methyl]]-3′-[*S*-(*R*)-homocysteinyl]-3′-deoxy-(*S*)-glycerol (**1**, 2′,3′-acyclic L-SAH). Several related SAH analogues were also converted to their 2′,3′-acyclic derivatives (Chart I). The SAH analogues which were chosen for this study have well-recognized inhibitory activity toward specific methyltransferases [e.g., D-SAH,[12] histamine *N*-methyltransferase (HMT); L-SAHO,[12] catechol *O*-methyltransferase (COMT); 2-aza-SAH,[13] phenylethanolamine *N*-methyltransferase (PNMT); and 8-aza-SAH,[14] hydroxyindole *O*-methyltransferase (HIOMT)]. By converting these SAH analogues to their corresponding 2′,3′-acyclic derivatives, we could then evaluate the importance of the intact ribofuranosyl ring in binding to several methyltransferases. If these acyclic analogues exhibit inhibitory activity similar to the parent ribonucleoside, then the 2′,3′-acyclic ribosyl moiety might have general utility in the design of methyltransferase inhibitors.

Experimental Section

Melting points (uncorrected) were obtained on a calibrated Thomas-Hoover Uni-melt apparatus. Unless otherwise stated, the IR, NMR, and UV data were consistent with the assigned structures. IR data were recorded on a Beckman IR-33 spectrophotometer, NMR data on a Perkin-Elmer R-24B spectrophotometer (Me_4Si), and UV data on a Cary Model 14 spectrophotometer. Scintillation counting was done on a Beckman LS-150 scintillation counter. TLC were run on Analtech silica gel GF (250 μm) and Avicel F (250 μm). Spots were detected by visual examination under UV light and/or ninhydrin for compounds containing amine moieties.

Materials. SAM-$^{14}CH_3$ (New England Nuclear, 55.0 mCi/mmol) was diluted to a concentration of 10 μCi/mL and stored at −20 °F. SAM chloride (Sigma) was stored as a 10-mM aqueous stock solution. The following compounds were commercially available from the indicated sources: 3,4-dihydroxybenzoate (Aldrich), DL-β-phenylethanolamine, histamine hydrochloride, *N*-acetylserotonin, and L-SAH (Sigma). The SAH analogues were synthesized according to published procedures as cited below: D-SAH,[12] L-SAHO,[12] L-SAC,[15] 2-aza-SAH,[13] and 8-aza-SAH.[15]

General Procedure for Reduction of Ribonucleoside 2′,3′-Dialdehydes to 2′,3′-Acyclic Ribonucleosides. The ribonucleosides (e.g., L-SAH, D-SAH, SAM, etc.) were oxidized to the corresponding 2′,3′-dialdehydes using paraperiodic acid as previously described.[16] To a stirred solution of the nucleoside 2′,3′-dialdehyde (0.25 mmol) in 0.1 M phosphate buffer, pH 8.4 (6 mL), at ambient temperature was added slowly $NaBH_4$ (0.60 mmol) over a 30-min period. After 5 h the solution was adjusted to pH 5 with 5% HCl and then readjusted to pH 7 with 0.2 N NaOH. The product was purified by thick-layer chromatography on cellulose eluting with H_2O or EtOH–H_2O mixtures. The desired 2′,3′-acyclic ribonucleoside was recovered from the cellulose by extraction with H_2O, followed by lyophilization. The 2′,3′-acyclic ribonucleosides were not obtained in crystalline form, since their instability prohibited crystallization by classical techniques. The 2′,3′-acyclic ribonucleosides were characterized by their chromatographic properties (see Table I for the chromatographic systems used and the R_f values observed) and their spectral

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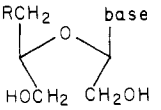
Chart I. 2′,3′-Acyclic Analogues of SAH as Potential Inhibitors of SAM-Dependent Methyltransferases



no.	compd abbreviations	R	base
1	2′,3′-acyclic L-SAH	-S(CH$_2$)$_2$CH(NH$_2$)CO$_2$H (L)	adenine
2	2′,3′-acyclic D-SAH	-S(CH$_2$)$_2$CH(NH$_2$)CO$_2$H (D)	adenine
3	2′,3′-acyclic L-SAHO	-S(→O)(CH$_2$)$_2$CH(NH$_2$)CO$_2$H (L)	adenine
4	2′,3′-acyclic SAC	-SCH$_2$CH(NH$_2$)CO$_2$H	adenine
5	2′,3′-acyclic 2-aza-SAH	-S(CH$_2$)$_2$CH(NH$_2$)CO$_2$H	2-azaadenine
6	2′,3′-acyclic 8-aza-SAH	-S(CH$_2$)$_2$CH(NH$_2$)CO$_2$H	8-azaadenine
7	2′,3′-acyclic SAM	-S$^+$(CH$_3$)(CH$_2$)$_2$CH(NH$_2$)CO$_2$H	adenine
8	2′,3′-acyclic adenosine	-OH	adenine

Table I. R_f Values for Ribonucleosides and 2′,3′-Acyclic Ribonucleosides on Thin-Layer Chromatography

compd	R_f values in chromatography system[a] A	B
SAH (L or D)	0.73	0.73
2′,3′-acyclic SAH (L or D) (1, 2)	0.65	0.72
L-SAC	0.74	0.79
2′,3′-acyclic L-SAC (4)	0.63	0.75
L-SAHO	0.72	0.74
2′,3′-acyclic L-SAHO (3)	0.60	0.74
2-aza-SAH	0.70	0.79
2′,3′-acyclic 2-aza-SAH (5)	0.69	0.66
8-aza-SAH	0.71	0.72
2′,3′-acyclic 8-aza-SAH (6)	0.69	0.58
SAM	0.34	0.20
2′,3′-acyclic SAM (7)	0.46	0.11

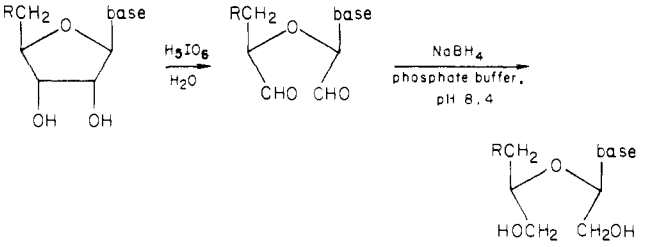
[a] The chromatography systems had the following compositions: system A, 5% Na_2HPO_4 on silica gel GF; system B, 9 parts of EtOH-HOAc-H_2O (20:2:2) and 1 part of 0.1 M phosphate buffer, pH 7.4, on silica gel GF.

properties (UV and NMR). In the NMR spectra several characteristic signals were consistently observed: e.g., δ 6.35–6.00 (t, 1 H, $J_{1'-2'} \approx 6$ Hz, $H_{1'}$) and 4.37–4.16 (d, 2 H, $H_{2'}$). The mass spectral data were consistent with fragmentation patterns reported for structurally related 5′-alkyl-5′-thioadenosine derivatives:[21,22] 2′,3′-acyclic L-SAH (1), 2′,3′-acyclic D-SAH (2), and 2′,3′-acyclic SAC (4) all exhibited m/e 284 [M$^+$ - (CH$_2$)$_{1-2}$CH(NH$_2$)CO$_2$H], 252 [M$^+$ - S(CH$_2$)$_{1-2}$CH(NH$_2$)CO$_2$H], 235 [M$^+$ - CH$_2$S(CH$_2$)$_{1-2}$CH(NH$_2$)CO$_2$H], 135 (adeninyl + H), and 136 (adeninyl + 2H); 2′,3′-acyclic 2-aza-SAH (5) and 2′,3′-acyclic 8-aza-SAH (6) both exhibited m/e 285 [M$^+$ - CH$_2$CH$_2$CH(NH$_2$)CO$_2$H], 253 [M$^+$ - SCH$_2$CH$_2$CH(NH$_2$)CO$_2$H], 136 (azaadeninyl + H), and 137 (azaadeninyl + 2H); 2′,3′-acyclic SAM (7) exhibited m/e 354 (M$^+$ - SCH$_3$), 299 [M$^+$ - CH$_2$CH$_2$CH(NH$_2$)CO$_2$H], 252 [M$^+$ - S(CH$_3$)CH$_2$CH$_2$CH(NH$_2$)CO$_2$H], 135 (adeninyl + H), and 136 (adeninyl + 2H); and 2′,3′-acyclic L-SAHO (3) exhibited m/e 135 (adeninyl + H) and 136 (adeninyl + 2H).

Reduction of Adenosine Dialdehyde to [2′,3′-$^2H_2$]-2′-[*O*-[(*R*)-Hydroxymethyl(adeninyl-9-yl)methyl]]glycerol. Adenosine dialdehyde[16] (265 mg, 1 mmol) in 5 mL of 2.5 μM NaOD in D_2O (~pH 8.4) was preincubated at ambient temperature for 1 h and then reduced with $NaBD_4$ (164 mg, 4 mmol) at ambient temperature for 4 h. The reaction mixture was adjusted to pH 5 with 5% HCl and then readjusted to pH 7.0 with 0.2 N NaOH. The resulting solution was applied to a cellulose column and the product eluted with H_2O. Lyophilization afforded an amorphous powder (259 mg, 96%): NMR (D_2O) δ 8.19, 8.32 (2 s, 2 H, H_2 and H_8), 5.98 (d, 1 H, $J_{1'-2'} = 6$ Hz, $H_{1'}$), 4.06 (d, 1 H, $J_{1'-2'} = 6$ Hz, $H_{2'}$), 3.40–3.90 (m, 4 H, $H_{3'}$, $H_{4'}$, and $H_{5'}$).

Enzyme Purification and Assay. The enzymes used in this study were purified from the following sources according to previously described procedures: COMT,[17] rat liver (male, Sprague–Dawley, 180–200 g); PNMT,[18] bovine adrenal medulla (Pel-Freez Biologicals); HMT,[19] guinea pig brain (Pel-Freez Biologicals); HIOMT,[20] bovine pineal glands (Pel-Freez Biologicals). The enzyme activities were measured and the analogues of SAH evaluated as inhibitors using the radiochemical techniques described in the preceding papers of this series.[15] In the experiments for screening the SAH analogues as enzyme inhibitors (Table II), the assay mixtures contained SAM-$^{14}CH_3$ (0.05 μCi, 1.0 mM) and the appropriate acceptor molecules with their final concentrations as follows: COMT, 3,4-dihydroxybenzoate (2.0 mM); PNMT, DL-β-phenylethanolamine (1.0 mM); HMT, histamine (1.0 mM); and HIOMT, *N*-acetylserotonin (1.0 mM).

Scheme I. General Route for the Synthesis of 2′,3′-Acyclic Analogues of SAH



R = -S(CH$_2$)$_2$CH(NH$_2$)CO$_2$H (D or L), -SCH$_2$CH(NH$_2$)CO$_2$H, etc.; base = adenine, 2-azaadenine, 8-azaadenine

Results

Chemistry. The 2′,3′-acyclic ribonucleosides which were prepared as potential inhibitors of methyltransferases are listed in Chart I. These compounds were prepared by the initial oxidation of the corresponding ribonucleoside using 1 equiv of periodic acid in H_2O (Scheme I) as described earlier by Borchardt et al.[16] The ribonucleoside dialdehydes were subsequently reduced with $NaBH_4$ to the desired 2′,3′-acyclic ribonucleosides. The R_f values for the parent ribonucleosides and the corresponding 2′,3′-acyclic ribonucleosides using two thin-layer chromatography systems are listed in Table I.

To further confirm the structure of these 2′,3′-acyclic ribonucleosides, adenosine dialdehyde was prepared by periodic acid oxidation of adenosine.[16] When adenosine dialdehyde was preincubated with NaOD in D_2O and then reduced with $NaBD_4$, two deuterium atoms were incorporated: one each at the former $C_{2'}$ and $C_{3'}$ positions. Since deuterium was not incorporated at former $C_{1'}$ and $C_{4'}$, the existence of enolic structures for adenosine dialdehyde could be ruled out. Therefore, it was assumed that the configurations at former $C_{1'}$ and $C_{4'}$ were retained during the periodate oxidation of the ribonucleoside and reduction to the 2′,3′-acyclic ribonucleoside. Based on these observations, the *R* configuration was assigned to the former $C_{1'}$ and the *S* configuration to the former $C_{4'}$ position in all the 2′,3′-acyclic ribonucleosides except 2′,3′-acyclic adenosine.[8]

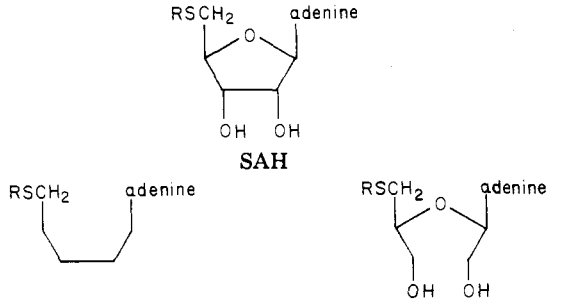
2′,3′-Acyclic Ribonucleosides as Inhibitors of Methyltransferases. The 2′,3′-acyclic ribonucleosides were

Table II. Inhibition of COMT, PNMT, HIOMT, and HMT by 2',3'-Acyclic Analogues of SAH[a]

compd	inhibitor concn, mM	% inhibition COMT	PNMT	HIOMT	HMT
L-SAH[b]	0.2	39	49	71	40
	2.0	87	92	94	89
2',3'-acyclic L-SAH (1)	0.2	0	8	9	0
	2.0	26	16	26	19
D-SAH[b]	0.2	5	14	1	73
	2.0	22	32	6	99
2',3'-acyclic D-SAH (2)	0.2	2	0	0	0
	2.0	25	0	6	0
SAHO[b]	0.2	14	10	0	0
	2.0	42	22	0	9
2',3'-acyclic L-SAHO (3)	0.2	5	9	7	0
	2.0	22	17	16	0
SAC[c]	0.2	0	11	12	14
	2.0	5	27	33	43
2',3'-acyclic L-SAC (4)	0.2	0	10	0	0
	2.0	12	15	0	3
2-aza-SAH[d]	0.4	3	38	23	0
	1.0	6	58	39	8
2',3'-acyclic 2-aza-SAH (5)	0.2	4	0	18	0
	2.0	5	15	25	0
8-aza-SAH[c]	0.2	5	8	49	4
	2.0	28	24	89	33
2',3'-acyclic 8-aza-SAH (6)	0.2	0	0	12	0
	2.0	17	10	31	0
2',3'-acyclic L-SAM (7)	0.2	7	1	0	0
	2.0	14	21	8	0

[a] The standard incubation mixture consisted of the inhibitor (0.2 or 2.0 mM), SAM (1.0 mM), methyl acceptor (1.0 mM), and buffer. Incubation was carried out at 37 °C and enzyme activity determined by extraction of the radiolabeled product. [b] Data taken from ref 12. [c] Data taken from ref 15. [d] Data taken from ref 13.

Chart II. Acyclic Analogues of SAH



R = -S(CH$_2$)$_2$CH(NH$_2$)CO$_2$H

tested as inhibitors of COMT, PNMT, HIOMT, and HMT, and the results are shown in Table II. For the sake of comparison, the inhibitory activities of the corresponding ribonucleosides are also listed. In each case the 2',3'-acyclic ribonucleosides were substantially less active as inhibitors of the methylases than the corresponding ribonucleosides. For example, L-SAH is a very potent inhibitor of all four enzymes, whereas 2',3'-acyclic L-SAH (1) is essentially inactive under identical assay conditions. Similarly, 2',3'-acyclic D-SAH (2) is substantially less active than D-SAH toward HMT, 2',3'-acyclic L-SAHO (3) is less active than L-SAHO toward COMT, 2',3'-acyclic 2-aza-SAH (5) is less active than 2-aza-SAH toward PNMT and HIOMT, and 2',3'-acyclic 8-aza-SAH (6) is substantially less active than 8-aza-SAH toward HIOMT. Consistent with these observations was the finding that 2',3'-acyclic L-SAM (7) was also devoid of methylase inhibitory activity (Table II).

Discussion

A potentially interesting type of sugar modification of SAH is acyclic analogues. Coward and Sweet[8] have reported the synthesis of 1',4'-acyclic SAH analogues (Chart II) which contain adenine, a 5-C bridge, and the thioether residue. In this study we prepared a series of 2',3'-acyclic SAH analogues where the 2',3' carbon–carbon bond of the ribonucleoside was cleaved. The 2',3'-acyclic SAH analogues retain major structural features of the corresponding ribonucleosides except for the rigidity of the ribosyl ring system. Considering the poor inhibitory activity of these 2',3'-acyclic ribonucleosides as compared to the corresponding ribonucleosides, it must be concluded that the rigidity of the ribosyl ring contributes significantly to enzymatic binding. The ribosyl ring of SAH, in addition to direct binding interactions with the enzyme[10] (e.g., through the 2'- or 3'-hydroxyl group), must provide a rigid framework which is necessary to properly orient the adenine and thioether residues for maximum interaction with protein.

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References and Notes

(1) Established Investigator of the American Heart Association.
(2) Abbreviations used are L-SAM, *S*-adenosyl-L-methionine; L-SAH, *S*-adenosyl-L-homocysteine; D-SAH, *S*-adenosyl-D-homocysteine; L-SAHO, *S*-adenosyl-L-homocysteine sulfoxide; L-SAC, *S*-adenosyl-L-cysteine; 2-aza-SAH, *S*-(2-azaadenosyl)-L-homocysteine; 8-aza-SAH, *S*-(8-aza-adenosyl)-L-homocysteine; 2',3'-acyclic L-SAM (7), 2'-[*O*-[(*R*)-hydroxymethyl(adenin-9-yl)methyl]]-3'-[*S*-(*R*)-methioninyl]-3'-deoxy-(*S*)-glycerol; 2',3'-acyclic L-SAH (1), 2'-[*O*-[(*R*)-hydroxymethyl(adenin-9-yl)methyl]]-3'-[*S*-(*R*)-homocysteinyl]-3'-deoxy-(*S*)-glycerol; 2',3'-acyclic D-SAH (2), 2'-[*O*-[(*R*)-hydroxymethyl(adenin-9-yl)methyl]]-3'-[*S*-(*S*)-homocysteinyl]-3'-deoxy-(*S*)-glycerol; 2',3'-acyclic L-SAHO (3), 2'-[*O*-[(*R*)-hydroxymethyl(adenin-9-yl)-methyl]]-3'-[*S*-(*R*)-homocysteinyl sulfoxide]-3'-deoxy-(*S*)-glycerol; 2',3'-acyclic SAC (4), 2'-[*O*-[(*R*)-hydroxymethyl(adenin-9-yl)methyl]]-3'-[*S*-(*R*)-cysteinyl]-3'-deoxy-(*S*)-glycerol; 2',3'-acyclic 2-aza-SAH (5), 2'-[*O*-[(*R*)-hydroxymethyl(2-azaadenin-9-yl)methyl]]-3'-[*S*-(*R*)-

homocysteinyl]-3′-deoxy-(S)-glycerol; 2′,3′-acyclic 8-aza-SAH (6), 2′-[O-[(R)-homocysteinyl(8-azaadenin-9-yl)methyl]]-3′-[S-(R)-homocysteinyl]-3′-deoxy-(S)-glycerol; HMT, histamine N-methyltransferase; COMT, catechol O-methyltransferase; PNMT, phenylethanolamine N-methyltransferase; HIOMT, hydroxyindole O-methyltransferase.
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Inhibition of Beef-Brain and Dog-Heart ($Na^+ + K^+$) Activated Adenosine Triphosphatase by Carbon-3 Branched Cardenolides

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Twenty-two C-3 branched cardenolides were investigated as inhibitors of beef-brain and dog-heart ($Na^+ + K^+$) activated adenosine triphosphatase. The synthetic compounds had lower inhibitory strength than digitoxigenin, and there was no indication of an improved safety index. Structure–activity relationships show that increased steric shielding of the 3-OH group results in reduced inhibition.

During our continued interest in the design and synthesis of cardiac glycosides with improved safety indices, a series of C-3 branched cardenolides was synthesized.[1-3] It was then of interest to evaluate if there exists any structure–activity relationship for these compounds.

Following the first report by Schatzmann[4] that ouabain binds to the ($Na^+ + K^+$) ATPase enzyme responsible for the active transport of potassium and sodium, a large amount of evidence was gathered which showed that the mode of action of cardiac glycosides is their inhibition of a membrane or microsomal ($Na^+ + K^+$) activated adenosine triphosphatase (E.C. 3.6.1.3).[5] We first selected beef-brain microsomal ATPase for our investigations, since it was reported that the receptor site of ATPase for cardiac glycosides is the same in all organs of the same species.[6,7] Thereafter, microsomal ATPase from dog hearts was also used.

Experimental Section

Beef brains were obtained fresh from the municipal slaughterhouse, Mannheim, and dog hearts were kindly supplied by Dr. Engelmann from our pharmacology department. The organs were frozen immediately and only partially thawed prior to use. Microsomal ATPase was isolated from beef brains according to Klodas et al.,[8] while for the isolation from dog hearts a slightly different procedure was used.[9] In accordance with Schwartz et al.,[10] the enzymatic activity of ATPase was followed in a linked enzyme system by reaction of primarily liberated ADP with pyruvate kinase and subsequent reaction of the pyruvate with NADH in the presence of lactate dehydrogenase. The monitoring of the oxidation of NADH in the photometer is a direct measure for the activity of ATPase. In the case of the inhibition of beef-brain ATPase, the linked enzyme system was incubated with the inhibitor for 5 min prior to addition of the substrate (ATP). The inhibitor was either dissolved in 1.0 mL of water or first in a few drops of dimethyl sulfoxide which was then made-up with water to a final volume of 1.0 mL. Under these conditions dimethyl sulfoxide did not effect the enzymatic reaction. The final volume of the reaction mixture was 2.95 mL. For inhibition studies with dog-heart ATPase, the microsomal enzyme preparation in a magnesium chloride, potassium chloride, and sodium chloride containing Tris-HCl solution (pH 7.4) was incubated with inhibitor solution for 5 min, after which time were added pyruvate kinase, lactate dehydrogenase, phosphoenol pyruvate, and NADH. The reaction was then started by adding ATP.

The enzymes, substrates, and reagents were obtained from Boehringer Mannheim and were of analytical grade. The photometer used was an Eppendorff Model 1101 equipped with an automatic cuvette programmer (Model 2702) and a Model 4412 recorder.

The inhibition of ATPase by the C-3 branched cardenolides was determined at at least four different inhibitor concentrations which were about equidistant on the log scale, and each determination was run in duplicate or triplicate. The linear regression of the percent inhibition and the logarithm of the inhibitor concentration were calculated on a Hewlett-Packard Calculator 9100 B.

Results

The isolation of microsomal ($Na^+ + K^+$) activated ATPase from beef brain resulted in preparations with enzymatic activities of about 50 μmol of ATP used per milligram of protein per hour. More than 95% of this activity was sensitive to ouabain or digitoxigenin inhibition. The enzymatic reaction was linear for at least 20 min. The ID_{50} values of the inhibitors with their confidence limits in parentheses are given in Table I. In Figure 1 the

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