Enzyme Inhibitors XXIV: Bridging Hydrophobic and Hydrophilic Regions on Adenosine Deaminase

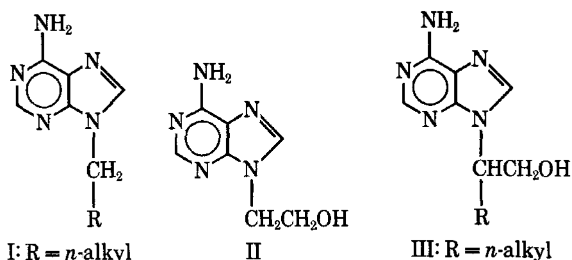
HOWARD J. SCHAEFFER* and CHARLES F. SCHWENDER†

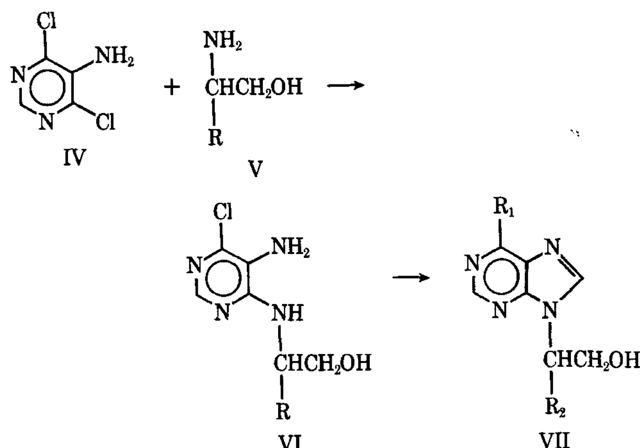
Abstract □ Previous studies have shown that adenosine deaminase has a large hydrophobic region which is important for enzyme inhibitor complex formation with some 9-*n*-alkyladenines. Further, the enzyme has a hydroxyl binding site which makes a contribution to enzyme inhibitor complex formation in a compound such as 9-(2-hydroxyethyl)adenine. In an attempt to gain information concerning the spatial relationship of these two binding regions, a variety of 9-(1-hydroxy-2-alkyl)adenines were prepared and found to be potent inhibitors of adenosine deaminase. These data argue strongly for the presence of a single binding site for the adenine moiety of the inhibitors and for the close spatial relationship of the hydrophobic region and the hydroxyl binding region on the enzyme.

Keyphrases □ Adenosine deaminase inhibitors—9-(1-hydroxy-2-alkyl)adenines, single binding site-bridging mechanism, theoretical □ Enzyme inhibitors—single binding site-hydrophobic-hydrophilic bridging, theoretical □ 9-(1-Hydroxy-2-alkyl)adenines—synthesis, adenosine deaminase inhibitors

Calf intestinal mucosal adenosine deaminase was found to possess several different types of binding regions for the 9-substituent of some 6,9-disubstituted purines (1). By the use of different inhibitors, it was possible to show the presence on adenosine deaminase of a specific methyl binding region (2), a hydroxyl binding site (3, 4), and a large hydrophobic region (5) which are important in forming complexes with the 9-substituent of the 9-substituted adenines.

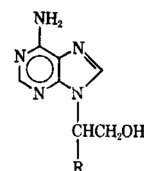
Since there are several different binding regions for the 9-substituent, it is possible that there are, in fact, three different binding sites for the three classes of inhibitors. However, the possibility also exists that the adenine moiety of these inhibitors always complexes to the same site on the enzyme and the 9-substituent bridges to the appropriate accessory binding region. It should be possible to obtain information concerning the identity or nonidentity of inhibitor binding sites by incorporating in one inhibitor the moieties that contribute to binding in two different inhibitors. 9-*n*-Alkyladenines (I) constitute a class of inhibitors in which the 9-substituent forms complexes with the large hydrophobic region on adenosine deaminase (5) and the hydroxyl group of 9-(2-hydroxyethyl)adenine is important in the formation of complexes with the hydroxyl binding site on the enzyme (2, 4). If the adenine moiety of these two types of inhibitors (I and II) complexes to the same site on the enzyme, and the accessory binding regions (hydrophobic and hydroxyl binding sites) are spatially closely associated with the site to which the adenine moiety binds, one would predict that an inhibitor bearing an alkyl chain and the 2-hydroxyethyl group at the 9-position of adenine (III) would be a potent inhibitor of adenosine deaminase. The purposes of this paper are: (*a*) to describe the synthesis of some 9-(1-hydroxy-2-alkyl)-





Scheme I

Table I—Inhibition of Adenosine Deaminase by:



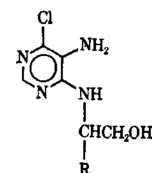
Compound[a]	R	$(I/S)_{0.5}$[b]	$\Delta G/CH_2$[c] (Calc.)
II[d]	H	1.1 ± 0.05[e]	—
VIII	CH_3	1.2 ± 0.03	—
IX	C_2H_5	0.49 ± 0.02	−521
X	C_3H_6	0.071 ± 0.004	−1140
XI	C_4H_9	0.033 ± 0.006	−451
XII	C_5H_{11}	0.015 ± 0.001	−467
XIII	C_6H_{13}	0.0062 ± 0.0002	−521
XIV	C_7H_{15}	0.0047 ± 0.00003	−163
XV	C_8H_{17}	0.0039 ± 0.00002	−110
XVI	C_9H_{19}	0.0030 ± 0.00006	−155

[a] None of these compounds served as substrates of adenosine deaminase. [b] The inhibition index $(I/S)_{0.5}$ is the ratio of the millimolar concentration of the inhibitor for 50% inhibition to the millimolar concentration of the substrate. The concentration of adenosine in all experiments was 0.066 m*M*. [c] Calculated by the equation

$$\Delta G = -RT \ln \left[\frac{(I/S)_{0.5} \text{ for Compound A}}{(I/S)_{0.5} \text{ for Compound B}}\right]$$

where A and B differ by one methylene group. [d] Data taken from *Reference 3.* [e] Average of two determinations.

Table II—Physical Constants and Analytical Data of:



Compound[a]	R	Melting Point	Yield, %	Recrystallization Solvent	Formula	Analysis, % Calc.	Analysis, % Found
XVII	CH_3	74–77°	82	H_2O	$C_7H_{11}ClN_4O$	C, 41.50 H, 5.47 Cl, 17.50 N, 27.66	C, 41.34 H, 5.51 Cl, 17.33 N, 27.63
XVIII	C_2H_5	164°[b]	47	H_2O	$C_8H_{13}ClN_4O$	C, 44.34 H, 6.05 Cl, 16.36 N, 25.86	C, 44.19 H, 6.15 Cl, 16.15 N, 25.88
XIX	C_3H_7	89–90°	48	MeOH–H_2O	$C_9H_{15}ClN_4O$[c]	C, 43.46 H, 6.87 Cl, 14.26 N, 22.53	C, 43.33 H, 6.85 Cl, 14.66 N, 22.40
XX	C_4H_9	102–109°	88	MeOH–H_2O	$C_{10}H_{17}ClN_4O$[d]	C, 46.67 H, 7.44 Cl, 13.15 N, 20.75	C, 47.08 H, 7.50 Cl, 13.37 N, 20.68
XXI	C_6H_{13}	102–105°	75	MeOH–H_2O	$C_{12}H_{21}ClN_4O$	C, 52.83 H, 7.77 Cl, 12.99 N, 20.54	C, 52.62 H, 7.73 Cl, 13.23 N, 20.61
XXII	C_7H_{15}	105–108°	71	MeOH–H_2O	$C_{13}H_{23}ClN_4O$	C, 54.44 H, 8.08 Cl, 12.36 N, 19.61	C, 54.34 H, 7.83 Cl, 12.52 N, 19.36
XXIII	C_8H_{17}	103–107°	70	MeOH–H_2O	$C_{14}H_{25}ClN_4O$	C, 55.90 H, 8.37 Cl, 11.79 N, 18.63	C, 56.00 H, 8.30 Cl, 11.66 N, 18.82
XXIV	C_9H_{19}	109–113°	64	MeOH–H_2O	$C_{15}H_{27}ClN_4O$	C, 57.21 H, 8.63 Cl, 11.26 N, 17.79	C, 57.26 H, 8.68 Cl, 11.41 N, 17.56

[a] These compounds were all prepared by minor modification of Method A from 5-amino-4,6-dichloropyrimidine and the appropriate 2-amino-1 alkanol. [b] Melting point taken on Kofler Heizbank. [c] Isolated as the monohydrate. [d] Isolated as $^1/_2$ $H_2O \cdot {}^1/_2$ CH_4O.

adenines (III), and (*b*) to provide evidence that, for adenosine deaminase, there is a single inhibitor binding site for, at least, several classes of inhibitors.

CHEMISTRY

The compounds required for this study were synthesized by the general procedure (1–7) of condensation of an appropriate amino alcohol (V) with 5-amino-4,6-dichloropyrimidine (IV) (Scheme I). The resultant substituted pyrimidine VI was cyclized with triethyl orthoformate to the 6-chloropurine intermediate (VII: R_1 = Cl), which, upon treatment with a variety of nucleophilic agents, gave the desired 6-substituted purines (VII). In all cases, the adenine derivative was prepared. In selected cases, the 6-substituent was methylamino, dimethylamino, hydroxy, mercapto, methoxy, hydrazino, or hydrogen. The amino alcohols (V) employed were the homologs from 2-amino-1-propanol through 2-amino-1-undecanol; *i.e.*, R equals CH_3 through C_9H_{19}.

DISCUSSION

Previous studies have shown that the hydroxyl binding site and the large hydrophobic site of adenosine deaminase cannot be bridged by adenine derivatives which are substituted at the 9-position by a straight-chain alkyl group bearing a hydroxyl group on carbon 2 (8). Examination of Table I, however, clearly demonstrates the dramatic increase in potency of inhibition that can result by incorporation of several moieties previously found to facilitate enzyme inhibitor complex formation into a single inhibitor molecule. For example, II has an $(I/S)_{0.5}$ of 1.1 and 9-*n*-heptyladenine (5) has an $(I/S)_{0.5}$ of 0.32, but the $(I/S)_{0.5}$ for XIII is 0.0062. The enhanced inhibitory properties of the 9-(1-hydroxy-2-alkyl)adenines are interpreted to be the results of a single binding site for the adenine moiety in compounds of general structure I, II, or III and of the close spatial relationship of the large hydrophobic region and the hydroxyl binding site to the site to which adenine is bound on the enzyme[1].

For this potent series of compounds, it was hoped that the effect of the 6-substituent on the magnitude of inhibition of adenosine deaminase could be accurately assessed. Therefore, several series were prepared with different substituents at the 6-position of the purine nucleus, particularly the 9-(1-hydroxy-2-octyl)- and the 9-(1-hydroxy-2-undecyl)-6-substituted purines. Unfortunately, those analogs substituted at the 6-position by dimethylamino, methoxy, mercapto, hydroxy, or chloro were either inactive or too weakly inhibitory to allow an accurate evaluation of $(I/S)_{0.5}$. The 6-hydrazino and 6-hydrogen analogs were sufficiently insoluble that reliable data on inhibition could not be obtained, although both substituents did cause weak inhibition when compared to the 6-amino derivative. The 6-methylamino derivatives exhibited 9–15 times less inhibition of adenosine deaminase when compared to the corresponding 6-amino analogs.

Two other points require brief comment. Normally, hydrophobic forces are considered to be relatively weak interactions. If one considers a single methyl or methylene, it is generally true that hydrophobic forces are relatively weak (9, 10). However, if the macromolecular system possesses a large hydrophobic region, the sum of

[1] The close spatial relationship of the hydrophobic region and the hydroxyl binding region may be native or may be the result of a conformational change in the enzyme induced by the enzyme inhibitor complex.

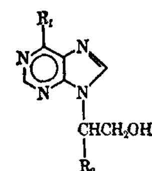


Table III—Physical Constants and Analytical Data of:

Compound	R_1	R_2	Melting Point	Yield, %	Recrystallization Solvent[a]	Method	Formula	Analysis, % Calc.	Analysis, % Found
XXV	Cl	CH_3	201–204°	47	$CHCl_3$	B	$C_8H_9ClN_4O$	C, 45.19 H, 4.22 Cl, 16.68 N, 26.35	C, 45.20 H, 4.40 Cl, 16.58 N, 26.13
VIII	NH_2	CH_3	209–212°	50	EtOH	C	$C_8H_{11}N_5O$	C, 49.73 H, 5.74 N, 36.25	C, 50.06 H, 6.02 N, 36.31
XXVI	NHMe	CH_3	158–160°	60	A	D	$C_9H_{13}N_5O$	C, 52.17 H, 6.33 N, 33.80	C, 51.91 H, 6.39 N, 34.07
XXVII	NMe_2	CH_3	106.5–108.5°	78	B	E	$C_{10}H_{15}N_5O$	C, 54.27 H, 6.83 N, 31.65	C, 54.35 H, 6.97 N, 31.90
XXVIII	OH	CH_3	232.5–235.5°	72	H_2O	F	$C_8H_{10}N_4O_2$	C, 49.47 H, 5.18 N, 28.85	C, 49.48 H, 5.37 N, 29.00
XXIX	SH	CH_3	272–274°[b]	44	iso-PrOH	G	$C_8H_{10}N_4OS$	C, 45.69 H, 4.81 N, 26.64 S, 15.24	C, 45.92 H, 5.00 N, 26.52 S, 15.30
XXX	Cl	C_2H_5	136°[c]	26	A	B	$C_9H_{11}ClN_4O$	C, 47.68 H, 4.89 Cl, 15.64 N, 24.71	C, 47.89 H, 4.69 Cl, 15.43 N, 24.83
IX	NH_2	C_2H_5	185–188°	26	EtOH	C	$C_9H_{13}N_5O$	C, 52.16 H, 6.32 N, 33.80	C, 52.22 H, 6.43 N, 33.93
XXXI	NHMe	C_2H_5	123–125°	52	B	D	$C_{10}H_{15}N_5O$	C, 54.27 H, 6.83 N, 31.65	C, 54.06 H, 6.86 N, 31.60
XXXII	NMe_2	C_2H_5	112–115°	73	B	E	$C_{11}H_{17}N_5O$	C, 56.14 H, 7.28 N, 29.77	C, 56.19 H, 7.29 N, 29.63
XXXIII	OH	C_2H_5	196–198°	44	MeOH	F	$C_9H_{12}N_4O_2$	C, 51.92 H, 5.81 N, 26.91	C, 51.63 H, 5.52 N, 26.70
XXXIV	SH	C_2H_5	280–285°	47	*n*-PrOH	G	$C_9H_{12}N_4OS$	C, 48.19 H, 5.39 N, 24.98 S, 14.30	C, 48.40 H, 5.49 N, 24.76 S, 14.47
XXXV	Cl	C_3H_7	112–114°	62	A	B	$C_{10}H_{13}ClN_4O$	C, 49.90 H, 5.43 Cl, 14.73 N, 23.28	C, 50.04 H, 5.52 Cl, 15.05 N, 23.29
X	NH_2	C_3H_7	147–148°	58	C	C	$C_{10}H_{15}N_5O$	C, 54.27 H, 6.83 N, 31.65	C, 54.03 H, 6.51 N, 31.59
XXXVI	Cl	C_4H_9	77–81°	70	A	B	$C_{11}H_{15}ClN_4O$	C, 51.87 H, 5.88 Cl, 13.92 N, 22.00	C, 51.85 H, 5.93 Cl, 14.09 N, 21.82
XI	NH_2	C_4H_9	149–152°	44	A	C	$C_{11}H_{17}N_5O$	C, 56.10 H, 7.28 N, 29.77	C, 56.36 H, 7.11 N, 29.65
XII	NH_2	C_5H_{11}	158–161°	47	D	C	—[d]	C, 53.05 H, 6.87 N, 23.80	C, 52.91 H, 6.86 N, 23.68
XXXVII	Cl	C_6H_{13}	72–74°	87	A	B	$C_{13}H_{19}ClN_4O$	C, 55.21 H, 6.77 Cl, 12.54 N, 19.81	C, 55.09 H, 6.84 Cl, 12.79 N, 20.08
XIII	NH_2	C_6H_{13}	150–152°	49	E	C	$C_{13}H_{21}N_5O$	C, 59.30 H, 8.04 N, 26.60	C, 59.31 H, 7.93 N, 26.54
XXXVIII	NHMe	C_6H_{13}	108–112°	68	B	D	$C_{14}H_{23}N_5O$	C, 60.61 H, 8.37 N, 25.21	C, 60.45 H, 8.45 N, 25.10
XXXIX	NMe_2	C_6H_{13}	183–184°	85	D	E	—[e]	C, 53.57 H, 7.19 N, 18.35	C, 53.31 H, 7.03 N, 18.28
XL	OH	C_6H_{13}	223–227°	54	E	F	$C_{13}H_{20}N_4O_2$	C, 59.07 H, 7.65 N, 21.16	C, 58.88 H, 7.58 N, 20.99

Table III—(*Continued*)

Compound	R_1	R_2	Melting Point	Yield, %	Recrystallization Solvent[a]	Method	Formula	Analysis, % Calc.	Analysis, % Found
XLI	SH	C_6H_{13}	278–280°[b]	20	F	G	$C_{13}H_{20}N_4OS$	C, 55.68 H, 7.22 N, 19.98 S, 11.44	C, 55.67 H, 7.40 N, 19.79 S, 11.19
XLII	OMe	C_6H_{13}	132–134°	68	A	H	$C_{14}H_{22}N_4O_2$	C, 60.42 H, 7.99 N, 20.13	C, 60.40 H, 8.04 N, 20.27
XLIII	$NHNH_2$	C_6H_{13}	141–144°	88	F	I	$C_{13}H_{22}N_6O$	C, 56.08 H, 7.98 N, 30.19	C, 56.10 H, 8.03 N, 30.30
XLIV	H	C_6H_{13}	65–67°	64	B	J	$C_{13}H_{20}N_4O$	C, 62.88 H, 8.14 N, 22.57	C, 63.05 H, 8.27 N, 22.51
XLV	Cl	C_7H_{15}	85–89°	65	A	B	$C_{14}H_{21}ClN_4O$	C, 56.65 H, 7.13 Cl, 11.95 N, 18.88	C, 56.48 H, 7.01 Cl, 12.11 N, 18.60
XIV	NH_2	C_7H_{15}	150–153°	77	F	C	$C_{14}H_{23}N_5O$	C, 60.61 H, 8.38 N, 25.25	C, 60.70 H, 8.65 N, 25.56
XLVI	OH	C_7H_{15}	224–228°	72	F	F	$C_{14}H_{22}N_4O$	C, 60.42 H, 7.98 N, 20.13	C, 60.27 H, 7.99 N, 20.34
XLVII	Cl	C_8H_{17}	87–90°	75	C_6H_{14}	B	$C_{15}H_{23}ClN_4O$	C, 57.93 H, 7.46 Cl, 11.41 N, 18.03	C, 58.17 H, 7.50 Cl, 11.52 N, 17.94
XV	NH_2	C_8H_{17}	148–152°	70	F	C	$C_{15}H_{25}N_5O$	C, 61.82 H, 8.66 N, 24.04	C, 61.58 H, 8.61 N, 23.93
XLVIII	NHMe	C_8H_{17}	107–110°	85	B	D	$C_{16}H_{27}N_5O$	C, 62.92 H, 8.91 N, 22.93	C, 62.78 H, 8.90 N, 22.90
XLIX	OH	C_9H_{17}	226–230°	63	F	F	$C_{15}H_{24}N_4O_2$	C, 61.61 H, 8.28 N, 19.16	C, 61.87 H, 8.47 N, 19.25
L	Cl	C_9H_{19}	91–93°	68	C_6H_{14}	B	$C_{16}H_{25}ClN_4O$	C, 59.16 H, 7.76 Cl, 10.92 N, 17.25	C, 59.26 H, 7.90 Cl, 11.13 N, 17.02
XVI	NH_2	C_9H_{19}	147–150°	73	F	C	$C_{16}H_{27}N_5O$	C, 62.92 H, 8.92 N, 22.93	C, 63.16 H, 9.17 N, 22.63
LI	NHMe	C_9H_{19}	105–110°	81	F	D	$C_{17}H_{29}N_5O$	C, 63.92 H, 9.15 N, 21.93	C, 64.02 H, 9.12 N, 22.02
LII	NMe_2	C_9H_{19}	182–185°	75	D	E	—[f]	C, 56.72 H, 7.85 N, 16.54	C, 56.66 H, 7.84 N, 16.71
LIII	OH	C_9H_{19}	229–231°	60	F	F	$C_{16}H_{16}N_4O_2$	C, 62.71 H, 8.56 N, 18.29	C, 62.54 H, 8.70 N, 18.31
LIV	SH	C_9H_{19}	233–235°	39	F	G	$C_{16}H_{26}N_4OS$	C, 59.58 H, 8.14 N, 17.37 S, 9.94	C, 59.37 H, 7.88 N, 17.19 S, 10.06
LV	$NHNH_2$	C_9H_{19}	138–140°	74	F	I	$C_{16}H_{28}N_6O$	C, 59.98 H, 8.82 N, 26.23	C, 59.78 H, 8.68 N, 25.99

[a] Solvents: A, chloroform and hexane; B, benzene and hexane; C, acetone and hexane; D, isopropyl alcohol containing 1% oxalic acid; E, ethanol and water; and F, methanol and water. [b] Melts with decomposition. [c] Melting point taken on Kofler Heizbank. [d] Isolated as oxalate salt, $C_{12}H_{19}N_5O \cdot {}^1/_2\ C_2H_2O_4$. [e] Isolated as oxalate salt, $C_{15}H_{25}N_5O \cdot C_2H_2O_4$. [f] Isolated as oxalate salt, $C_{18}H_{31}N_5O \cdot C_2H_2O_4$.

the interaction forces due to hydrophobic binding can be considerable. For example, comparison of VIII with XIII; *i.e.*, the change of R from methyl to hexyl results in a change of ΔG of -3.1 kcal. In terms of the enzyme inhibitor dissociation constant, the change in free energy corresponds to a *Ki* of approximately 5×10^{-3} *M*, a considerable effect due mainly to the summation of hydrophobic forces.

Finally, the large hydrophobic region on adenosine deaminase, which is important in enzyme inhibitor complex formation for the hydrocarbon chain in compounds of type III, is most probably the same region involved in complex formation of the 9-*n*-alkyl group in compounds of type I. This statement is based on the observation that the dimension of the hydrophobic region on the enzyme is the same for both sets of compounds. Both sets of compounds (I and III) exhibit significant changes in $\Delta G/CH_2$ as the hydrocarbon chain is lengthened from propyl through heptyl[2]. Further extension of the hydrocarbon chain in either series resulted in minimal changes in $\Delta G/CH_2$. Note the unusually large change in free energy for the additional methylene group when comparing IX to X. The magnitude of this change is beyond simple hydrophobic transfer forces (10) and may reflect a conformational change in the enzyme (1, 11).

[2] For the purpose of comparing chain length of the 9-(1-hydroxy-2-alkyl)adenines to the 9-*n*-alkyladenines, the hydroxymethyl group has been ignored; *i.e.*, XIII has an alkyl chain length of seven. The listing of $\Delta G/CH_2$ for compounds of type I is found in *Reference 5* and for compounds of type III in Table I of this paper.

In summary, the combination of a properly positioned hydrophobic group and hydroxyl group at the 9-position of adenine has resulted in inhibitors of adenosine deaminase with greatly enhanced inhibitory activities.

EXPERIMENTAL[3,4]

Method A: 5-Amino-4-chloro-6-(1-hydroxy-2-propylamino)pyrimidine (XVII)—A mixture of 322 mg. (1.97 mmoles) of 4,6-dichloro-5-aminopyrimidine, 174 mg. (2.29 mmoles) of 1-hydroxy-2-propylamine, and 303 mg. (3.00 mmoles) of triethylamine in 15 ml. of 1-butanol was heated at reflux temperature for 22.5 hr. The reaction mixture was evaporated *in vacuo* to a liquid residue, which crystallized upon trituration with water. The crude solid material was recrystallized from water, giving the pure product.

Method B: 6-Chloro-9-(1-hydroxy-2-propyl)purine (XXV)—To a suspension of 626 mg. (3.08 mmoles) of 5-amino-4-chloro-6-(1-hydroxy-2-propylamino)pyrimidine in 25 ml. of triethyl orthoformate was added 53.4 mg. (0.485 mmole) of ethanesulfonic acid; the mixture was stirred at room temperature for 19 hr. The reaction mixture was cooled, and the precipitate was collected by filtration; the filtrate was evaporated *in vacuo* to a liquid residue which was dissolved in chloroform. *n*-Hexane was added, which gave a second crop of crude material. Recrystallization of the combined fractions from chloroform gave the pure product.

Method C: 9-(1-Hydroxy-2-propyl)adenine (VIII)—A mixture of 367 mg. (1.72 mmoles) of 6-chloro-9-(1-hydroxy-2-propyl)purine and 23 ml. of methanolic ammonia was heated at 90° for 18 hr. in a steel bomb. The reaction mixture was evaporated *in vacuo* and gave a residual solid which was extracted with hot acetone. The acetone extract was evaporated *in vacuo* to give the crude product, which gave the pure crystalline product upon recrystallization from ethanol.

Method D: 6-Methylamino-9-(1-hydroxy-2-propyl)purine (XXVI)—A steel bomb containing 404 mg. (1.90 mmoles) of 6-chloro-9-(1-hydroxy-2-propyl)purine and 20 ml. of aqueous methylamine (40%) was heated at 92° for 16 hr. The reaction mixture was evaporated *in vacuo* and gave a glassy residue which was extracted with hot chloroform. The chloroform extract was concentrated to a small volume, and hexane was added. Cooling gave the white crystalline product, which gave the analytically pure product upon recrystallization from chloroform–hexane.

Method E: 6-Dimethylamino-9-(1-hydroxy-2-propyl)purine (XXVII)—In a steel bomb was heated a mixture of 490 mg. (2.30 mmoles) of 6-chloro-9-(1-hydroxy-2-propyl)purine and 20 ml. of aqueous dimethylamine (25%) for 17 hr. at 100°. The reaction mixture was evaporated *in vacuo* and gave a liquid residue which was extracted with hot benzene. Upon addition of hexane to the extract and cooling, a crystalline precipitate formed. The solid material was recrystallized from benzene–hexane, and a white needlelike solid was obtained as the analytical sample.

Method F: 6-Hydroxy-9-(1-hydroxy-2-propyl)purine (XXVIII)—A reaction mixture of 510 mg. (2.24 mmoles) of 6-chloro-9-(1-hydroxy-2-propyl)purine and 10 ml. of 3 *N* hydrochloric acid was heated at reflux for 10 min. After the reaction mixture had been evaporated *in vacuo* to a residual oil, concentrated ammonium hydroxide (3 ml.) was added to the crude material. This mixture was evaporated *in vacuo*, giving a white solid material which was recrystallized from water, yielding the white crystalline product.

Method G: 6-Mercapto-9-(1-hydroxy-2-propyl)purine (XXIX)—A mixture of 325 mg. (1.52 mmoles) of 6-chloro-9-(1-hydroxy-2-propyl)purine, 116 mg. (1.52 mmoles) of thiourea, and 10 ml. of 1-propanol was heated at reflux for 15 min. The reaction mixture was allowed to cool, and the precipitate which formed was collected by filtration. The material was recrystallized from 1-propanol, giving a white crystalline product as the analytical sample.

Method H: 6-Methoxy-9-(1-hydroxy-2-octyl)purine (XLII)—To a solution of 1.06 g. (3.74 mmoles) of 6-chloro-9-(1-hydroxy-2-octyl)purine in 20 ml. of methanol, 0.540 g. (10.0 mmoles) of sodium methoxide was added, and the mixture was refluxed 1 hr. The reaction mixture was then cooled in an ice bath and adjusted to neutral pH with 6 *N* hydrochloric acid. The mixture was then evaporated *in vacuo* to a residual solid which was triturated in water (10 ml.). The remaining solid material was collected by filtration. The product was recrystallized from chloroform–hexane, and the analytical product was obtained.

Method I: 6-Hydrazino-9-(1-hydroxy-2-octyl)purine (XLIII)—A mixture of 12 mg. (3.22 mmoles) of 6-chloro-9-(1-hydroxy-2-octyl)purine and 15 ml. of hydrazine (95%) was cooled at 0° for 10 min. and stirred at room temperature for 2 hr. Cooling the reaction mixture gave a solid precipitate which was collected by filtration. The material was recrystallized from methanol–water and gave the analytical sample.

Method J: 9-(1-Hydroxy-2-octyl)purine (XLIV)—A mixture of 1.01 g. (3.56 mmoles) of 6-chloro-9-(1-hydroxy-2-octyl)purine, 202 mg. (5.00 mmoles) of magnesium oxide, and 200 mg. of 5% palladium-on-charcoal in 200 ml. of ethanol was hydrogenated over 2 hr. until an uptake of the theoretical amount of hydrogen had occurred. The mixture was filtered through a Celite pad, and the catalyst was washed with 100 ml. of hot ethanol. The combined ethanol filtrates were evaporated *in vacuo* to 50 ml. in volume, and 20 ml. of sodium carbonate solution (5%) was added. The mixture was evaporated *in vacuo* to an oily residue. The crude product was dissolved in hot hexane. Cooling gave a white crystalline product. Further recrystallizations of the material from benzene–hexane gave the analytical sample.

2-Amino-1-alkanols—Most of the amino alcohols required for this study were either commercially available or had been described in the literature. The following general procedure was employed for the new amino alcohols.

2-Amino-1-decanol—To a suspension of 13.4 g. (71.7 mmoles) of 2-aminodecanoic acid in 250 ml. of absolute ethanol, cooled at 0°, was added 10.7 g. (90.0 mmoles) of thionyl chloride. The resulting mixture was heated at reflux temperature for 8 hr. The reaction mixture was evaporated *in vacuo* and gave crude solid ethyl 2-aminodecanoate hydrochloride, 17.0 g. (94.2%), which was dissolved in 100 ml. of chloroform and washed with excess cold 5% sodium hydroxide solution and with water (1 × 5 ml.). After the chloroform solution was dried with anhydrous magnesium sulfate, it was evaporated *in vacuo* and gave a quantitative yield of crude ethyl 2-aminodecanoate; ν in $cm.^{-1}$ (film): 1735 (ester).

To a suspension of 3.79 g. (100 mmoles) of lithium aluminum hydride in 2 l. of anhydrous ether was added an ethereal solution (1 l.) of ethyl 2-aminodecanoate (67.5 mmoles). The reaction mixture was refluxed for 7 hr., after which time 25 ml. of water was added slowly. The suspension was filtered, and the solid residue was washed with ether (500 ml.). The ether solutions were combined and evaporated *in vacuo* to give a liquid residue, which was then crystallized and recrystallized from hexane to give white crystalline 2-amino-1-decanol; yield 7.81 g. (67.2%), m.p. 41–46°.

Anal.—Calc. for $C_{10}H_{23}NO$: C, 69.30; H, 13.38; N, 8.09. Found: C, 69.19; H, 13.35; N, 8.17.

By a similar procedure, the following amino alcohols were synthesized.

2-Amino-1-heptanol—This was isolated as the oxalate salt and recrystallized from 2-propanol, m.p. 150–151°.

Anal.—Calc. for $C_7H_{17}NO \cdot C_2H_2O_4$: C, 48.86; H, 8.67; N, 6.34. Found: C, 48.57; H, 8.48; N, 6.55.

2-Amino-1-nonanol—This was isolated as the hydrochloride salt and recrystallized from chloroform–hexane, m.p. 94–96°.

Anal.—Calc. for $C_9H_{21}NO \cdot HCl$: C, 55.15; H, 11.33; N, 7.15; Cl, 18.11. Found: C, 55.40; H, 11.28; N, 6.98; Cl, 18.23.

2-Amino-1-undecanol—This was isolated as the free base and recrystallized from hexane, m.p. 48–52°.

Anal.—Calc. for $C_{11}H_{25}NO$: C, 70.53; H, 13.40; N, 7.48. Found: C, 70.60; H, 13.42; N, 7.29.

Enzyme Assay—Adenosine deaminase (Type I, calf intestinal mucosa) was purchased[5]. The assay procedure for the study of reversible inhibitors was previously described (2–5) and is a modification of the procedure of Kaplan (12) based on the method of Kalckar (13).

[3] General procedures are given for the synthesis of the various types of compounds. The physical constants and analytical data for the compounds are given in Tables II and III.

[4] The melting points, unless noted otherwise, were taken in open capillary tubes on a Mel-Temp apparatus and are uncorrected. All analytical samples had IR and UV spectra compatible with their assigned structures and moved as a single spot on TLC on Brinkmann silica gel. Analyses were performed by Galbraith Microanalytical Laboratories, Knoxville, Tenn.

[5] Sigma Chemical Co.

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* Present address: Wellcome Research Laboratories, Burroughs Wellcome Co, Research Triangle Park, NC 27709

† Present address: Warner-Lambert Research Institute, Morris Plains, NJ 07950

Chemical and Pharmacological Evaluation of *Banistereopsis argentea* Spring ex Juss

S. GHOSAL*, U. K. MAZUMDER, and S. K. BHATTACHARYA†

Abstract ☐ Seven simple indole bases—*viz.*, *N,N*-dimethyltryptamine (I), its N_b-oxide, harmine (II), harmaline (III), (+)-N_b-methyltetrahydroharman (IV), (+)-tetrahydroharmine (V), and (+)-5-methoxytetrahydroharman (VI)—were isolated from the leaves and stems of *Banistereopsis argentea* Spring ex Juss (Malpighiaceae) and their identity established by physical and chemical methods. In addition to these alkaloids, two partially characterized minor indole-3-alkylamines, together with choline and betaine, were obtained from the same parts of the plant. This was the first demonstration of the occurrence of N_b-methyltetrahydroharman in the family Malpighiaceae and of a 5-methoxytetrahydro-β-carboline alkaloid in nature. The total alkaloids from the leaves have shown three main pharmacological actions: nonspecific spasmolytic action against acetylcholine-, histamine-, and serotonin-induced spasm; histamine release-mediated depressor response; and pronounced behavioral effects.

Keyphrases ☐ *Banistereopsis argentea* Spring ex Juss—isolation, identification, pharmacological evaluation of seven indole alkaloids ☐ N_b-Methyltetrahydroharman—isolation, identification from *B. argentea* ☐ 5-Methoxytetrahydro-β-carboline alkaloid—occurrence in nature, *B. argentea* ☐ CNS activity—indole alkaloids in *B. argentea* ☐ TLC—separation, isolation, *B. argentea* alkaloids ☐ Mass spectrometry—identification, structure

The use of members of the genus *Banistereopsis* (family Malpighiaceae) in the preparation of narcotic and hallucinogenic decoctions by certain Colombian and Peruvian Indian tribes is well known (1). Earlier chemical investigations with a number of *Banistereopsis* species showed (2–5) the presence of the following four indole bases in their various parts: *N,N*-dimethyltryptamine (I), harmine (II), harmaline (III), and (+)-tetrahydroharmine (V). Since then, there has been much speculation (6, 7) as to the contribution of the individual alkaloidal entities to the hallucinogenic activity of the crude preparations. Although the hallucinogenic activity of harmine was demonstrated earlier, the crude preparations of plants containing harmine have, however, appeared to produce greater psychotropic action than pure harmine (6). Moreover, the well-documented hallucinogenic activity of *N,N*-dimethyltryptamine (7) cannot alone account for the strong behavioral changes caused by the plant extracts, because the indole base is only present as a minor constituent in the genus *Banistereopsis*. It seems likely that the strong hallucinogenic activity of the crude plant preparations is due to some unidentified, more potent, extraneous materials introduced or generated chemically during preparation, to other natural products, or to a synergistic action of the mixture.

To extend the knowledge of the content and distribution of the aforementioned and related alkaloids in the genus *Banistereopsis*, the phytochemical and pharmacological evaluation of a rare species, *B. argentea* Spring ex Juss[1], was undertaken. Some recent investigations were directed toward the study of Indian medicinal plants used as psychotherapeutic agents in the Ayurvedic system of medicine. The results, with a number of species belonging to the families Leguminosae and Gramineae, showed the presence of about two dozen indole-3-alkylamines and β-carbolines, several of which were either hallucinogens or MAO inhibitors (8–14). The

[1] The plant material was obtained through Mr. R. Biswas, Indian Botanic Gardens, Howrah, India.