substance ${\bf P}$ also significantly contributes to its function at a site.

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Synthesis and Hypocholesterolemic Activities of Eritadenine Derivatives

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More than 100 derivatives of eritadenine were synthesized and their hypocholesterolemic activities were evaluated. The most active derivatives were carboxylic acid esters with short-chain monohydroxy alcohols, which were 50 times more active than eritadenine and effective in lowering serum cholesterol of rats at the dose of 0.0001% in the diet. The carboxyl function and at least one hydroxy group appear to be essential for activity. The intact adenine structure seems to be required for activity except for the N^1 position where an oxygen or an alkoxy group could be attached without loss of activity.

Eritadenine is a hypolipidemic substance isolated from the Japanese mushroom shiitake (*Lentinus edodes*) and its chemical structure has been identified as 2(R),3(R)dihydroxy-4-(9-adenyl)butyric acid by two independent groups of workers.^{1,2} Eritadenine lowers all the lipid components of plasma lipoproteins (cholesterol, triglyceride, and phospholipid) and is active in several animal species including man.³ Its hypocholesterolemic property has been studied in detail in the rat.⁴⁻⁶

In order to study the structure-activity relationship of this naturally occurring hypolipidemic agent, and also in the hope of finding a derivative with the best possible therapeutic index, numerous derivatives and analogs of eritadenine were synthesized in these laboratories and their hypocholesterolemic activities were evaluated in normal rats.

Chemical Procedures. Preparation of the eritadenine esters and amides (compounds 1-50) was carried out by the common methods, which are described in the Experimental Section.

Syntheses of the stereoisomers of eritadenine (compounds 51-56), noreritadenine (compound 70), homoeritadenine (compounds 73-77), α -O-alkyleritadenine (compound 68b) and the reversed nucleosides (compounds 78-82) have been reported by Kawazu, *et al.*^{7,8}

Compounds 57 and 58 were synthesized by direct alkylation of adenine with an O-protected ethyl 4-bromo-2,3dioxybutyrate. The latter was obtained by warming Derythronolactone in ethanol saturated with hydrogen bromide and, after removal of the ethanol, by treating the residue with a protecting agent such as ethyl orthoformate, acetone, or acetaldehyde. Compounds 59, 60, and 61 were prepared by condensation of the eritadenine ester with the cyclic ketones in the presence of an acid.

Compound 63 was prepared by reaction of the sodium salt of adenine with pantolactone.

Compounds 64 and 65 were synthesized from the pyrimidine derivatives employing the usual synthetic method for the purine skeleton as reported for the synthesis of eritadenine.^{1,9}

The amino acids (compounds 66-68) were synthesized from the reversed nucleoside of adenine, 5-(adenine-9H-9-yl)-3-acylamino-D-ribose, by oxidation with oxygen under conditions similar to those for synthesis of homoerit-





adenine series.^{8,10} The hypoxanthine analog 83 was synthesized from eritadenine by diazotization. The 6-methylmercaptopurine derivative 84 was prepared from 4-(6-methylmercapto-9*H*-purin-9-yl)-2(*R*),3(R)-(cyclohexylidenedioxy)butyric acid.¹¹ Compounds 85-99 (except 96 and 97) were prepared by the usual methods shown in the Experimental Section.

The N^6 -formyl derivatives 90-94 were obtained by treating eritadenine with dimethylformamide dialkylacetal and purified by silica gel chromatography. These compounds were probably produced via an intermediate, N^{6} dimethylaminomethylene eritadeninate, which could be hydrolyzed owing to the acidity of silica to the formyl derivatives during the purification process.

Preparation of compounds 96, 121, and 123 was carried out by oxidation of corresponding reversed nucleosides according to the method by Kanno, et al.¹²

 N^1 -Oxyeritadenine (compound 100) was prepared from eritadenine by oxidation with hydrogen peroxide in acetic acid, and N^1 -alkoxyeritadenine derivatives (compounds 112-116) were obtained by alkylation of the N^1 -oxyeritadenine derivatives with alkyl halides (Scheme I).

The structures of these compounds were confirmed by means of elemental analysis and uv spectroscopy. Absorption maxima in water at 261 (pH 2), 235, 264 (pH 6.8), 232, and 268 nm (pH 13) for compound 114 were compared with those for N^1 -oxyadenine-9H-9-yl derivatives reported by Stevens, et al., ¹³ and with those for N^1 -alkoxyadenine-9H-9-yl derivatives reported by Fujii, et al.¹⁴

The N^7 isomers (compounds 119 and 120) were obtained via the synthetic route shown in Scheme II. After removing the protective groups of XII, oxidation with oxygen in dilute alkali solution was successfully carried out.

Synthesis of the N³ isomers (compounds 117 and 118) has been reported by Okumura, et al.¹¹

Biological Results. The hypocholesterolemic rates of eritadenine at the doses of 5 and 10 mg % in the diet were 18 (mean of seven experiments) and 25% (mean of 23 experiments), respectively, under the present conditions.

Scheme II



				ACH ₂ CHC	CHCOY"			
				RÓ C)R			
No.	Ϋ́	R	Method	Yield, $\%$	${ m Recrystn} { m solvent}^a$	Mp, °C	MED, mg %	${ m Formula}$
Eri	tadenine						5	
-	0CH ₃	Н	\mathbf{A}^{d}	85.6	Α	228 - 230	0.2, >0.05	C ₁₀ H ₁₅ N ₅ O ₄
21	$0C_2H_5$	Н	Α	93.5	в	191 - 193	0.1, > 0.05	C ₁₁ H ₁₅ N ₅ O ₄
က	$0-n-C_3H_7$	Н	V	85.5	C	176-178	0.1 - 0.05	$C_{12}H_{17}N_5O_4$
4	OCH2CH-CH2	Н	Α	68.5	В	140 - 142	0.3, > 0.1	$C_{12}H_{15}N_5O_4^e$
r0	$OCH_2C \equiv CH^b$	Н	Α	25.8	Α	190 - 192	0.3, >0.05	$\mathbf{C}_{12}\mathbf{H}_{13}\mathbf{N}_{5}\mathbf{O}_{4}^{\prime}$
9	$0-i-C_3H_7$	Н	V	42.9	D	182 - 185	0.2, >0.05	$\mathbf{C}_{12}\mathbf{H}_{17}\mathbf{N}_{5}\mathbf{O}_{4}^{o}$
2	$O-n-C_4H_9$	Н	Α	88.9	E	156 - 158	0.2, >0.05	C ₁₅ H ₁₉ N ₅ O ₄
×	$0-i-C_4H_9$	Н	V	80.5	Ŀ	183 - 185	0.1-0.05	C ₁₃ H ₁₉ N ₅ O ₄
6	$0-n-C_5H_{11}$	Н	Α	77.6	C	153 - 155	0.15	$C_{14}H_{21}N_5O_4$
10	0- <i>i</i> -C ₅ H ₁₁	Н	V	53.2	C	161 - 163	0.1	$C_{14}H_{21}N_5O_4$
11	$0-sec-C_{5}H_{11}$	Н	Α	39.0	B^{-G}	146 - 151	0.1	$\mathbf{C}_{14}\mathbf{H}_{21}\mathbf{N}_5\mathbf{O}_4$
12	$0-n-C_{6}H_{13}$	Н	Α	66.0	Ĺ4	151 - 153	0.3, > 0.2	$C_{15}H_{23}N_5O_4$
13	$0-n-C_8H_{17}$	Н	Α	61.6	Α	159 - 161	0.2-0.3	$\mathbf{C}_{17}\mathbf{H}_{27}\mathbf{N}_{5}\mathbf{O}_{4}$
14	$0-n-C_{12}H_{25}$	Н	Α	14.3	Α	165 - 166	2, >0.3	$C_{21}H_{35}N_{5}O_{4}$
15	$0-n-C_{18}H_{37}$	Н	Α	9.6	Α	165 - 167	1, >0.25	$C_{27}H_{47}N_{5}O_{4}$
16	OCH ₂ C ₆ H ₅	Н	Α	65.5	HI	195 -197	1, >0.5	$C_{16}H_{17}N_{5}O_{4}$
17	$OCH_{2-\alpha}$ -furyl	Н	в	3.6	В	168 - 170	1, >0.2	$C_{14}H_{15}N_5O_5$
18	$O(CH_2)_3CI$	Н	A	65.5	В	148 - 150	>0.2	$C_{12}H_{16}N_5O_4CI$
19	0(CH ₂)5Cl	Н	В	16.0	D	131133	0.1	$C_{14}H_{20}N_5O_4Br$
20	$0(CH_2)_2OH$	Н	V	80.0	A-H	214 - 215	5, >0.5	$\mathbf{C}_{11}\mathbf{H}_{15}\mathbf{N}_{5}\mathbf{O}_{5}$
21	$0(CH_2)_3OH$	Н	Α	68.0	B-H	161 - 165	>1	$\mathbf{C}_{12}\mathbf{H}_{17}\mathbf{N}_{5}\mathbf{O}_{5}h$
22	OCH2C(OH)HCH2OH	Η	A	27.5	A-B	154-156	>5	$\mathbf{C}_{12}\mathbf{H}_{17}\mathbf{N}_{5}\mathbf{O}_{6}$
ę			•	:		dec	,	(; ; ; ; ;
23	OCH2CH2O-Ent	н	А	40.6	Α	219-221 dec	0.1	$\mathbf{C}_{20}\mathbf{H}_{24}\mathbf{N}_{10}\mathbf{O}_{8}$
24	0(CH ₃) ₂ OCH ₃	Н	Α	57.0	D	112-115	5. > 1	$C_{13}H_{17}N_{3}O_{5}$
25	O(CH2)2OC2H5	Η	A	0.69	D	126 - 128	>0.2	C ₁₃ H ₁₅ N ₅ O.
26	$O(CH_2)_2OC_5H_6$	Н	V	43.0	В	162 - 164	>0.2	$C_{17}H_{19}N_{5}O_{5}$
27	OCH ₂ CO ₂ C ₂ H ₅	Н	B	55.5	D	173 - 175	1, >0.5	$C_{13}H_{17}N_sO_6$
28	OCH2COC6H5	Η	В	56.0	H.L	177 dec	<0.5	$C_{17}H_{17}N_{2}O_{5} \cdot 0$. 25 $H_{2}O$
29	OCH2COC6H4NO2	Η	В	65.0	Н Ц	169 - 170	\geq 1	$C_{17}H_{16}N_6O_{17}$
30	$OCH_2OC(=0)(CH_2)_4CH_3$	Н	В	7.0	H-L	153	>2	$C_{16}H_{23}N_5O_6$
31	4-N-Methylpiperidyl	Н	С	21.2	Н	222 - 223	>1	$\mathrm{C}_{15}\mathrm{H}_{22}\mathrm{N}_6\mathrm{O}_4$
32	O-Cholesteryl	Н	В	4.7	B⊶H	234 - 235	10, >2	$\mathbf{C}_{38}\mathbf{H}_{59}\mathbf{N}_{5}\mathbf{O}_{4}$
33	$O-\beta$ -Sitostery]	Н	В	3.5	H-L	233 - 235	>2	$\mathrm{C}_{40}\mathrm{H}_{63}\mathrm{N}_{5}\mathrm{O}_{4}$
34	2,3,4-Tri-O-acetyl-D-xylose	Н	В	20.0	H-I	$200 \mathrm{dec}$	>2	$\mathbf{C}_{20}\mathbf{H}_{23}\mathbf{N}_{3}\mathbf{O}_{11}$
35	2,3,4,6-Tetra-O-acetyl-D-	Н	в	38.0	I-H	209	>10	$\mathbf{C}_{23}\mathbf{H}_{29}\mathbf{N}_{3}\mathbf{O}_{13}$
36	glucose OCH.	COCH.	ц	84 4		915-917	2	C.H.N.O.
2	BTTOO	STO 200	1		ì	110-010	5/	0/141 n1/1 × 3//16

Table I

37	0C2H5	COCH,		81.0	В	198-201	0.2	C ₁₅ H ₁₉ N ₅ O ₆
38	$0-n-C_3H_7$	COCH,		69.4	В	180 - 182	0.1	CitH31N50
39	$0-i-C_4H_9$	COCH,		82.4	n eq	182 - 184	0.2	CriH, NO
40	$0-n-C_8H_{17}$	COCH,		64.5	B	162 - 164	>0.25	C ₂₁ H ₃₁ N ₅ O ₆
41	$0C_2H_5$	COC ₃ H ₅		70.0	B_{-J}	130 - 132	0.1. > 0.05	$C_{17}H_{23}N_5O_6$
4	$0-n-C_3H_7$	COC ₂ H,		53.3	B-K	118-121	0.1	C ₁₈ H ₂₅ N ₅ O ₆
43	$0-i-C_4H_9$	COC ₃ H	ы	55.0	B-K	128 - 130	0.2	$C_{19}H_{27}N_4O_6$
44	$0-n-C_8H_{17}$	COC ₂ H	E	36.8	A_K	105 - 106	>0.25	$C_{23}H_{35}N_5O_6$
45	HO	n -COC $_3$ H $_7$	E	50.0	В	202 - 204	5	$C_{17}H_{23}N_5O_6$
46	$0C_2H_5$	$n-\mathrm{COC}_3\mathrm{H}_7$	E	65.0	В-J	110-112	0.2	C ₁₉ H ₂₇ N ₅ O ₆
47	$\rm NH_2$	Н	ы	73.5	L	253 - 255	>5	$C_9H_{12}N_6O_3$
48	NHCH ₂ C ₆ H ₅	Н	Γų	81.8	В	193 - 195	>5	$C_{16}H_{18}N_6O_3^k$
49	$S_i - C_5 H_{11}$	Η	D	43.0	Ċ	166 - 168	0.1	$C_{14}H_{21}N_5O_3S$
50	SCH ₂ CH ₂ CI	Н	D	10.0	D	162 - 164	0.2, >0.1	C ₁₁ H ₁₄ N ₅ O ₅ SCl ¹
as as as ar and a searchaile and a searc	A, MeOH; B, EtOH; C, n -C ₃ H ₇ OH; D t catalyst. e Erit = $-COC(OH)HC(OH)$:d, 46.30; found, 45.88. N: calcd, 22.50 = aden-9-vl.), <i>i</i> -C ₃ H ₇ OH; E, diox H)HCH ₂ A. ^d See Exp 0; found, 22.09. ⁱ N: _i	ane; G, CH ₃ C erimental Sec calcd, 19.77; f	N; H, DMF; I, ction. ⁴ N: calcd, found, 20.19. ^j C:	Et ₂ O; J, petrolei 23.88; found, 2 calcd, 56.11; fo	um ether; K, isopro 23.37. /N: calcd, 24 und, 56.60. [*] N: calc	pyl ether; L, H ₂ O. ⁶ Es 4.05; found, 23.62. ^ø N: 3d, 24.55; found, 23.68	tterified in benzene using TsOH : calcd, 23.72; found, 23.28. ^h C: . 'N: calcd, 21.11; found, 20.52.

Eritadenine Derivatives

Table II

	NH2 NNN	>	
	Ċ	H2CH(OH)CH(OH)COOR	
No.	R	Stereoisomerism	MED, mg $\%$
51 52 53 54 55 55 56	$egin{array}{c} H \ C_2 H_3 \ Na \ C_2 H_5 \ Na \ C_2 H_5 \end{array}$	D-Threo D-Threo L-Erythro L-Erythro L-Threo L-Threo	20 5, >0.2 >20 5, >0.2 >20 5, >0.2 >20 >5

This activity is about ten times as active as clofibrate, the most widely used synthetic hypocholesterolemic agent. The hypocholesterolemic activity of eritadenine was immensely augmented by esterifying the carboxyl group with lower (C_1-C_5) monohydroxy alcohols (1-3, 6-11). These compounds were 25 to 50 times as active as the parent compound and their minimal effective doses in the diet were as low as 0.1-0.2 mg/kg of body weight/day when calculated from the amount of food consumption.

Esters with alcohols possessing an aliphatic chain of six or more carbon atoms (12-15) or aromatic rings (16 and 17) were less active than the lower esters, but they were more active than eritadenine. The effect of substitution of a halogen atom at the ω carbon of lower linear alcohols in the eritadenine esters was inconsistent (18 and 19).

Introduction of a double bond (4) or a triple bond (5) in the aliphatic chain seems to be somewhat detrimental. Substitution of OH (20-22) or OR (24-26) on the aliphatic chain appears to diminish markedly the activity of the parent ester, sometimes to levels lower than that of eritadenine. The ethylene glycol bisester 23 of eritadenine showed an activity comparable to those of unsubstituted lower alkyl esters. Carbonyl- or carbonyloxy-substituted methyl esters (27-30) were less active than the unsubstituted methyl ester, although some of them (e.g., 27 and 28) may be more active than eritadenine.

Esterification with a hydroxyl function which is directly attached to a bulky ring structure seems to give no advantage over unesterified eritadenine (31-35). Amide derivatives are probably less active than eritadenine (47 and 48). Esters with mercaptans are probably as active as those with ordinary alcohols (49 and 50). Acylation of the two hydroxyl groups of the eritadenine side chain with shortchain aliphatic carboxylic acids does not seem to affect the activities of eritadenine and its esters (36-46) (Table I).

Eritadenine is the D-erthro isomer of four possible optical isomers resulting from the two asymmetric centers of the side chain. Among the three other isomers of eritadenine, the D-threo isomer (51, 52) was the most active, but it was less active than eritadenine. The L-threo isomer (55,56) was the least active (probably totally inactive), and the L-erythro isomer (53, 54) was intermediate between the above two isomers (Table II).

Ether bond formation at one (68b) or both (58-60) of the hydroxyl groups abolished the activity except for the ethoxymethylene derivative 57, which is chemically unstable. Displacement of the hydroxyl groups by hydrogen (64), or by a phenyl group at 3-C and by hydrogen at 2-C (65), or substitution of two methyl groups at 3-C (62) led to loss of activity. Substitution of an amino or acetylamino group for the hydroxyl group at 2-C (66, 68) weakened



^aA, MeOH; I, Et₂O; N, CCl₄; O, AcOEt; P, benzene; M, CHCl₃. ^bC: calcd, 55.32; found, 55.74.

the activity considerably but apparently not to zero as judged by the activity of the ethyl ester of 66 (67) (Tables III and IV).

Substitution of a formylmethyl group (69), 5-deoxypentoses (78-80) and 6-deoxyhexoses (81, 82) for the entire side chain, and reduction of the carboxyl function (71, 72) gave compounds without activity. Deletion of one hydroxymethylene group (70), or insertion of one with either configuration between 2-C and COOH (73, 76), did not nullify the activity as judged by the activities of their lower alkyl esters (74, 75, 77), although the activity was reduced substantially in comparison with eritadenine (Table V).

Substitution of OH at C⁶ (83), SCH₃ at C² (85, 86), C⁶ (84), or C⁸ (89), or Br at C⁸ (87, 88) on the purine ring gave inactive compounds. Formylation of the amino group at C⁶ did not affect the activity (90–94). Gastric acidity is expected to liberate the formyl group after ingestion. Substitution of an alkyl (95, 96), phenylalkyl (97), or N-substituted carbamoyl (98, 99) group on the C⁶ amino nitrogen seems to impair the activity (Table VI).

The N^1 -oxide of eritadenine (100) and its lower alkyl esters (101-107) were nearly as active as eritadenine and its corresponding esters. The benzyl (108) and 2,3-dihydroxypropyl (111) esters of eritadenine N^1 -oxide were much less active than the lower alkyl esters. The 2-chloroethyl ester of the N^1 -oxide (110) was less active than the unsubstituted ethyl ester. The cyclohexyl ester (109) showed an activity equal to or higher than that of eritadenine (Table VII).

Lower alkyl esters of N^1 -alkoxy- (C₁-C₅) eritadenine (112-116) appear to be as active as the corresponding esters of eritadenine (Table VIII).

A position isomer of eritadenine with the side chain at N^3 (117) seems to retain some activity while the other N^7 isomer (119) was inactive as judged by the activities of their respective lower alkyl esters (118, 120) (Table IX).

Substitution of benzimidazole (121), uracil (123), or ammonia (124) for the adenine moiety of eritadenine resulted in an entire loss of activity. The isoamyl ester of a 4,5-substituted imidazole analog of eritadenine (122) was unexpectedly active, although less active than eritadenine (Table X).

In conclusion, the minimum structural requirements for the hypocholesterolemic activity of eritadenine include the carboxy function, at least one hydroxy group on the side chain, and the intact adenine nucleus where an oxygen or alkoxy group could be attached at N^1 without loss of activity. Esterification of the carboxy group with lower monohydroxy alcohols invariably amplifies the activities of parent compounds.

Discussion

The most noteworthy of the above results is the marked augmentation of the hypocholesterolemic property observed upon esterification of eritadenine with lower alcohols. One of the factors that determine the activities of these esters is absorbability from the intestinal tract, since Meshi, et al., † in these laboratories found that only about 10% of an oral dose of eritadenine was absorbed in the rat, whereas the bulk of an oral dose of lower alkyl esters of eritadenine was absorbed. They also showed that the longer the alkyl chain of the alcohol the more susceptible to hydrolysis in the intestine and, hence, the less permeable through the intestinal barrier. This explains the relatively diminished activities of the esters with higher alcohols. Whether these esters act per se at the receptor site or only after hydrolysis to unesterfied eritadenine is unknown. In this respect it is of interest that Iwai, et al., 15 in these laboratories have observed that some of the eritadenine esters are more potent inhibitors than eritadenine in cell-free cyclic AMP-dependent protein kinase systems from rat liver and fat tissue. In view of the extremely low minimal effective doses of the eritadenine esters and structural similarity with cyclic AMP that functions in minute concentrations in the cells, an effect on some cyclic AMP-mediated system would be an attractive hypothesis as the mechanism of action of eritadenine derivatives.

Determination of Hypocholesterolemic Activity. Male Sprague-Dawley rats were purchased from Nihon CLEA Co., Ltd., Tokyo, and maintained on commercial laboratory chow (Nihon CLEA CE-2 pellets) for at least 1 week before the experiments. At the start of each experiment rats weighing 120–140 g were anesthetized with ether, blood samples of about 0.5 ml were taken from the tail tip, and serum cholesterol levels were determined by the method of Zak, *et al.*¹⁶ After the rats showing extremely low or high serum cholesterol levels had been eliminated, the rest were divided into groups of five rats in such a way that the average serum cholesterol levels and the average body weights of all the groups were as close to each other as possible. In this way experimental errors arising from

[†]T. Meshi, et al., unpublished results.



^aA, MeOH; H, DMF; L, H₂O; M, CHCl₃; O, AcOEt. ^bCompounds **64** and **65** were obtained by thermal cyclization of 4-(4-amino-5-formylaminopyrimidine-6-yl)aminobutyric acid derivatives in diphenyl ether. ^cThis compound was independently synthesized by N. J. Leonard and K. Ito, J. Amer. Chem. Soc., **95**, 4010 (1973).

the variation of the natural serum cholesterol level of individual rats were minimized.

Each test compound was mixed with the powder chow of Nihon CLEA CE-2 in a mortar and fed *ad libitum* from a powder-feeding dish (Natsume Seisakusho Co., Ltd., Tokyo) for 7 days. Control rats were fed the CE-2 powder. Calculation from the consumed amount of the diet showed that the concentration of the test compound expressed in mg % (mg per 100 g powder chow) could be taken in approximation as the dose expressed in mg/kg of body weight/day.

After the feeding period of 7 days serum cholesterol levels of all the rats were determined as described above. A test compound in a given dosage was judged to be effective when the hypocholesterolemic rate calculated by the following formula was 15% or more.

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hypocholesterolemic rate = 1 - \frac{\text{mean serum cholesterol of treated rats}}{\text{mean serum cholesterol of control rats}} \times 100\%
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The hypocholesterolemic activities of the test compounds are listed in the tables as minimal effective doses (MED) which represent the lowest effective concentration (mg%) examined of each compound in the diet.

Experimental Section

General Procedures for the Preparation of the Eritadenine Ester and Amide Derivatives (Compounds 1-50). Method A. By the Reaction with Alcohols in the Presence of Acid Catalyst. A mixture of eritadenine, an equal amount of concentrated H_2SO_4 , and an alcohol (50 times) was heated at 70-100°. After cooling at 60°, the mixture was neutralized with Amberlite IR-45 and the resin was removed by filtration. The filtrate was concentrated to dryness *in vacuo*, and the residue was crystallized from an appropriate solvent to afford the product.

Method B. By Alkylation of the Metal Salt of Eritadenine. A suspension of the metal (sodium or silver) salt of eritadenine and a small excess of an alkyl halide (or O-tosylate in some cases) in DMF (20 times) was heated at 100° for 10-20 hr. After cooling, the reaction mixture was filtered to remove the inorganic precipitate and the unchanged material, and the filtrate was concentrated to dryness *in vacuo*. Purification by recrystallization or, if necessary, by column chromatography on silica gel gave the product.

Method C. By the Ester-Exchange Reaction. Sodium (46 mg) was dissolved in anhydrous N-methyl-4-piperidinol (46 g). To this solution was added eritadenine ethyl ester (8 g), and the mixture was heated at $130 \pm 5^{\circ}$ for 24 hr. After cooling, the reaction mix-



ture was diluted with ether and the precipitate was collected by filtration. Recrystallization from DMF gave compound 31 (2.1 g).

Method D. By the DCCD Method (Example). To a cooled solution of 2,3-(O-cyclohexylidene)eritadenine (1.0 g) in DMF (10 ml) were added isoamyl mercaptan (830 mg) and DCCD (825 mg), and the mixture was stirred at 25° for 5 hr. The reaction mixture was diluted with ether (10 ml). The precipitate was removed by filtration and the filtrate was concentrated to dryness *in vacuo*. The residue was purified by column chromatography on silica gel (20 g) to give 2,3-(O-cyclohexylidene)eritadenine isoamyl thioester (640 mg, 51%): mp 123-125°; nmr (CDCl₃) δ 0.93 (d, 6 H), 2.9 (t, 2 H), 4.0-4.75 (m, 4 H), 6.09 (s, 2 H), 7.95 (s, 1 H), 8.37 (s, 1 H). A portion (420 mg) of this sample was treated with 70% HCO₂H (10 ml) at 25° for 4 days. The solution was concentrated to dryness *in vacuo*, and the residual solid was recrystal-lized from MeCN to afford compound 49 (290 mg, 85%).

Method E. By Acylation of Eritadenine Esters. A mixture of an eritadenine ester, pyridine (30-40 times), and an acid anhydride (4 molar equiv) was warmed at $40-45^\circ$ for 3 hr. The pyridine was removed by evaporation *in vacuo* and the residue was crystallized by trituration with isopropyl ether. The crystals were collected by filtration and recrystallized from an appropriate solvent to give the pure product.

Method F. By Aminolysis of Eritadenine Ethyl Ester. A mix-

				а <u>,</u> -					
				R					
					CH_CH(OH)CH(O)	H)COOR			
N0.	\mathbf{R}_{I}	\mathbf{R}_{2}	\mathbf{R}_{s}	R	Yield, %	${f Recrystn} {f solvent}^a$	$M_{ m p, \ ^{\circ}C}$	MED, mg %	Formula
8	H	HO	H	NH4	82.0	B-L	222-224	>20	$C_9H_{13}N_sO_5$
84	Н	SCH.	H	Н	83.5	F	156 - 159	>10	$\mathrm{C_{10}H_{12}N_4O_4S\cdot C_4H_8O_2}$
85	SCH.	NH.	Н	Н		Ľ	242	>20	$C_{10}H_{13}N_5O_4S$
86	SCH ₃	NH [*]	Н	C_2H_5	70.0	В	169 - 170	>10	$C_{12}H_1 \gamma N_5 O_4 S$
87	Н	NH ²	$_{\rm Br}$	Na	34.0	$\mathbf{B}^{-}\mathbf{L}$	217 - 219	>10	$C_9H_9N_5O_4BrNa \cdot 0.5H_2O_5$
88	Η	NH ²	Br	C_2H_5	70.0	В	182 - 183	>1	$\mathrm{C}_{11}\mathrm{H}_{14}\mathrm{N}_{\mathrm{s}}\mathrm{O}_{4}\mathrm{Br}$
89	Н	NH.	SCH_3	$\mathrm{C_2H_5}$	38.4	B	143 - 145	>10	$C_{12}H_{17}N_5O_4S \cdot H_2O$
06	Н	NHCHO	Н	CH_3	20.4	Α	169-171	$<\!10$	$C_{11}H_{13}N_sO_s$
16	Н	NHCHO	Н	C_2H_5	22.6	В	160 - 161	0.2	$C_{12}H_{15}N_5O_5$
92	Н	NHCHO	Н	n-C ₅ H ₁₁	11.4	В	132 - 134	0.2	$C_{15}H_{21}N_5O_5$
93	Н	NHCHO	Н	$i-C_5H_{11}$	21.4	В	167 - 168	0.2	$\mathbf{C}_{15}\mathbf{H}_{21}\mathbf{N}_{5}\mathbf{O}_{5}$
94	Н	NHCHO	Н	CH_2Ph	27.0	В	109 - 111	>0.2	$\mathbf{C}_{17}\mathbf{H}_{17}\mathbf{N}_{5}\mathbf{O}_{5}\cdot0$. 25 $\mathbf{H}_{2}\mathbf{O}$
95	Н	NHEt	Н	Н	6.0	B^{-I}	190 - 192	>2	$C_{11}H_{15}N_5O_4 \cdot 1$. $5H_2O$
96	Н	NH-n-Bu	Н	<i>i</i> -Bu				>0.2	
97	Н	NHCH ₂ Ph	Н	Н				>10	
98	Н	NHCONHEt	Н	C_2H_5	12.4	в	177 - 180	>1	$C_{14}H_{20}N_6O_5$
66	Н	NHCONHPh	Н	C_2H_5	13.6	æ	186-188	>1	$C_{18}H_{20}N_6O_5$

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ture of eritadenine ethyl ester and a large excess of an amine was heated at $80-85^{\circ}$ for 24 hr. After cooling, the reaction mixture was diluted with isopropyl ether and the precipitate was collected by filtration. Recrystallization from an appropriate solvent afforded the pure product. The reaction with an amine which has a low boiling point was run in ethanolic solution in a sealed tube.

Ethyl 4-Bromo-2,3-dihydroxybutyrate. A solution of 12 g of D-erythronolactone in 20 ml of EtOH was saturated with hydrogen bromide below 40° and allowed to stand overnight at room temperature.

The reaction mixture was evaporated in vacuo, then poured into ice-water, neutralized with 10% Na₂CO₃ solution, and extracted with ether. The extract was washed with NaCl-saturated water and dried over anhydrous sodium sulfate. After removal of the solvent, the residue was distilled in vacuo to give a colorless oil (10 g): bp 124-125° (4 mm); $\nu_{\rm max}$ (neat) 3380 (OH), 1725 (-C=O), 1210, 1090 cm⁻¹ (-CO-).

General Procedure for Preparation of the Masked Ethyl 4-Bromo-2,3-dihydroxybutyrate VIII. Ethyl 4-bromo-2,3-dihydroxybutyrate (0.1 mol) was dissolved in 200 ml of the masking reagent; then 5 ml of concentrated H₂SO₄ was added dropwise to this solution at 5°. The mixture was allowed to stand overnight at room temperature and poured into a saturated caustic baryta solution at 0° with stirring. The inorganic substance was removed by filtration, and the mother liquor was spin evaporated *in vacuo*. The residue was extracted with benzene. The extract was dried and evaporated. Distillation of the residue under reduced pressure gave a respective masked compound. For compound VIII with R = R' = CH₃, bp 80-81° (4 mm), ν_{max} (neat) 1740 cm⁻¹ (CO), yield 88%; R = H, R' = CH₃, bp 103-106° (4 mm), ν_{max} (neat) 1740 cm⁻¹ (CO), yield 75%; R = H, R' = OEt, bp 129-133° (4 mm), ν_{max} (neat) 1740 cm⁻¹ (CO), yield 78%.

General Procedure for Preparation of 4-(4-Amino-5-formylaminopyrimidin-6-yl)aminobutyric Acid Derivatives IV. A mixture of 4-amino-5-nitro-6-chloropyrimidine (0.02 mol), amino acid (0.022 mol), K2CO3 (0.035 mol), Me2CO (50 ml), and H2O (25 ml) was stirred for 3 hr at 40°, and then the acetone was removed by distillation. The residual solution was acidified with HCl to give a crystalline product II. The crude product was dissolved in a 5% KOH solution and reduced with H_2 in the presence of Raney nickel at room temperature. After removal of the catalyst by filtration, the filtrate was acidified with HCOOH to give a crystalline 4-(4,5-diaminopyrimidin-6-yl)aminobutyric acid derivative III. The crude amino acid was dissolved in formic acid (ca. 15 times volume) and refluxed for 4 hr. The formic acid was evaporated in vacuo to give the formyl derivative. Recrystallization from water gave pure IV: λ_{max} (MeOH) 270 (H+), 278 nm (OH^{-}) . For compound IV with R = H, mp 237-239°, total yield 51.5%, v_{max} (Nujol) 3360, 3160 (NH), 1670 (CO), 1630 cm⁻¹ (NHCO); R = Ph, mp 228-230°, total yield 70.5%, ν_{max} (Nujol) 3410, 3270 (NH), 1680 (CO), 1635 cm⁻¹ (NHCO).

D-4-(Aden-9-yl)pantoic Acid VI. To a mixture of adenine (5.4 g) and DMF (140 ml) was added sodium hydride (960 mg) at 10° with stirring. After heating at 55-60° for 1.5 hr, 8.6 g of pantolactone was added to the solution and the temperature was maintained for 18 hr. Then the solvent was removed by distillation under reduced pressure. To the residue was added 20 ml of $\mathrm{H_{2}O}$ and the mixture was adjusted at pH 3 with 0.1 N HCl and allowed to stand overnight in a refrigerator. The crystals which separated were collected on a funnel and washed with H₂O, then triturated in a NaHCO3 solution. After removal of the undissolved adenine by filtration, the filtrate was again adjusted to pH 3 with 0.1 NHCl and allowed to stand 2 days in a cold room to give the crude product as lightly brown precipitate. Recrystallization of the precipitate from MeOH gave 570 mg (5.4%) of pure D-4-(aden-9-yl)pantoic acid: mp 196-197.5°; $\bar{\nu}_{max}$ (Nujol) 3280 (OH), 3120 (NH), 1690 cm⁻¹ (CO).

Ethyl D-4-(Aden-9-yl)pantoate VII. A suspension of D-4-(aden-9-yl)pantoic acid (450 mg) in 50 ml of EtOH was saturated with HCl. After refluxing for 7 hr, EtOH was removed in vacuo. The residue was triturated with a NaHCO₃ solution and extracted with EtOAc. The extract was washed with H₂O and dried over anhydrous Na₂SO₄. The solvent was removed by distillation; then the residue was recrystallized from CHCl₃-EtOAc to give 270 mg (60.8%) of pure 62: mp 160.5-162°; ν_{max} (Nujol) 3280 (OH), 3100 (NH), 1732 cm⁻¹ (-CO).

Reaction of the Masked Ethyl 4-Bromo-2,3-dihydroxybutyrate with the Sodium Salt of Adenine IX. Sodium hydride (1 g. 60% oil suspension) was added to 60 ml of DMSO with stirring at 10°. After 1 hr, 4 g of adenine was added and the solution was

Table VII

		O N	NH_2 N N N N N $CH_2CH(OH$	l)CH(OH)COOR		
No.	R	Yield, %	${f Recrystn}\ {f solvent}^a$	Mp, °C	$\substack{\textbf{MED,}\\ \textbf{mg} \ \%}$	Formula
100	H	61.0	L	260-264	10	$C_9H_{11}N_5O_5$
101	C_2H_5	69.4	В	215 - 216	0.2	$C_{11}H_{15}N_5O_5 \cdot 0.5H_2O$
102	$n-C_3H_7$	35.0	В	171 - 172	0.2	$C_{12}H_{17}N_5O_5\cdot 0.5H_2O$
103	$n-C_4H_9$	50.0	н	198-199	0.2	$C_{13}H_{19}N_5O_5$
104	$i-C_4H_9$	65.0	H	216 - 217	0.2	$C_{13}H_{19}N_5O_5$
105	sec-C ₄ H ₉	28.4	\mathbf{H}	218 - 220	0.2	$C_{13}H_{19}N_{5}O_{5}\cdot 0.5H_{2}O_{5}$
106	<i>i</i> -C ₅ H ₁₁	87.0	H	204 - 205	0.2	$C_{14}H_{21}N_{5}O_{5}$
107	$n-C_8H_{17}$	12.4	Н	176–179	0.2	$C_{17}H_{27}N_{5}O_{5}$
108	$CH_2C_6H_5$	56.0	Н	192–194	>0.5	$C_{16}H_{17}N_{5}O_{5}$
109	Cyclohexyl	5 9 .0	н	230-232	2	$C_{15}H_{21}N_5O_5$
110	CH_2CH_2Cl	53.7	B-H	175 - 177	>0.2	$\mathrm{C_{11}H_{14}N_5O_5Cl} \cdot 0$, $\mathrm{5H_2O}$
111	$CH_2CH(OH)CH_2OH$	55.0	B-L	195–197	>1	$C_{12}H_{17}N_5O_7\cdot H_2O$

Table VIII

			R _i O N		DH)CH(OH)COO	R ₂	
No.	\mathbf{R}_1	\mathbf{R}_2	Yield, %	Recrystn solvent ^a	Mp, °C	$\begin{array}{c} \mathbf{MED,} \\ \mathbf{mg} \ \% \end{array}$	Formula
112	CH	C ₂ H ₅	15	м	80-82	10	$C_{10}H_{17}N_{1}O_{11}O_{17$
113	\widetilde{CH}_{3}	$i - C_5 H_{11}$	32.8	Ň	113-116	$\tilde{0.1}$	$C_{15}H_{92}N_{5}O_{5}$
114	$\mathbf{C}_{\mathbf{v}}\mathbf{H}_{\mathbf{v}}$	$C_{2}H_{5}$	33.7	F	140	5	$C_{12}H_{12}N_{5}O_{5}$
115	$i-C_5H_{11}$	C_2H_5	81.0	D	131-134	0.5	$C_{16}H_{25}N_{5}O_{5}$
116	i-C ₅ H ₁₁	$i-C_5H_{11}$	16.6	N	110-112	0.1	$C_{19}H_{31}N_5O_5$

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^aB, EtOH; D, *i*-C₃H₇OH; F, dioxane; H, DMF; I, Et₂O; L, H₂O; M, CHCl₃; N, CCl₄; A, MeOH. ^bThe sample contained dioxane as crystalline solvent.

Table IX

				NH2 N N N N N	-CH ₂ CH(OH)Cl	H(OH)COOR		
No.	Substn at	R		Yield, %	Recrystn solvent ^a	Mp, °C	MED, mg %	Formula
117 118 119 120	3 3 7 7	Na <i>i</i> -C₄H ₃ H C₂H₅	b c	70.0 d	B–I F–H H–L B	281–282 217–219 278–279 183–185	>10 10 >20 >10	$\begin{array}{c} C_{\vartheta}H_{10}N_{5}O_{4}Na\cdot 1.5H_{2}O\\ C_{13}H_{1\vartheta}N_{5}O_{4}\\ C_{\vartheta}H_{11}N_{5}O_{4}\\ C_{11}H_{15}N_{5}O_{4}\\ \end{array}$

^aSee footnote *a*, Table VIII. ^bK. Okumura, *et al.*, *J. Org. Chem.*, **36**, 1573 (1971). ^cEsterified with isobutyl alcohol in the presence of concentrated H₂SO₄. ^dSee Experimental Section.

stirred for 1 hr at 50°. To this mixture was added 3 mmol of the masked ethyl 4-bromo-2,3-dihydroxybutyrate, and the reaction mixture was heated at $85-95^{\circ}$ for 12 hr and then spin evaporated *in vacuo*. The residue was triturated with 100 ml of EtOH and the unchanged adenine precipitate was removed by filtration. The filtrate was spin evaporated *in vacuo*, and the residue was dissolved in CHCl₃, washed with water, and then dried over an-hydrous Na₂SO₄. The solvent was removed, and the residue was recrystallized from a respective solvent to give a pure sample of the eritadenine derivative.

General Procedure for Protection of the Hydroxy Groups of Eritadenine Esters (59-61). A mixture of eritadenine ester (0.01 mol), cyclic ketone (0.3 mol), TsOH·H₂O (0.015 mol), and CHCl₃ (200 ml) was heated under reflux until separation of H₂O was no longer observed (10-20 hr). The reaction mixture was concentrated *in vacuo* and the residue was dissolved in AcOEt. The solution was washed first with aqueous NaHCO₃ and then with H_2O and dried. Evaporation of the solvent gave the crude product, which was recrystallized from the respective solvent to give the pure product.

4-(Aden-9-yl)-1,2(S),3(R)-butanetriol (71). To a suspension of eritadenine ethyl ester (3.0 g, 0.011 mol) in EtOH (100 ml) was added portionwise NaBH₄ (1.75 g, 0.046 mol) with stirring at 25°, and stirring was continued for 5 hr. The reaction mixture was concentrated *in vacuo* to give the crude product. The crude product was dissolved in H₂O and the solution was passed through a column of Amberlite IR-120 (H⁺ form). The column was washed with H₂O and then eluted with 5% NH₄OH. The eluate was

Table X



^aThis compound was obtained as a by-product in the preparation of isopentyl N¹-ethoxyeritadenate, mp 151° (AcOEt). Anal. Calcd for $C_{16}H_{27}O_6N_5$: C, 49.86; H, 7.06; N, 18.17. Found: C, 50.02; H, 7.01; N, 18.13.

evaporated to dryness in vacuo. The residue was recrystallized from H_2O to give 4-(6-amino-9H-purin-9-yl)-1,2(S),3(R)-butanetriol‡ (2.05 g, 80.4 %) as colorless prisms: mp 222-224° dec. Anal. Calcd for C₉H₁₃N₅O₃: C, 45.17; H, 5.47; N, 29.27. Found: C, 45.03; H, 5.45; N, 29.30.

Preparation of the Compounds Listed in Table VI. Compound 83. Diazotization of Eritadenine. To a solution of eritadenine (0.5 g, 2 mmol) in 50% HNO₃ (14 ml) was added NaNO₂ (0.69 g, 0.01 mol) in small portions with stirring at 25°. After stirring for 5 hr, the reaction mixture was concentrated to dryness *in* vacuo and the residue was dissolved in H₂O. The aqueous solution was passed through a column of Diaion SKI-B resin (H⁻ form of a sulfonic acid-type ion-exchange resin). The column was thoroughly washed with H₂O and then eluted with 5% NH₄OH. The eluate was evaporated *in* vacuo to afford the crude product, which was recrystallized from aqueous MeOH to give 4-(6-hydroxy-9H-purin-9-yl)-2(R),3(R)-dihydroxybutyric acid (0.44 g, 82%): mp 218-220° dec.

Compound 84. Hydrolysis of the Protecting Group. A solution of 4-(6-methylmercapto-9H-purin-9-yl)-2(R),3(R)-(O-cyclohexylidenedioxy)butyric acid (1.0 g) in 50% aqueous HCO₂H (15 ml) was allowed to stand at 25° for 4 days. The solution was concentrated to dryness *in vacuo*, and the residue was recrystallized from dioxane to give 4-(6-methylmercapto-9H-purin-9-yl)-2(R),3(R)-dihydroxybutyric acid (0.65 g, 83.5%) as colorless needles.

Compound 88. Esterification with the Alcohol in the Presence of Acid Catalyst. A mixture of the sodium salt of 8-bromoeritadenine (hemihydrate, 1.09 g, 3 mmol), absolute EtOH (80 ml), and concentrated H_2SO_4 (1.1 g) was refluxed for 3 hr. After cooling, the reaction mixture was neutralized with Amberlite IR-45 (9 g). The resin was removed by filtration and the filtrate was concentrated *in vacuo*. The resulting crystals were recrystallized from EtOH to give 8-bromoeritadenine ethyl ester (755 mg, 70%) as colorless needles: mp 182-183°.

Compound 87. Bromination of Eritadenine. To a solution of bromine (2.4 g, 0.015 mol) in H₂O (150 ml) was added the sodium salt of eritadenine (hemihydrate, 2.84 g, 0.01 mol). The mixture was allowed to stand at 25° for 6 hr. The precipitate formed was collected by filtration, washed with H₂O, and dissolved in 1 N NaOH (20 ml). The solution was diluted with EtOH to precipitate the sodium salt of 8-bromoeritadenine. Recrystallization from 70% aqueous EtOH afforded the pure sample (1.24 g, 34%) as colorless plates: mp 217-219° dec.

Compounds 90-94. N⁶-Formyleritadenine Esters. To a sus-

Table XI	
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- 4,210		
Compd	$R_{ m f}$	λ_{max} (MeOH), nm (log $\varepsilon)$
90	0.42	274 (4.29), 282 (4.14)
91 92	$0.53 \\ 0.74$	274.5(4.29), 282(4.14) 274.5(4.28), 283(4.13)
93	0.74	274.5 (4.28), 283 (4.14)
94	0.76	274 (4.26), 282 (4.11)

pension of eritadenine (2.53 g, 0.01 mol) in DMF (25 ml) was added a dimethylformamide dialkylacetal (0.04 mol) and the mixture was stirred at 25° for 10 hr. The reaction mixture was concentrated to dryness *in vacuo*. The residue was dissolved in CHCl₃ and the CHCl₃ layer was passed through a column of silica gel (1.2 × 50 cm). The product was eluted with CHCl₃-EtOH (4:1 v/v). The fractions were monitored by tlc [silica gel GF 254 (Merck), which has solvent system] and the fractions which had the respective $R_{\rm f}$ values listed in Table XI were collected. Evaporation of the solvent and recrystallization of the residue gave the N^6 -formyleritadenine alkyl ester.

Compound 95. N^{6} -Ethyleritadenine. A solution of eritadenine ethyl ester (4.0 g, 0.015 mol) and ethyl iodide (8.0 g) in DMF (50 ml) was heated at 60° for 2 days. After cooling, ether was added to the reaction mixture to separate an oil, which was treated with 3% aqueous NaOH at 90° for 1 hr. The solution was passed through a column of Amberlite IR-120 (H⁻ form) and the column was thoroughly washed with H₂O. The product was eluted with dilute ammonium hydroxide. Concentration of the eluate afforded the crude product, which was recrystallized from EtOH-Et₂O to give N⁶-ethyleritadenine (0.3 g, 6.0 %): mp 190-192°.

Compounds 98 and 99. N⁶-Carbamoyl Derivatives (Example). A mixture of the sodium salt of 4-(6-amino-9H-purin-9-yl)-2(R), 3(R)-(O-cyclohexylidenedioxy) butyric acid (12.5 g, 0.035) mol), ethyl tosylate (14 g, 0.07 mol), and DMF (50 ml) was stirred at 70° for 2 hr. The mixture was concentrated to dryness in vacuo, and the residue was extracted with CHCl₃. The extract was concentrated and the residue was purified by column chromatography with silica gel to afford ethyl 4-(6-amino-9H-purin-9-yl)-2(R), 3(R)-(O-cyclohexylidenedioxy) butyrate (5.0 g, 37%): mp 80-82°. One gram (2.77 mmol) of this sample was treated with ethyl isocyanate (0.485 g, 6.85 mmol) in benzene (20 ml) at the reflux temperature for 24 hr. The mixture was concentrated to dryness in vacuo and the residue was recrystallized from isopropyl alcohol to give ethyl 4-[6-(ethylcarbamoyl)amino-9H-purin-9yl]-2(R),3(R)-(cyclohexylidenedioxy)butyrate (0.61 g, 51.5%): mp 113-115°. Anal. Calcd for $C_{20}H_{28}N_6O_5$: C, 55.54; H, 6.53; N, 19.43. Found: C, 55.47; H, 6.64; N, 19.14. Removal of the protecting group by treatment with 70% HCO_2H at 40° for 3 days gave ethyl 4-[6-(ethylcarbamoyl)amino-9H-purin-9-yl]-2(R),3(R)dihvdroxybutyrate in 64% vield.

In the case of compound 99, the intermediate, ethyl 4-[6-(phenylcarbamoyl)amino-9H-purin-9-yl]-2(R),3(R)-(O-cyclohexylidenedioxy)butyrate, was obtained in 50% yield: mp 182°. Anal. Calcd for C₂₄H₂₈N₆O₅: C, 59.99; H, 5.87; N, 17.49. Found: C, 59.89; H, 5.68; N, 17.10.

General Procedures for Preparation of the N^1 -Oxyeritadenine Ester Derivatives (Compounds 100-111). (I) By Oxidation with H₂O₂ in AcOH. To a solution of an eritadenine ester (0.01 mol) in AcOH (30 ml) was added dropwise 30% H₂O₂ (9 ml) at 25°. The mixture was heated at 40-50° for 26 hr and then concentrated to dryness below 40° in vacuo. The residue was crystallized with Et₂O. The crystals were purified by recrystallization from an appropriate solvent to afford the product.

(II) By Esterification of N^1 -Oxyeritadenine with Alcohols in the Presence of Acid Catalyst. A mixture of N^1 -oxyeritadenine (1.34 g, 5 mmol), concentrated H₂SO₄ (1.34 g), and an alcohol (70 ml) was heated at 60-70° for 4 hr. The reaction mixture was neutralized with Amberlite IR-45 (OH⁻ form) while hot, and the resin was removed by filtration. The filtrate was allowed to stand in a refrigerator. The crystals formed were collected by filtration to give the crude product, which was recrystallized from an appropriate solvent.

General Procedure for Preparation of N^1 -Alkoxyeritadenine Esters. A solution of a N^1 -oxyeritadenine alkyl ester and an alkyl iodide (2 molar equiv) in DMF (ten times) was stirred at 25° for 24 hr. The reaction mixture was concentrated *in vacuo*, and the residue was dissolved in MeOH. The methanolic solution was neutralized with Amberlite IR-45 (OH⁻ form) and concentrated to dryness *in vacuo*. The residue was extracted with CHCl₃ and

t4-(Aden-9-yl)-1,2(S),3(R)-butanetriol was independently prepared by Kamiya, et al.,¹⁷ by reduction of eritadenine methyl ester with NaBH₄ in 2-propanol: mp 219-220°; [α]p +30° (1 N HCl), 4-(6-Amino-9H-purin-9-yl)-1,2(R),3(S)-butanetriol (72) was prepared from D-glucose and adenine according to the method of Ikehara, et al.,¹⁸ mp 218-219°; [α]¹⁷D -32.4° (c 1.05, 1 N HCl).

the CHCl₃ layer was concentrated *in vacuo* to afford the crude product. Recrystallization from an appropriate solvent gave the pure product.

Synthesis of N^7 -Isoeritadenine Xa and Xb. Reaction of 4-Amino-5-cyanoimidazole with Methyl 2,3-O-Isopropylidene-5-O-tosyl-D-ribonucleoside. To a solution of 4-amino-5-cyanoimidazole (6.0 g, 0.055 mol) in DMF (150 ml) was added NaH (52% oil dispersion, 2.7 g, 0.055 mol) and the mixture was warmed at 50° for 30 min. A solution of methyl 2,3-O-isopropylidene-5-O-tosyl-D-ribonucleoside (18.0 g, 0.55 mol) in DMF (50 ml) was added to the mixture at 30°. The whole mixture was heated at 90-100° for 2 hr and concentrated to dryness in vacuo. The residue was extracted with EtOAc. Evaporation of the solvent from the dried EtOAc layer gave a mixture of the crude products (11.0 g). This crude mixture was extracted with CHCl₃ and the CHCl₃-insoluble crystals were recrystallized from EtOH to afford methyl 5-(4'-amino-5'-cyanoimidazol-3'-yl)-2,3-O-isopropylidene-D-ribonucleoside (II): 4.5 g (31%); mp 208-209°; uv max (2 × 10³) 247 nm (13.0) at pH 6.9; uv max 238 (11.2), 254 (8.2) at pH 1.4; uv max 247 (14.6) at pH 12.3. Anal. Calcd for C13H18N4O4: C, 53.05; H, 6.16; N, 19.04. Found: C, 52.98; H, 6.16; N, 19.19. The CHCl₃ extract was concentrated to dryness in vacuo, and the residue was recrystallized from EtOH to give methyl 5-(4'-amino-5'-cyanoimidazol-1'-yl)-2,3-Oisopropylidene-D-ribonucleoside (II', 4.3 g, 29%) as colorless leaflets: mp 190–191.5°; uv max ($\epsilon \times 10^3$) 258 nm (9.3) at pH 6.9; uv max 237 (8.2), 260 (7.6) at pH 1.4; uv max 258 (10.0) at pH 12.3. Anal. Calcd for C13H18N4O4: C, 53.05; H, 6.16; N, 19.04. Found: C, 52.90; H, 6.15; N, 19.30.

Methyl 5-(6'-Amino-9'H-purin-9'-yl)-2,3-O-isopropylidene-D-ribonucleoside XIa. A solution of Xa (0.43 g) and HC(OEt)₃ (10 ml) in DMF (20 ml) was refluxed for 10 hr. The reaction mixture was concentrated *in vacuo* to give an oily product. The oil was dissolved in 20% methanolic ammonia and the solution was stirred at 25° for 2 hr. The precipitate which formed was collected by filtration to give almost pure IV (0.2 g): mp 251.5°. This was identified with an authentic sample by the mixed fusion test and comparison of the ir spectra.

Methyl 5-(6'-Amino-7'H-purin-7'-yl)-2,3-O-isopropylidene-D-ribonucleoside XII. A solution of Xb (4.2 g) and HC(OEt)₃ (5 ml) in DMF (80 ml) was refluxed for 4 hr. The reaction mixture was concentrated *in vacuo*. The residue was recrystallized from isopropyl ether to afford methyl 5-(5'-cyano-4'-ethoxymethyleneiminoimidazol-1'-yl)-2,3-O-isopropylidene-D-ribonucleoside (XIb, 4.5 g, 92%): mp 89-91°. Anal. Calcd for $C_{10}H_{22}N_4O_5$: C, 54.84; H, 6.33; N, 15.99. Found: C, 54.82; H, 6.32; N, 16.21. This sample was treated with 20% methanolic ammonia (50 ml) at 25° for 2 hr. The solution was concentrated to dryness *in vacuo*, and the residue was recrystallized from MeOH to give XII (4.0 g, 89.7%) as colorless needles: mp 157-159°. Anal. Calcd for $C_{14}H_{19}N_5O_4$. 0.5H₂O: C, 50.90; H, 6.06; N, 21.2. Found: C, 50.72; H, 5.76; N, 21.05.

7-Isoeritadenine. A solution of XII (4.0 g) in 0.1 N HCl (100 ml) was heated at $80-90^\circ$ for 3 hr. After cooling, the solution was made alkaline with NaOH (1.7 g), and oxygen was bubbled into

the solution for 10 hr. The solution was neutralized with HCO_2H and concentrated to a volume of 30 ml and acidified to pH 3.0 with HCO_2H . The precipitate was collected by filtration to give 7-isoeritadenine XIII (1.6 g, 45.4%) as colorless prisms: mp 278-279°. Anal. Calcd for $C_9H_{11}N_5O_4$: C, 42.69; H, 4.38; N, 27.67. Found: C, 42.57; H, 4.39; N, 27.27.

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