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Synthetic Studies on Eritadenine. I.[1] Reactions of Some Purines with the 2,3-O-Protected Dihydroxybutyrolactone

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A convenient method for the synthesis of eritadenine (**8**) has been achieved *via* condensation of 2(*R*),3(*R*)-O-protected dihydroxybutyrolactone with the sodium salt of some purines: adenine (**1a**), 6-benzylamino- (**1b**), 6-(*N*,*N*-dimethylaminomethyleneamino)- (**1c**), 6-amino-8-methylthio- (**1d**), 6-amino-2-methylthio- (**1e**), and 6-methylthiopurine (**1f**). Reaction of 2(*R*),3(*R*)-cyclohexylidenedioxybutyrolactone (**2**) with the sodium salt of **1a** gave 4-(6-amino-9*H*-purin-9-yl)-2(*R*),3(*R*)-cyclohexylidenedioxybutyric acid (**3a**) in fairly good yield along with small amount of 4-(6-amino-3*H*-purin-3-yl)-2(*R*),3(*R*)-cyclohexylidenedioxybutyric acid (**4a**). Similarly, the purines, **1b** and **1e**, afforded a predominant yield of 9-substituted purine and a lesser amount of 3-substituted purine; however, the purine **1d** gave both substituted purines in the ratio of 1:1. The purines, **1c** and **1f**, yielded 9-substituted and 7-substituted purine; in the case of **1c** the 7-substituted product was a major product, and it turned out to be a minor product in the case of **1f**. Each product obtained in the reaction was converted into the corresponding isomeric eritadenine, **8**, **9**, and **10**, by reduction or reductive desulfurization or amminolysis, and by subsequent hydrolysis. Reactivity of the lactones, 2(*R*),3(*R*)-cyclohexylidenedioxy- (**2**), 2(*R*),3(*R*)-isopropylidenedioxy- (**6**), and 2(*R*),3(*R*)-cyclopentylidenedioxybutyrolactone (**7**), was also investigated. This study suggested that the lactone **7** has the highest reactivity among others.

Recently we have reported[2] the structure and hypocholesterolemic activity of eritadenine (**8**) isolated from *Lentinus edodes* Sing. Kamiya[3] and coworkers have also reported their work on this substance. In view of the utility of eritadenine (**8**) as a new hypolipidemic agent, it became necessary for us to search for the convenient method of preparation. The usual construction[4] of the 9-substituted adenine by the stepwise synthesis starting from the pyrimidine or imidazole derivatives seemed to be circuitous for present purpose. Therefore, direct alkylation at N^9 of adenine (**1a**) with the reagent that has the requisite functional groups was first attempted. It is well known that a reaction[5] of γ-butyrolactone with potassium phthalimide affords 4-phthalimidobutyric acid in fairly good yield. The fact that the cleavage of the CH_2–O bond of the γ-butyrolactone and simultaneous formation of the CH_2–N bond had occurred in the course of this reaction prompted us to investigate the alkylation of adenine (**1a**) with the 2,3-O-protected dihydroxybutyrolactone in the presence of base.[6]

A reaction of 2(*R*),3(*R*)-cyclohexylidenedioxybutyrolactone (**2**) with the sodium salt of adenine (**1a**) at 140–145° in dimethylformamide afforded two isomeric products: A, mp 231–232° dec, and B, mp 280–282° dec. Elementary analysis of the products showed that they have the same empirical formula, $C_{15}H_{19}N_5O_4$, indicating them to be the 1:1 adduct of adenine (**1a**) and the lactone **2** (Scheme I).

The presence of the carboxyl group in the molecule, which was proved by the fact that they are soluble in aqueous sodium bicarbonate solution and regenerated upon acidification of the resulting solution, eliminated the occurrence of amide bond formation in the reaction. The major product A exhibits absorption at 259.5 nm (ε 15,300, pH 1.3) and 262 nm (ε 15,700, pH 12.5) and was converted into eritadenine (**8**) by acidic hydrolysis. These data confirm the structure of A as 4-(6-amino-9*H*-purin-9-yl)-2(*R*),3(*R*)-cyclohexylidenedioxybutyric acid (**3a**). On the other hand, the structure of the minor product B was elucidated as 4-(6-amino-3*H*-purin-3-yl)-2(*R*),3(*R*)-cyclohexylidenedioxybutyric acid (**4a**) by the comparison of its ultraviolet spectrum [maximum at 276.5 nm (ε 18,800, pH 1.3), 276 nm (ε 12,600, pH 12.5), and difference (−4 nm) of the mini-

(1) Preliminary communication, *Chem. Commun.*, 1045 (1970).

(2) I. Chibata, K. Okumura, S. Takeyama, and K. Kotera, *Experientia*, **25**, 1237 (1969).

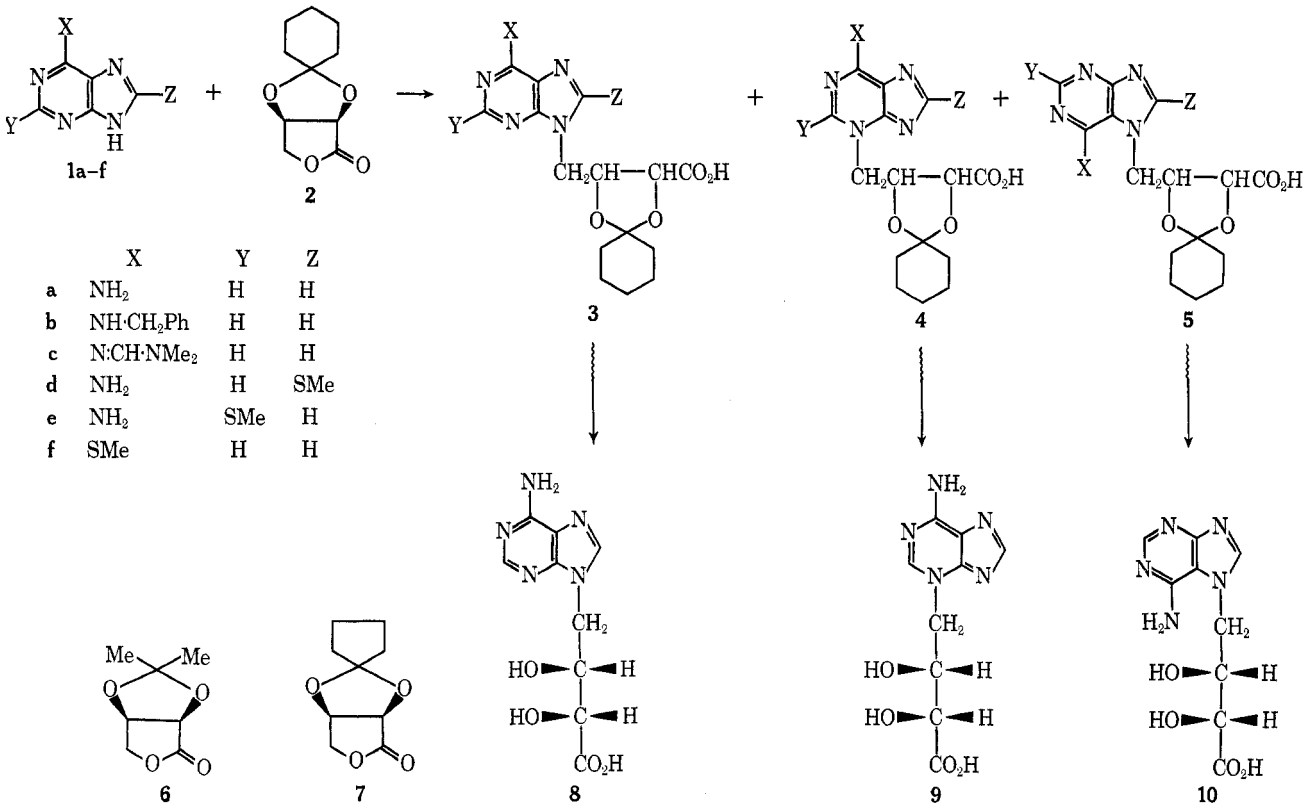
(3) (a) T. Kamiya, Y. Saito, M. Hashimoto, and H. Seki, *Tetrahedron Lett.*, 4729 (1969); (b) M. Hashimoto, Y. Saito, H. Seki, and T. Kamiya, *ibid.*, 1359 (1970); (c) T. Rokujo, H. Kikuchi, A. Tensho, Y. Tsukitani, T. Takenawa, K. Yoshida, and T. Kamiya, *Life Sci. (Oxford)*, **9**, 379 (1970).

(4) A. M. Michelson, "The Chemistry of Nucleosides and Nucleotides," Academic Press, London and New York, 1962, pp 61–68.

(5) G. Talbot, R. Gaudry, and L. Berlinguet, *Can. J. Chem.*, **36**, 593 (1958).

(6) While the author was preparing this manuscript, the similar investigation had been reported: T. Kamiya, Y. Saito, M. Hashimoto, and H. Seki, *Chem. Ind. (London)*, 652 (1970).

SCHEME I



	X	Y	Z
a	NH_2	H	H
b	$NH{\cdot}CH_2Ph$	H	H
c	$N{:}CH{\cdot}NMe_2$	H	H
d	NH_2	H	SMe
e	NH_2	SMe	H
f	SMe	H	H

mum from pH 1.0 to 7.0] and by the comparison of the $\Delta\delta$ value between the 2 and 8 protons in nmr spectrum (see Table IV) with those of 3-alkyladenines.[7] Acid hydrolysis of the crude mixture that was obtained by the reaction described above afforded a mixture of eritadenine (**8**) and 3-isoeritadenine (**9**), 4-(6-amino-3*H*-purin-3-yl)-2(*R*),3(*R*)-dihydroxybutric acid, in good yield. Chromatographic analysis by ion-exchange resin showed the ratio of the 9:3 isomer (**8**:**9**) to be approximately 10:1 and the absence of the other isomer. In an effort to improve the yield of this reaction, experiments were carried out under various conditions, change of solvent, base, and temperature, but gave no significant result. However, with the expectation that the alteration of alkylidene moiety of the ketal ring in the lactone might give preferable influence on the yield of the reaction, the lactones, 2(*R*),3(*R*)-isopropylidenedioxy- (**6**),[8] 2(*R*),3(*R*)-cyclopentylidenedioxy- (**7**), and 2(*R*),3(*R*)-cyclohexylidenedioxybutyrolactone (**2**), were subjected to the reaction at lower temperature (100°) at which the decomposition of the lactones would not affect evaluation of the relative reactivities. The higher reactivity of the lactone **7** can be deduced from the data described in Table I, and this would be explained by an assumption that the cyclopentylidene moiety would exert its higher strain on the lactone ring to make the γ carbon of the lactone more susceptible to the nucleophile.

However, at higher temperatures (140–145°), the lactones **2** and **6** gave better results than the lactone **7**.

(7) (a) N. J. Leonard and J. A. Deyrup, *J. Amer. Chem. Soc.*, **84**, 2148 (1962); (b) B. C. Pal, *Biochemistry*, **1**, 558 (1962); (c) N. J. Leonard and R. A. Laursen, *J. Amer. Chem. Soc.*, **85**, 2026 (1963); (d) J. A. Montgomery and H. J. Thomas, *J. Heterocycl. Chem.*, **1**, 115 (1964); (e) L. B. Townsend, R. K. Robins, R. N. Loeppky, and N. J. Leonard, *J. Amer. Chem. Soc.*, **86**, 5320 (1964).

(8) D. L. Mitchell, *Can. J. Chem.*, **41**, 214 (1963).

TABLE I

COMPARISON OF THE YIELDS OF THE ERITADENINE (**8**) IN THE REACTION OF THE LACTONES WITH THE SODIUM SALT OF ADENINE

No.	Lactone	Reaction temp, °C	Time, hr	Yield[a] of eritadenine, % Analyzed[b]	Isolated
1	**6**	100	50	20	10
2	**2**	100	50	22	12
3	**7**	100	50	37	24.5

[a] Approximately 10% of 3-isoeritadenine (**9**) is contaminated. [b] An aliquot of the acid hydrolysate of the concentrated reaction mixture, after neutralization, was submitted to paper partition chromatography [Toyo No. 51A filter paper, solvent system 1-butanol-AcOH-H_2O (18:8:5), by the ascending method]. Eritadenine, which is visualized on the developed chromatogram under uv light, was extracted with Clark-Lubs buffer solution (pH 1.4) and determined by means of uv spectrometry.

This inconsistency would be due to the lower stability of the lactone **7**.

Then, the investigation was extended to the reactions of the lactone **2** with other substituted purines **1b–f** that would be convertible into adenine. The reactions were carried out under similar conditions to that described for adenine (**1a**), and thus isomerically substituted products were obtained in the varying ratio which depends on the directive influence of the substituent on the purine ring (Table II).

The attaching sites of the side chain of the products were established by an analogy of uv spectra (Table III) with those of suitable model compounds reported in the literature and confirmed by conversion of the intermediates into the isomeric eritadenine, **8**, **9**, and **10**, respectively.

The previous proposal[7e] for differentiating N^3-substituted adenine between isomerically substituted adenines by nmr spectra and melting points holds also for the sets of our products (see Table IV).

TABLE II

PRODUCT COMPOSITION IN THE REACTIONS OF THE PURINES (1a–f) WITH THE LACTONE (2)

Expt no.	Purine	Product ratio 9 isomer	3 isomer	7 isomer	Total yield, %
1	1a	100	9[a]		44.2
2	1b	100	34		31.2
3	1c	100[b]	Trace[d]	184	36.5
4	1d	100	103[c]		26.8
5	1e	100	Trace[a]		52.0
6	1f	100[c]		9	67.3

[a] Determined by uv spectrometric analysis of the mixture obtained from the hydrolysate of the crude product. [b] As the intermediate **3c** could not be isolated owing to the instability of the N^6-protecting group, the value was estimated from the amount of the final product **8**. [c] Determined by nmr spectrometric analysis of the mixture. [d] Determined by paper electrophoresis of the mixture.

TABLE III

ULTRAVIOLET ABSORPTION DATA OF ISOMERICALLY SUBSTITUTED PURINES

Compd	pH 1.3 Max, nm	pH 1.3 ε × 10^{-3}	pH 7 Max, nm	pH 7 ε × 10^{-3}	pH 12.5 Max, nm	pH 12.5 ε × 10^{-3}	Ref[a]
3a	259.5	15.3	262	15.7	262	15.7	2
4a	276.5	18.8	276	14.1	276	12.6	7a–d
3b	268.5	21.8	271.5	21.8	271.5	22.0	17
4b	288	23.3	291	17.8	292	17.1	17
3d	286	22.0	280.5	21.6	280.5	19.6	*b*
4d	303	25.8	308	19.3	308	25.8	
3e	271.5	17.1	277.5	15.2	277.5	15.2	*c*
5a	275	13.6	272.5	9.8	272.5	9.8	7a, *d*
3f	286	Sh[e]	286	21.0	286	21.2	*f, g*
	294	19.3	294	21.0	294	21.0	
5f	303	12.4	295	13.8	295	13.8	*g, h*
	296	Sh	302	Sh	302	Sh	

[a] The spectral data of the model compounds shown in these references were compared with those of the compounds listed in Table III. [b] R. E. Holmes and R. K. Robins, *J. Amer. Chem. Soc.*, **86**, 1242 (1964). [c] Y. Ishido, T. Sato, and Y. Kikuchi, *Nippon Kagaku Zasshi*, **86**, 240 (1965); H. J. Schaeffer and H. J. Thomas, *J. Amer. Chem. Soc.*, **80**, 3738 (1958). [d] J. A. Montgomery and H. J. Thomas, *ibid.*, **85**, 2672 (1963). [e] Sh = shoulder. [f] D. E. O'Brien, J. D. Westover, R. K. Robins, and C. C. Cheng, *J. Med. Chem.*, **8**, 182 (1965). [g] Z. Neiman and F. Bergmann, *Israel J. Chem.*, **3**, 161 (1965); B. Pullman, H. Berthod, F. Bergmann, Z. Neiman, H. Weiler-Feilchenfeld, and E. D. Bergmann, *Tetrahedron*, **26**, 1483 (1970). [h] R. J. Rousseau, R. P. Panzica, S. M. Reddick, R. K. Robins, and L. B. Townsend, *J. Org. Chem.*, **35**, 631 (1970).

TABLE IV

MELTING POINTS AND DIFFERENCES OF THE CHEMICAL SHIFT (IN D_2O–NaOD)[a] BETWEEN THE SIGNALS OBSERVED FOR THE 2- AND 8-AROMATIC PROTONS OF THE COMPOUNDS

Compd	Mp, °C, dec	Δδ, cps	Compd	Mp, °C, dec	Δδ, cps
8	261–263	3	**3a**	231–232	7[b]
9	297–299	30	**4a**	280–282	15
10	278–279	5	**5a**	242–244	2

[a] Solute concentration is about 0.2 mol. [b] Determined in DMSO-d_6.

In the case of N^6-benzyladenine[9] (**1b**), the 9 isomer **3b** [4-(6-benzylamino-9*H*-purin-9-yl)-2(*R*),3(*R*)-cyclohexylidenedioxybutyric acid] was a major product, though the relative yield of the 3 isomer **4b** [4-(6-benzylamino-3*H*-purin-3-yl)-2(*R*),3(*R*)-cyclohexylidenedioxybutyric acid] increased slightly. Mild hydrolysis (at 80–90°, 1 hr) of **3b** and **4b** with dilute hydrochloric acid gave N^6-benzyleritadenine (**11**) and N^6-benzyl-3-isoeritadenine (**12**), respectively, in good yield. Both **11** and **12** resisted stubbornly hydrogenolytic debenzylation. However, reductive debenzylation of **11** with sodium in liquid ammonia yielded **8** in low yield (13.7%).

The *N*,*N*-dimethylaminomethylene group had been widely used[10] in the chemistry of nucleotides to protect the reactive amino functions of heterocyclic moieties. However, the alkylation of 6-(*N*,*N*-dimethylaminomethyleneamino)purine (**1c**) has not been attempted up to the present. Therefore, it was interesting for us to study the directive influence of the *N*,*N*-dimethylaminomethyleneamino group on the purine in our alkylation reaction.

Interestingly, the 7 isomer **5c** {4-[6-(*N*,*N*-dimethylaminomethyleneamino)-7*H*-purin-7-yl]-2(*R*),3(*R*)-cyclohexylidenedioxybutyric acid}, maximum at 332 nm (ε 38,100, pH 1.3) and 316 nm (ε 28,100, at pH 12.5), was obtained predominantly, and the 9 isomer **3a**, which would arise from the intermediary product **3c** by deblocking of the protective group in the course of separation, was also obtained as the second product. Chromatographic analysis of the partially purified mixture that was obtained from the acid hydrolysate of the reaction product showed the presence of a minute amount of **9**. However, this does not necessarily indicate the occurrence of the alkylation at N^3 of **1c**, because the partial decomposition, though very small, of **1c** into adenine under the reaction condition was observed in a preliminary experiment by which the stability of **1c** was checked, and the resultant adenine would react with the lactone **2**, yielding **3a** and **4a**. Treatment of **5c** with 5% ammonium hydroxide caused hydrolysis of the N^6-protective group selectively to give 4-(6-amino-7*H*-purin-7-yl)-2(*R*),3(*R*)-cyclohexylidenedioxybutyric acid (**5a**), which on acid hydrolysis afforded the 7-isoeritadenine (**10**) [4-(6-amino-7*H*-purin-7-yl)-2(*R*),3(*R*)-dihydroxybutyric acid]. The structure of 7-isoeritadenine (**10**) was assigned by the elemental analysis and ultraviolet spectra [maximum at 275 nm (ε 14,100, pH 1.3), 272.5 nm (ε 10,400, pH 12.5) and difference (+7 nm) of the minimum from pH 1.0 to 7.0] and finally confirmed by the direct comparison of its spectrometric data with those of authentic sample that was prepared unambiguously *via* methyl 5-(5′-amino-4′-cyanoimidazol-3′-yl)-2,3-*O*-isopropylidene ribofuranoside.[11]

The alkylation of the sodium salt of 6-amino-8-methylthiopurine (**1d**)[12] with the lactone **2** gave a mixture of the 9 isomer **3d** [4-(6-amino-8-methylthio-9*H*-purin-9-yl)-2(*R*),3(*R*)-cyclohexylidenedioxybutyric acid] and the 3 isomer **4d** [4-(6-amino-8-methylthio-3*H*-purin-3-yl)-2(*R*),3(*R*)-cyclohexylidenedioxybutyric acid]. Both isomers were obtained pure by chromatographic separation with silica gel in the ratio of approximately 1:1. The ratio was supported by the integration of each ring proton signal (δ 8.13 ppm of **3d** and 8.25 ppm of **4d**) in the nuclear magnetic resonance (nmr) spectrum of the mixture.

(9) C. W. Whitehead and J. J. Travrso, *J. Amer. Chem. Soc.*, **82**, 3973 (1960).

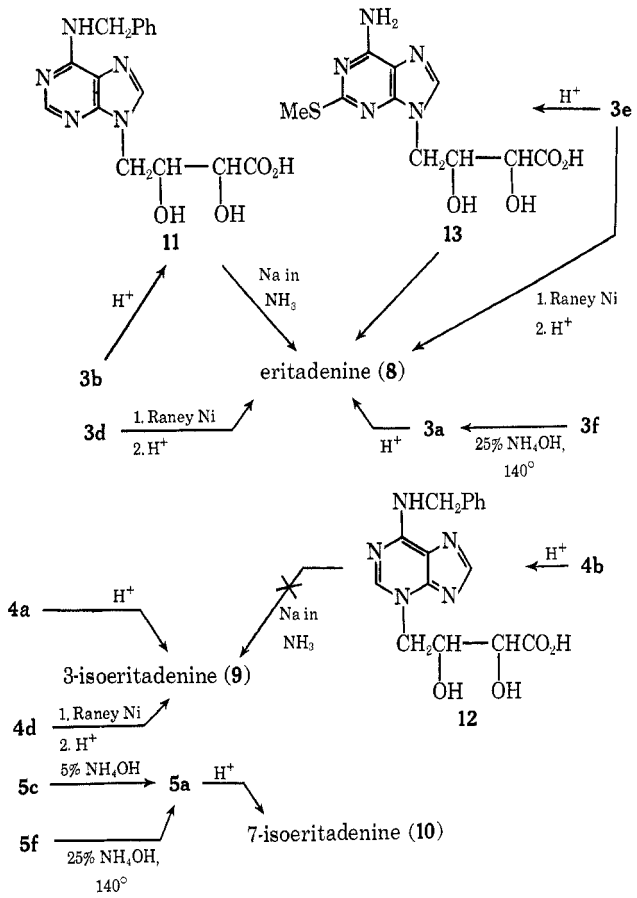
(10) A. Holy and J. Zemlicka, *Collect. Czech. Chem. Commun.*, **34**, 2449 (1969), and references therein.

(11) S. Ohoshiro, T. Nagura, and K. Okumura, unpublished work.

(12) R. K. Robins, *J. Amer. Chem. Soc.*, **80**, 6671 (1958).

Desulfurization of **3d** and **4d** with Raney nickel, after acid hydrolysis, afforded the isomeric eritadenine, **8** and **9**, respectively (Scheme II).

SCHEME II



In the case of 6-amino-2-methylthiopurine (**1e**),[13] the 9 isomer **3e** [4-(6-amino-2-methylthio-9*H*-purin-9-yl)-2(*R*),3(*R*)-cyclohexylidenedioxybutyric acid] was again a major product, and concomitant yield of the 3 isomer **4e** [4-(6-amino-2-methylthio-3*H*-purin-3-yl)-2-(*R*),3(*R*)-cyclohexylidenedioxybutyric acid] diminished so markedly that the formation of **4e** was detected only by chromatographic analysis of the product which was obtained after desulfurization and acid hydrolysis of the crude reaction product. The high yield of **4d** from **1d** and the extremely low yield of **4e** from **1e** are attributable to steric hindrance by the methylthio groups at C_2 or C_8 of the purine (**1d** or **1e**).

The alkylation of the sodium salt of 6-methylthiopurine (**1f**)[14] with the lactone **2** gave the 9 isomer **3f** [4-(6-methylthio-9*H*-purin-9-yl)-2(*R*),3(*R*)-cyclohexylidenedioxybutyric acid] in fairly good yield along with small amounts of the 7 isomer **5f** [4-(6-methylthio-7*H*-purin-7-yl)-2(*R*),3(*R*)-cyclohexylidenedioxybutyric acid] which was isolated by chromatographic separation with silica gel. Ammonolysis of **3f** and **5f** with 25% ammonium hydroxide at 140–145° yielded **3a** and **5a**, respectively.

Some of the reactions described here provide a convenient method for the preparation of eritadenine because of simplicity of the reaction and availability of the material used.

(13) E. C. Taylor, O. Vogl, and C. C. Cheng, *J. Amer. Chem. Soc.*, **81**, 2442 (1959).

(14) G. B. Eliton, E. Burgi, and G. H. Hitchings, *ibid.*, **74**, 411 (1952).

Experimental Section

Melting points were determined on a Yamato apparatus MP-21 and are uncorrected. The nmr spectra were determined on a Hitachi Perkin-Elmer R-20A instrument with tetramethylsilane as internal standard. Uv spectra were determined on a Hitachi EPS-2U instrument.

2(*R*),3(*R*)-Cyclohexylidenedioxybutyrolactone (2).—A mixture of 2(*R*),3(*R*)-dihydroxybutyrolactone[15] (59 g), cyclohexanone (50 g), *p*-toluenesulfonic acid (2.5 g), and benzene (350 ml) in a flask equipped with Dean-Stark separator was refluxed for 5 hr (the water that had formed was removed from the separator). The mixture was diluted with 150 ml of benzene and cooled; then the diluted mixture was washed with H_2O, saturated aqueous sodium bicarbonate solution, and again with H_2O. The benzene layer was dried over anhydrous sodium sulfate and concentrated to dryness *in vacuo*. The residue was triturated with 100 ml of hexane and collected by filtration to give **2**, yield 93 g (90%). Recrystallization from cyclohexane gave analytically pure **2** as colorless leaflets: mp 76–78°; $[\alpha]^{20}D$ −104° (*c* 1.0, $CHCl_3$); ir (Nujol) 1764 cm^{-1} ($\nu_{C=O}$).

Anal. Calcd for $C_{10}H_{14}O_4$: C, 60.59; H, 7.12. Found: C, 60.51; H, 7.03.

2(*R*),3(*R*)-Cyclopentylidenedioxybutyrolactone (7).—A mixture of 2(*R*),3(*R*)-dihydroxybutyrolactone (59 g), cyclopentanone (44 g), *p*-toluenesulfonic acid (5 g), and benzene (500 ml) was refluxed for 7 hr. Similar treatment to that described above gave **7**, yield 67 g (69.5%). Recrystallization from isopropyl ether gave analytically pure **7** as colorless prisms: mp 47–49°; $[\alpha]^{20}D$ −107.2° (*c* 1.0, $CHCl_3$); ir (Nujol) 1773 cm^{-1} ($\nu_{C=O}$).

Anal. Calcd for $C_9H_{12}O_4$: C, 58.69; H, 6.57. Found: C, 58.54; H, 6.37.

6-(*N*,*N*-Dimethylaminomethyleneamino)purine (1c).—A mixture of adenine (**1a**, 91 g, 0.67 mol), DMF (1680 ml), and dimethylformamide dimethyl acetal[16] (101 g) was stirred at 50° for 6 hr. The mixture was evaporated to dryness *in vacuo* and the residue was triturated with EtOH. The resulting crystals were filtered and dried. Recrystallization from a 1:1 mixture of DMF and EtOH gave **1c**: yield 109.5 g (86.2%); mp 252–255°; nmr (DMSO-d_6) δ 3.36 (s, 3 H), 3.42 (s, 3 H), 8.61 (s, 1 H), 8.73 (s, 1 H), 9.19 (s, 1 H); uv max ($\epsilon \times 10^{-3}$) 222.5 nm (16.0), 287 (13.1), and 324 (21.7) at pH 1.6, 225 (14.4) and 310 (33.8) at pH 7.0, 227 (15.9) and 308 (23.9) at pH 12.6.

Anal. Calcd for $C_8H_{10}N_6$: C, 50.51; H, 5.30; N, 44.19. Found: C, 50.41; H, 5.19; N, 44.41.

General Procedure for the Reactions of the Sodium Salts of the Purines (1a–f) with 2(*R*),3(*R*)-Cyclohexylidenedioxybutyrolactone (2).—The sodium salt of the purine was prepared by stirring a suspension of an equimolar amount of the purine and sodium hydride (in mineral oil) in DMF (4 ml/mmol of the purine) at 100° for 1 hr. To this suspension was added the lactone **2** (equimolar amount) and stirring was continued at 140–145° for 15 hr. After cooling, the insoluble solid that had formed was filtered off and the filtrate was evaporated to dryness *in vacuo*. The resulting residue was treated in the appropriate manner for the respective reaction.

This procedure was employed for the reaction, unless otherwise stated.

Reaction of the Sodium Salt of Adenine (1a).—A mixture of the sodium salt of adenine (**1a**, 50 mmol), the lactone **2** (6.0 g, 50 mmol), and DMF (200 ml) was treated in the manner described in the general procedure. The resulting residue was dissolved in 50 ml of H_2O, and the solution was treated with charcoal and filtered. The filtrate was passed through a column of Amberlite IRC-50 (H form, 80 ml) and the column was washed with 1 l. of H_2O. The eluate and washing was evaporated to dryness *in vacuo*. The residue was dissolved in H_2O (30 ml) and the solution was acidified to pH 3.0 with 2% formic acid. The crude product that had precipitated was collected by filtration. The crude product was dissolved in H_2O (30 ml) containing 2 g of $NaHCO_3$, and the solution was treated with charcoal and filtered. The filtrate was acidified to pH 3.5 with formic acid and the precipitate that had formed was collected by filtration to yield 5.9 g of an approximately 10:1 mixture of 4-(6-amino-9*H*-purin-9-yl)-2(*R*),3(*R*)-cyclohexylidenedioxybutyric acid (**3a**) and 4-(6-

(15) (a) A. Jeanes and C. S. Hudson, *J. Org. Chem.*, **20**, 1565 (1955); (b) A. S. Perlin and C. Brice, *Can. J. Chem.*, **33**, 1216 (1955); (c) R. Barker and D. L. MachDonald, *J. Amer. Chem. Soc.*, **82**, 2301 (1960).

(16) Z. Aronild and M. Kornilov, *Collect. Czech. Chem. Commun.*, **29**, 645 (1961).

amino-3*H*-purin-3-yl)-2(*R*),3(*R*)-cyclohexylidenedioxybutyric acid (**4a**). The mixture was suspended in 20 ml of DMF and the suspension was boiled for 10 min. Insoluble material was removed by filtration while still hot and the filtrate was cooled. The colorless prisms that had formed were collected by filtration to give **3a**, yield 5.0 g (35.4%), mp 227–229° dec. The analytical sample that was recrystallized from DMF melted at 231–232° dec: $[\alpha]^{25}D$ +97.8° (*c* 1.0, 0.1 *N* NaOH); nmr (DMSO-d_6) δ 1.1–1.9 (m, 10 H), 4.1–4.3 (m, 2 H), 4.6–4.9 (m, 2 H), 8.20 (s, 1 H), 8.27 (s, 1 H).

Anal. Calcd for $C_{15}H_{19}N_5O_4$: C, 54.04; H, 5.75; N, 21.01. Found: C, 53.93; H, 5.74; N, 20.90.

The solid that was insoluble in hot DMF was dissolved in 10 ml of 2% aqueous sodium bicarbonate solution. After filtration, the solution was acidified to pH 3.5 to yield crude **4a** (0.4 g) which was collected by filtration. A suspension of **4a** in 10 ml of DMF was boiled for 5 min and the insoluble solid was collected by filtration while hot. This solid was dissolved again in 8 ml of 2% aqueous sodium bicarbonate solution and the solution was acidified to pH 3.5 to give pure **4a**, yield 370 mg (2.6%). The analytical sample was dried at 110° over P_2O_5 for 40 hr *in vacuo*: mp 280–282° dec; $[\alpha]^{25}D$ +145.6° (*c* 1.0, 0.1 *N* NaOH); nmr (D_2O–NaOD) δ (dioxane as internal reference) 0.8–1.9 (m, 10 H), 3.9–4.9 (m, 4 H), 7.77 (s, 1 H), 8.0 (s, 1 H).

Anal. Calcd for $C_{15}H_{19}N_5O_4$: C, 54.04; H, 5.75; N, 21.01. Found: C, 54.14; H, 5.70; N, 20.87.

Direct isolation of eritadenine (**8**) and 3-isoeritadenine (**9**) from the acid hydrolysate of the crude reaction product was carried out in the following manner.

The reaction of the sodium salt of adenine (**1a**, 8.1 g, 60 mmol) with the lactone **2** (9.9 g, 50 mmol) was carried out in the manner described in the general procedure. The resulting residue was dissolved in 10% hydrochloric acid (50 ml) and the solution was heated at 80° for 1 hr. After cooling, the solution was treated with charcoal and filtered. The filtrate was diluted with H_2O (40 ml) and neutralized with 25% ammonium hydroxide. To this solution was added 3.5 g of $MgCl_2 \cdot 6H_2O$, and the mixture was allowed to stand at room temperature overnight. A mixture (8.0 g) of the magnesium salt of eritadenine (**8**) and 3-isoeritadenine (**9**) that had separated was collected by filtration. The mixture of the magnesium salts was dissolved in dilute hydrochloric acid and the solution was treated with charcoal and then filtered. The filtrate was adjusted to pH 3.0 with 10% aqueous sodium hydroxide to give a mixture (5.0 g, 39%) of **8** and **9**; the mixture was shown to be a 100:8 mixture of **8** and **9** by ultraviolet spectral analysis. The mixture (5.0 g) was dissolved in H_2O (35 ml) containing $NaHCO_3$ (1.7 g) with slight warming. The solution was treated with charcoal and filtered. To the hot filtrate was added 170 ml of boiling ethanol. The mixture was allowed to stand at room temperature overnight and the resulting crystals of the sodium salt of **8** were collected by filtration. Further crystallization from 80% EtOH gave the pure sodium salt of **8** as colorless leaflets: yield 5.1 g; mp 265–266° dec; $[\alpha]^{25}D$ +39.4° (*c* 2.0, H_2O). The analytical sample was dried at 50° for 7 hr.

Anal. Calcd for $C_9H_{10}N_5O_4Na \cdot 2^1/_2H_2O$: C, 33.75; H, 4.72; N, 21.87. Found: C, 33.99; H, 4.73; N, 21.74.

From the sodium salt, pure eritadenine (**8**) was obtained, mp 265–266° dec.

Anal. Calcd for $C_9H_{11}N_5O_4$: C, 42.69; H, 4.38; N, 27.67. Found: C, 42.78; H, 4.52; N, 27.78.

This product was identical in all respects, decomposition point, uv and ir spectra, behavior on paper electrophoresis, and specific rotation, with natural eritadenine (**8**).

The mother liquors from the purification of the sodium salt were combined and evaporated to dryness *in vacuo*. The residue was recrystallized twice from 70% EtOH to give the sodium salt of 3-isoeritadenine (**9**, 0.2 g) as colorless prisms, mp 281–282° dec.

This sodium salt was dissolved in H_2O (2 ml), and the solution was acidified to pH 4 with 2% formic acid to give pure **9**.

Recrystallization from H_2O gave the analytically pure sample as colorless prisms: mp 297–299° dec; $[\alpha]^{25}D$ +86.7° (*c* 1.0, 0.1 *N* NaOH); uv max (ε × 10^{-3}) 276 nm (19.7) at pH 1.3, 276 (15.4) at pH 7, 276 (14.0) at pH 12.5; nmr (D_2O–NaOD) δ 4.2–4.6 (m, 4 H), 7.90 (s, 1 H), 8.20 (s, 1 H).

Anal. Calcd for $C_9H_{11}N_5O_4$: C, 42.69; H, 4.38; N, 27.67. Found: C, 42.81; H, 4.38; N, 27.45.

Reaction of the Sodium Salt of 6-Benzylaminopurine (1b).—A mixture of the sodium salt of **1b** (0.25 mol), the lactone **2** (49.5 g, 0.25 mol), and DMF (1250 ml) was treated in the manner described in the general procedure. The resulting residue was added to 750 ml of 5% aqueous sodium bicarbonate solution and the insoluble solid, which was found to be unchanged **1b** (21.6 g, recovery of 38.4%), was collected by filtration. The filtrate was acidified to pH 3 with 300 ml of 10% formic acid and the crystals that had precipitated were collected by filtration. These crystals were dissolved in EtOH (1000 ml), and the solution was allowed to stand at room temperature overnight. The deposited crystals were collected by filtration and dried to give a crude sample of 4-(6-benzylamino-3*H*-purin-3-yl)-2(*R*),3(*R*)-cyclohexylidenedioxybutyric acid (**4b**), yield 8.4 g (7.9%), mp 226–229°. Recrystallization from DMF afforded the analytically pure sample as colorless prisms, mp 260–261° dec, $[\alpha]^{25}D$ +87.1° (*c* 1.0, 0.1 *N* NaOH).

Anal. Calcd for $C_{22}H_{25}N_5O_4$: C, 62.40; H, 5.95; N, 16.54. Found: C, 62.64; H, 6.07; N, 16.76.

The ethanol filtrate was evaporated to dryness *in vacuo* and the residue was crystallized from a smaller amount of EtOH to give 4-(6-benzylamino-9*H*-purin-9-yl)-2(*R*),3(*R*)-cyclohexylidenedioxybutyric acid (**3b**), yield 24.7 g (23.3%), mp 187–189° dec. Recrystallization from MeOH afforded the analytically pure sample as colorless leaflets; mp 187–189° dec; $[\alpha]^{25}D$ +71.8° (*c* 1.0, 0.1 *N* NaOH); nmr (DMSO-d_6) δ 1.10–2.10 (m, 10 H), 4.30 (m, 2 H), 4.85 (m, 4 H), 7.31 (m, 5 H), 8.12 (s, 1 H), 8.23 (s, 1 H), 8.28 (m, 1 H).

Anal. Calcd for $C_{22}H_{25}N_5O_4$: C, 62.40; H, 5.95; N, 16.54. Found: C, 62.36; H, 5.92; N, 16.68.

Reaction of the Sodium Salt of 6-(*N*,*N*-Dimethylaminomethyleneamino)purine (1c).—A mixture of the sodium salt of **1c** (0.15 mol), the lactone **2** (29.7 g, 0.15 mol), and DMF (1000 ml) was treated in the manner described in the general procedure. (In this experiment, the reaction was carried out at 120° for 12 hr.)

The resulting residue was triturated with ether and the insoluble solids were collected by filtration and redissolved in MeOH. The methanol solution was passed through a column of Amberlite IRC-50 (H form, 500 ml, in MeOH). The eluate and methanolic washing (total volume, 2.4 l.) was evaporated to dryness *in vacuo*, and to the residue was added 500 ml of H_2O. The insoluble solid, which was found to be unchanged starting material **1c**, was removed by filtration, recovery 9.4 g (31.6%), mp 82–84°. Magnesium chloride hexahydrate (70 g) was added to the filtrate and the solid that had formed was collected by filtration to give the magnesium salt of 4-[6-(*N*,*N*-dimethylaminomethyleneamino)-7*H*-purin-7-yl]-2(*R*),3(*R*)-cyclohexylidenedioxybutyric acid (**5c**). The magnesium salt was dissolved in dilute hydrochloric acid and the pH of the solution was adjusted to 3.5 with 10% sodium hydroxide solution. The crystals that had precipitated were collected by filtration and dried to give crude **5c** (22 g, mp 205–208° dec). Recrystallization from EtOH gave pure **5c** as colorless needles: yield 13.8 g (23.7%); mp 211–213°; $[\alpha]^{25}D$ +150.5° (*c* 1.0, 0.1 *N* NaOH); mass spectrum M^+ 388; nmr (DMSO-d_6) δ 1.0–2.0 (m, 10 H), 3.05 (s, 3 H), 3.13 (s, 3 H), 4.55–5.05 (m, 4 H), 8.24 (s, 1 H), 8.39 (s, 1 H), 8.99 (s, 1 H); uv max (ε × 10^{-3}) 332 nm (38.1) at pH 1.3, 318 (28.1) at pH 7, 316 (28.1) at pH 12.5.

Anal. Calcd for $C_{18}H_{24}N_6O_4$: C, 55.66; H, 6.23; N, 21.64. Found: C, 56.10; H, 6.18; N, 21.71.

The mother liquor from filtration of the magnesium salt of **5c** was evaporated to dryness *in vacuo*. The residue was triturated with 2-propanol and the insoluble solid was collected by filtration. The solid was dissolved in dilute hydrochloric acid and the pH of the solution was adjusted to 3.5 with 10% sodium hydroxide solution. The precipitate that had formed was collected by filtration and dried to give 4-(6-amino-9*H*-purin-9-yl)-2(*R*),3(*R*)-cyclohexylidenedioxybutyric acid (**3a**), yield 6.4 g (12.8%), mp 218–220° dec. This product was identical in its uv and ir spectra with those of the sample obtained in the case of adenine (**1a**).

Reaction of the Sodium Salt of 8-Methylthio-6-aminopurine (1d).—A mixture of the sodium salt of **1d** (30 mmol), the lactone **2** (6.0 g, 31 mmol), and DMF (100 ml) was treated in the manner described in the general procedure. The resulting residue was dissolved in 100 ml of H_2O, and the solution, after treatment with charcoal, was passed through a column of Amberlite IRC-50 (H form, 100 ml). The column was washed with H_2O, and the eluate and washing (total 1500 ml) was evaporated to dryness *in vacuo*. The residue was shown by tlc (on silica gel, solvent $CHCl_3$–MeOH–AcOH 85:15:3) to be a mixture of two major products. This was chromatographed on silica gel (0.2–0.5 mm, 150 g, solvent $CHCl_3$–MeOH–AcOH, 80:20:3).

By suitable combination of fractions (on the basis of their tlc characteristics), two pure components were obtained. The first fraction to be eluted from the column was dissolved in 15 ml of 5% aqueous sodium bicarbonate solution, and the solution was acidified to pH 3 with 10% formic acid. The crystals that had precipitated were collected by filtration and dried. Recrystallization of the crystals from 50% MeOH gave 4-(6-amino-8-methylthio-9*H*-purin-9-yl)-2(*R*),3(*R*)-cyclohexylidenedioxybutyric acid (**3d**) as colorless prisms: yield 0.49 g (13.2%); mp 173–175° dec; $[\alpha]^{25}D$ +65.0° (*c* 1.0, 0.1 *N* NaOH); nmr (DMSO-d_6) δ 0.9–2.1 (m, 10 H), 2.73 (s, 3 H), 4.20 (m, 2 H), 4.62–5.10 (m, 2 H), 8.20 (s, 1 H).

Anal. Calcd for $C_{16}H_{21}N_5O_4S \cdot {}^1/_2H_2O$: C, 49.47; H, 5.71; N, 18.03; S, 8.25. Found: C, 49.82; H, 5.48; N, 17.80; S, 8.28.

The second component was recrystallized from 60% MeOH to give 4-(6-amino-8-methylthio-3*H*-purin-3-yl)-2(*R*),3(*R*)-cyclohexylidenedioxybutyric acid (**4d**) as colorless needles: yield 0.47 g (13.6%); mp 216–218° dec; $[\alpha]^{25}D$ +145° (*c*, 1.0, 0.1 *N* NaOH); nmr (DMSO-d_6) δ 1.05–2.0 (m, 10 H), 2.62 (s, 3 H), 3.95–4.60 (m, 2 H), 4.60–5.05 (m, 2 H), 8.22 (s, 1 H).

Anal. Calcd for $C_{16}H_{21}N_5O_4S \cdot H_2O$: C, 48.35; H, 5.83; N, 17.62. Found: C, 48.46; H, 5.58; N, 17.31.

Reaction of the Sodium Salt of 6-Amino-2-methylthiopurine (1e).—A mixture of the sodium salt of **1e** (26 mmol), the lactone **2** (8.6 g, 43 mmol), and DMF (150 ml) was treated in the manner described in the general procedure. The resulting residue was dissolved in H_2O and the insoluble solid, which was found to be unchanged **1e**, was filtered off. The filtrate was treated with charcoal and filtered.

This decolorized solution was acidified to pH 3 with 80% formic acid. The solid that had formed was collected by filtration, washed with H_2O, and dried. Recrystallization from acetone gave 4-(6-amino-2-methylthio-9*H*-purin-9-yl)-2(*R*),3(*R*)-cyclohexylidenedioxybutyric acid (**3e**) as colorless granules: yield 5.2 g (52%); mp 172–173°; $[\alpha]^{20}D$ +84.8° (*c* 1.0, 0.1 *N* NaOH); nmr (DMF-d_7) δ 1.30–1.80 (m, 10 H), 2.52 (s, 3 H), 4.1–4.5 (m, 2 H), 4.85–5.0 (m, 2 H), 7.30 (s, 2 H), 8.08 (s, 1 H).

Anal. Calcd for $C_{16}H_{21}N_5O_4S$: C, 50.65; H, 5.58; N, 18.46. Found: C, 50.64; H, 5.44; N, 18.01.

In another run, 4-(6-amino-2-methylthio-9*H*-purin-9-yl)-2(*R*),-3(*R*)-dihydroxybutyric acid (**13**) was obtained directly by the following treatment described below. The resulting residue was treated with 10% hydrochloric acid at 80–85° for 30 min. This hydrolysate was evaporated to dryness *in vacuo*. The residue was dissolved in 30 ml of H_2O, and the solution was filtered with charcoal. After having been concentrated to one-third of its volume, the filtrate was adjusted to pH 8 with sodium bicarbonate and the unchanged material **1e** was recovered by filtration. The mother liquor was acidified to pH 3 with 80% formic acid at 60°. After cooling, the solid that had formed was collected by filtration and washed with H_2O. Recrystallization from H_2O afforded **13** as colorless fine needles: yield 3.6 g (47.4%); mp 242° dec; $[\alpha]^{20}D$ +52.0° (*c* 1.0, 0.1 *N* NaOH); nmr (DMF-d_7) δ 2.48 (s, 3 H), 3.9–4.25 (m, 4 H), 4.5–6.5 (broad, 2 H), 7.21 (s, 2 H), 7.9 (s, 1 H); uv max ($\epsilon \times 10^{-3}$) 271.5 nm (15.6) at pH 1.3, 235 (22.4), 278 (14.4) at pH 7, 235 (22.4), 278 (14.4) at pH 12.5.

Anal. Calcd for $C_{10}H_{13}N_5O_4S$: C, 40.13; H, 4.38; N, 23.40. Found: C, 40.15; H, 4.42; N, 23.09.

Reaction of the Sodium Salt of 6-Methylthiopurine (1f).—A mixture of the sodium salt of **1f** (50 mmol), the lactone **2** (9.9 g, 50 mmol), and DMF (200 ml) was treated in the manner described in the general procedure. The resulting residue was dissolved in 40 ml of H_2O, and the solution was filtered with charcoal. The filtrate was passed through a column of Amberlite IRC-50 (H form, 80 ml), and the column was washed with H_2O (700 ml). The eluate and washing was concentrated to a volume of 40 ml and acidified to pH 3. Crystals that had formed were collected by filtration, weighing 8.5 g; it decomposed at 162–167°. The crude product was dissolved in 80 ml of H_2O containing sodium bicarbonate (2 g). After treatment with charcoal, the solution was acidified to pH 3 and the precipitate that had separated was collected by filtration, yield 8.1 g, mp 165–168° dec. Recrystallization from 50% EtOH gave 4-(6-methylthio-9*H*-purin-9-yl)-2(*R*),3(*R*)-cyclohexylidenedioxybutyric acid (**3f**) as colorless fine needles: mp 167–170° dec; $[\alpha]^{25}D$ +91.8° (*c* 1.0, 0.1 *N* NaOH); nmr ($CDCl_3$) δ 1.1–2.0 (m, 10 H), 2.64 (s, 3 H), 4.0–5.3 (m, 4 H), 8.23 (s, 1 H), 8.78 (s, 1 H).

The analytical sample was obtained by recrystallization from 50% EtOH, mp 170–172° dec.

Anal. Calcd for $C_{16}H_{20}N_4O_4S$: C, 52.74; H, 5.53; N, 15.38. Found: C, 52.60; H, 5.55; N, 15.27.

The combined acidic (pH 3.0) mother liquors were adjusted to pH 1.5 and the precipitate that had separated was collected by filtration. This solid (3.0 g) was dissolved in 3% aqueous sodium bicarbonate solution (30 ml), and the solution was acidified to pH 3.0. The additional fraction of **3f** (1.7 g), mp 168–169° dec, that had precipitated was removed by filtration. Acidification of the filtrate to pH 1.0 gave 0.8 g of solid which was shown to be a mixture of two major products on tlc.

This mixture was chromatographed with silica gel (solvent $CHCl_3$–MeOH–AcOH 90:10:3) to give **3f** (240 mg) and 4-(6-methylthio-7*H*-purin-7-yl)-2(*R*),3(*R*)-cyclohexylidenedioxybutyric acid (**5f**, 140 mg). Recrystallization of **5f** from 50% EtOH gave colorless needles: mp 200–201°; $[\alpha]^{25}D$ +154.5° (*c* 1.0, 0.1 *N* NaOH); nmr ($CDCl_3$) δ 1.1–2.1 (m, 10 H), 2.80 (s, 3 H), 4.5–5.2 (m, 4 H), 8.41 (s, 1 H), 8.87 (s, 1 H).

Anal. Calcd for $C_{16}H_{20}N_4O_4S$: C, 52.74; H, 5.53; N, 15.38. Found: C, 52.79; H, 5.50; N, 15.14.

The total yield of **3f** was 10.04 g (54.6%) and the yield of **5f** was determined to be approximately 5.3% by integration of the nmr spectrum of the crude mixture of **3f** and **5f**. In other run, the whole yield of **3f** and **5f** was 67%.

4-(6-Amino-9*H*-purin-9-yl)-2(*R*),3(*R*)-cyclohexylidenedioxybutyric Acid (3a). A. From 4-(6-Methylthio-9*H*-purin-9-yl)-2(*R*),3(*R*)-cyclohexylidenedioxybutyric Acid (5f).—A solution of **5f** (1.0 g) in concentrated ammonium hydroxide (50 ml) was heated in a sealed tube at 140–145° for 15 hr. The reaction mixture was evaporated to dryness *in vacuo*, and the residue was dissolved in dilute ammonium hydroxide (5 ml). The solution was passed through a column of Amberlite IRC-50 (H form, 20 ml) and the column was washed with H_2O (200 ml). The eluate and washing was concentrated to a volume of 10 ml *in vacuo*. The concentrated solution was acidified to pH 3.5 with 2% formic acid and the solid that had formed was collected by filtration to give **3a** as colorless needles, yield 0.52 g (57%), mp 226–229° dec. This product was identical in its uv and ir spectra with those of **3a** obtained by the reaction of the sodium salt of adenine with the lactone **2**.

B. From 4-(6-Amino-2-methylthio-9*H*-purin-9-yl)-2(*R*),3(*R*)-cyclohexylidenedioxybutyric Acid (3e).—A suspension of **3e** (2 g, 5.3 mmol) and freshly prepared Raney nickel (10 g) in EtOH (100 ml) was refluxed with stirring for 3 hr. The Raney nickel was filtered off and washed with 80% EtOH. The filtrate, after decolorization with charcoal, was concentrated to a small volume *in vacuo* and allowed to stand at room temperature overnight. The solid that had formed was collected by filtration, washed with cold EtOH, and dried. The compound (mp 226–228° dec), thus obtained, was identified with an authentic sample of **3a**.

4-(6-Amino-7*H*-purin-7-yl)-2(*R*),3(*R*)-cyclohexylidenedioxybutyric Acid (5a). A. From 4-[6-(*N*,*N*-Dimethylaminomethyleneamino)-7*H*-purin-7-yl]-2(*R*),3(*R*)-cyclohexylidenedioxybutyric Acid (5c).—A solution of **5c** (3.0g) in 5% ammonium hydroxide (30 ml) was heated at 80–90° for 45 min. After cooling, the solution was acidified to pH 3.5 with 10% hydrochloric acid and the solid that had precipitated was removed by filtration. The solid was found to be unchanged starting material **5c** (recovery of 1.4 g, 46.7%). The filtrate was allowed to stand at room temperature for 2 hr, and the precipitate that had formed was collected by filtration to give **5a** (1.1 g, mp 235–237° dec). Recrystallization from DMF–H_2O (1:1) gave analytically pure **5a** as colorless prisms, yield 0.4 g (15.5%), mp 242–244° dec.

Anal. Calcd for $C_{15}H_{19}N_5O_4$: C, 54.04; H, 5.75; N, 21.01. Found: C, 53.88; H, 5.81; N, 21.06.

B. From 4-(6-Methylthio-7*H*-purin-7-yl)-2(*R*),3(*R*)-cyclohexylidenedioxybutyric Acid (5f).—A solution of **5f** (200 mg) in concentrated ammonium hydroxide (10 ml) was heated in a sealed tube at 140–145° for 15 hr, and the reaction mixture was treated as described above for the 9 isomer. Recrystallization of the crude product (110 mg) from DMF–H_2O (1:1) gave pure **5a** as colorless prisms, mp 239–241° dec. The uv and ir spectra of this compound were identical with those of **5a** derived from **5c**.

N^6-Benzyleritadenine (11) from 4-(6-Benzylamino-9*H*-purin-9-yl)-2(*R*),3(*R*)-cyclohexylidenedioxybutyric Acid (3b).—A mixture of **3b** (10.0 g), 10% hydrochloric acid (40 ml), and dioxane (10 ml) was heated at 80–90° for 1 hr. The reaction mixture was evaporated to dryness *in vacuo* and the residue was dissolved in water (about 20 ml). The solution was adjusted to pH 3–4 with

10% sodium hydroxide solution and the precipitate that had formed was collected by filtration and dried to give crude **11** (8.0 g, 98.8%). The crude product was recrystallized from H_2O to yield colorless prisms: mp 208–210° dec; $[\alpha]^{25}D$ +32.0° (*c* 1.0, 0.1 *N* NaOH); nmr (DMSO-d_6) δ 4.0 (m, 2 H), 4.22 (m, 2 H), 4.79 (m, 2 H), 7.30 (m, 5 H), 8.04 (s, 1 H), 8.20 (s, 1 H), 8.25 (s, 1 H); uv max (ε × 10^{-3}) 268.5 nm (21.0) at pH 1.3, 271.5 (22.3) at pH 7, 271.5 (22.9) at pH 12.5.

The uv spectrum of the product was identical with that of 9-benzyl-6-benzylaminopurine.[17]

Anal. Calcd for $C_{16}H_{17}N_5O_4$: C, 55.97; H, 4.99; N, 20.40. Found: C, 56.03; H, 4.92; N, 20.45.

N^6-Benzyl-3-isoeritadenine (12) from 4-(6-Benzylamino-3*H*-purin-3-yl)-2(*R*),3(*R*)-cyclohexylidenedioxybutyric Acid (4b).—A mixture of **4b** (2.0 g), 10% hydrochloric acid (10 ml), and dioxane (10 ml) was heated at 80–90° for 1 hr and treated as in the above experiment. Recrystallization of the product from aqueous EtOH gave **12** (1.0 g, 61.8%) as colorless prisms: mp 237–239° dec; $[\alpha]^{25}D$ +50.7° (*c* 1.0, 0.1 *N* NaOH); uv max (ε × 10^{-3}) 288 nm (23.7) at pH 1.3, 291 (18.8) at pH 7, 292 (17.9) at pH 12.5.

Anal. Calcd for $C_{16}H_{17}N_5O_4$: C, 55.97; H, 4.99; N, 20.40. Found: C, 55.69; H, 4.97; N, 20.43.

The uv spectrum of the product was identical with that of 3-benzyl-6-benzylaminopurine.[17]

3-Isoeritadenine (9). A. From 4-(6-Amino-3*H*-purin-3-yl)-2(*R*),3(*R*)-cyclohexylidenedioxybutyric Acid (4a).—A solution of **4a** (130 mg) in 10% hydrochloric acid (1 ml) was heated at 80° for 30 min. The reaction mixture was evaporated to dryness *in vacuo* and the residue was dissolved in H_2O (2 ml). This solution was adjusted to pH 3.0 with 10% aqueous NaOH solution and the resulting solids were collected by filtration, yield 70 mg (71%). Recrystallization from H_2O gave 3-isoeritadenine (**9**) as colorless prisms, mp 295–297° dec. The ir and uv spectra of this sample were identical with those of **9** obtained *via* direct hydrolysis of the mixture (**3a** and **4a**).

The ethyl ester of 3-isoeritadenine (**9**) was prepared by esterification of **9** with EtOH in the presence of H_2SO_4, mp 218–220° (EtOH).

Anal. Calcd for $C_{11}H_{15}N_5O_4$: C, 46.97; H, 5.38; N, 24.98. Found: C, 47.03; H, 5.42; N, 24.98.

B. From 4-(6-Amino-8-methylthio-3*H*-purin-3-yl)-2(*R*),3(*R*)-cyclohexylidenedioxybutyric Acid (4d).—A suspension of **4d** (100 mg) and freshly prepared Raney nickel (1 ml) in 10 ml of 5% aqueous sodium bicarbonate solution was refluxed for 2.5 hr. The Raney nickel was removed by filtration and the clear filtrate was acidified with 10% hydrochloric acid. The acidic solution was heated at 80–90° for 1 hr and concentrated to a volume of 2 ml *in vacuo*. This concentrated solution was adjusted to pH 3, and the solid that had separated was collected by filtration to give crude 3-isoeritadenine (**9**). Purification of the crude product was unsuccessful; so identification was made by the criteria of uv spectra and the behaviors on paper electrophoresis and ion-exchange chromatography.[18]

7-Isoeritadenine (10) from 4-[6-(*N*,*N*-Dimethylaminomethyleneamino)-7*H*-purin-7-yl]-2(*R*),3(*R*)-cyclohexylidenedioxybutyric Acid (5c).—A solution of **5c** (1.0 g) in 10% hydrochloric acid (10 ml) was heated at 80–90° for 1 hr. After cooling, the solution was adjusted to pH 3.5, and the solid that had precipitated was collected by filtration, yield 0.6 g (90.8%), mp 278–279° dec. Recrystallization from DMF–H_2O (1:2, 180 ml) gave pure **10** as colorless prisms: yield 0.35 g; mp 278–279° dec; $[\alpha]^{20}D$ +59.1° (*c* 1.0, 0.1 *N* NaOH); nmr (D_2O–NaOD) δ 4.55–5.0 (m, 4 H), 8.55 (s, 1 H), 8.60 (s, 1 H); uv max (ε × 10^{-3}) 275 nm (14.1) at pH 1.3, 272.5 (10.3) at pH 6.5, 272.5 (10.4) at pH 12.5.

Anal. Calcd for $C_9H_{11}N_5O_4$: C, 42.69; H, 4.38; N, 27.67. Found: C, 42.70; H, 4.32; N, 27.54.

This compound was identical in its ir and uv spectra with an authentic sample of 7-isoeritadenine (**10**) that was prepared unambiguously *via* methyl 5-(5′-amino-4′-cyanoimidazol-3′-yl)-2,3-*O*-isopropylidene ribofuranoside.[11] The ethyl ester of 7-isoeritadenine (**10**) was prepared by esterification of **10** with EtOH in the presence of H_2SO_4, mp 183–185°, colorless leaflets from EtOH.

Anal. Calcd for $C_{11}H_{15}N_5O_4$: C, 46.97; H, 5.38; N, 24.90. Found: C, 46.95; H, 5.51; N, 25.06.

Eritadenine (8). A. From 4-(6-Amino-9*H*-purin-9-yl)-2(*R*),-3(*R*)-cyclohexylidenedioxybutyric Acid (3a).—A solution of **3a** (400 mg) in 10% hydrochloric acid (3 ml) was heated at 80° for 30 min. The reaction mixture was evaporated to dryness *in vacuo* and the residue was dissolved in H_2O (3 ml). This solution was adjusted to pH 3.0 with 10% aqueous NaOH solution and the resulting solids were collected by filtration, yield 270 mg (89%), mp 259–260° dec.

B. From 4-(6-Benzylamino-9*H*-purin-9-yl)-2(*R*),3(*R*)-dihydroxybutyric Acid (3b).—To a solution of **3b** (1.0 g) in liquid ammonia was added with stirring sodium (0.2 g) in pieces during the course of 30 min. The reaction mixture was stirred at room temperature until ammonia distilled away. The residue was dissolved in a few milliliters of H_2O and the solution was neutralized with 10% hydrochloric acid. This neutralized solution was passed through a column of Amberlite IR-120 (H form, 30 ml) and the column was washed with H_2O. The adsorbed substance was eluted with 5% ammonium hydroxide and the eluate was evaporated to dryness *in vacuo*. The residue was dissolved in a small amount of H_2O and the solution was adjusted to pH 3.5. The solid that had formed was collected by filtration. The crude product was dissolved in dilute aqueous sodium hydroxide solution, and the solution was acidified to pH 3.5 with 10% hydrochloric acid. The crystals that had precipitated were collected by filtration and dried to give eritadenine (**8**), yield 100 mg (13.7%).

C. From 4-(6-Amino-2-methylthio-9*H*-purin-9-yl)-2(*R*),3(*R*)-dihydroxybutyric Acid (13).—A suspension of **13** (2 g, 6.7 mmol) and freshly prepared Raney nickel (5 g) in 5% ammonium hydroxide (100 ml) was refluxed with stirring for 3 hr. The Raney nickel was filtered off and washed with H_2O. The filtrate and washing was evaporated to dryness *in vacuo*, and the residue was dissolved in 40 ml of H_2O. The solution was treated with charcoal and filtered. Acidification of the filtrate to pH 3 with 80% formic acid gave **8**, which was collected by filtration and washed H_2O. Recrystallization from H_2O gave eritadenine (**8**), yield 800 mg (47%), mp 262° dec.

D. From 4-(6-Amino-8-methylthio-9*H*-purin-9-yl)-2(*R*),3(*R*)-cyclohexylidenedioxybutyric Acid (3d).—A suspension of **3d** (100 mg) and freshly prepared Raney nickel (1 ml) in 5% aqueous sodium bicarbonate solution was refluxed for 2.5 hr. The Raney nickel was filtered off and the filtrate was acidified with 10% hydrochloric acid. The acidic solution was heated at 80–90° for 1 hr and concentrated to a volume of 2 ml *in vacuo*. This concentrated solution was adjusted to pH 3, and the solid that had separated was collected by filtration to give crude eritadenine (**8**). An attempt to purify the crude product was unsuccessful, so identification was made by the criteria of uv spectra and the behaviors on paper electrophoresis and ion-exchange chromatography.

The eritadenine (**8**) that was obtained in these experiment was identical in its uv and ir spectra with natural eritadenine (**8**).

Registry No.—**1c**, 28856-55-5; **2**, 28875-69-6; **3a**, 28875-70-9; **3b**, 28875-71-0; **3d**, 28875-72-1; **3e**, 28875-73-2; **3f**, 28875-74-3; **4a**, 28875-75-4; **4b**, 28875-76-5; **4d**, 28875-77-6; **5a**, 28875-78-7; **5c**, 28875-79-8; **5f**, 28875-80-1; **7**, 28875-81-2; **8**, 25486-40-2; **8** Na salt, 28875-83-4; **9**, 28875-84-5; **9** Na salt, 28875-85-6; **9** ethyl ester, 28875-86-7; **10**, 28875-87-8; **10** ethyl ester, 28875-88-9; **11**, 28875-89-0; **12**, 28875-90-3; **13**, 28875-91-4.

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(17) N. J. Leonard, K. L. Carraway, and J. P. Helgeson, *J. Heterocycl. Chem.*, **2**, 291 (1965).

(18) The details of the chromatographic analysis of the isomeric eritadenine will be reported later.