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Hypocholesterolemic Alkaloids of *Lentinus edodes* (Berk.) Sing. III.[1] Preparation of Analogous Compounds related to Eritadenine[2]

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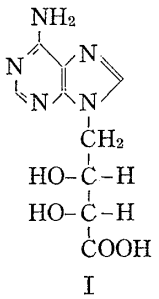
Various compounds related to the hypocholesterolemic alkaloid eritadenine (I) were synthesized for the evaluation of structure-activity relationships in this series. 6-Substituted purine analogs were prepared by reacting the 6-chloro-purine intermediate (II) with the corresponding nucleophilic reagents. 2,6-Di-substituted purine derivatives were prepared by utilizing a route involving an imidazole ring closure step, starting from the appropriately substituted pyrimidine precursors and the amino-acid (VI). The preparation of 6,8-di-substituted purine analogs was achieved by cyclization of the 5-amino-pyrimidine intermediate (XXIX) derived for the synthesis of I.

Eritadenine (I), a hypocholesterolemic alkaloidal substance isolated from *Lentinus edodes* (Berk.) Sing.,[4] was synthesized as reported previously.[1,4] Its excellent activity in rats prompted us to continue additional studies on it and its analogs. As part of a program for the preparation of analogous compounds related to eritadenine, we attempted to replace the adenine moiety of the eritadenine molecule by some other purine bases. In connection with various studies on nucleosides in the literature, this approach to the structural alteration might be of interest for the evaluation of structure-activity relationships in this series. In this paper, we wish to report the synthesis of some mono- and di-substituted purine analogs.



I

Chart 1

For the synthesis of compounds having several different substituents at the 6-position, the preparations of 4-(6-chloro-9H-purin-9-yl)-4-deoxy-D-erythronic acid (II) and its 2,3-O-isopropylidene derivative (III) which might serve as intermediates for our purpose were examined according to the general method derived by Temple, Jr., *et al.*[5] The compound (II) was found to be obtained in a good yield by condensation of 5-amino-4,6-dichloropyrimidine (IV)[6] and 4-amino-4-deoxy-D-erythronic acid (V),[4] followed by ring closure of the resulting product (VII) with ethyl orthoformate-hydrochloric acid. On the other hand, the preparation of III was found to be somewhat troublesome. Condensation of the pyrimidine precursor (IV) and 4-amino-4-deoxy-2,3-O-isopropylidene-D-erythronic acid (VI)[4] furnished a low yield of the desired product (VIII) owing to the lactamization of the amino-acid (VI) itself under the condition effecting the condensation reaction, although cyclization of the resulting product (VIII) with ethyl orthoformate-hydrochloric acid proceeded smoothly to give III. Therefore, II was chosen as the mother compound for preparing 6-substituted analogs.

1) Part II: T. Kamiya, Y. Saito, M. Hashimoto, and H. Seki, *J. Heterocyclic Chem.*, **9**, 359 (1972).
2) Presented at the 91st Annual Meeting of Pharmaceutical Society of Japan, Fukuoka, April 1971.
3) Location: *Kashima-cho, Higashiyodogawa-ku, Osaka.*
4) T. Kamiya, Y. Saito, M. Hashimoto, and H. Seki, *Tetrahedron*, **28**, 899 (1972).
5) C. Temple, Jr., C.L. Kussner and J.A. Montgomery, *J. Med. Chem.*, **5**, 866 (1962).
6) D.J. Brown, *J. Appl. Chem.* (London), **4**, 72 (1954).

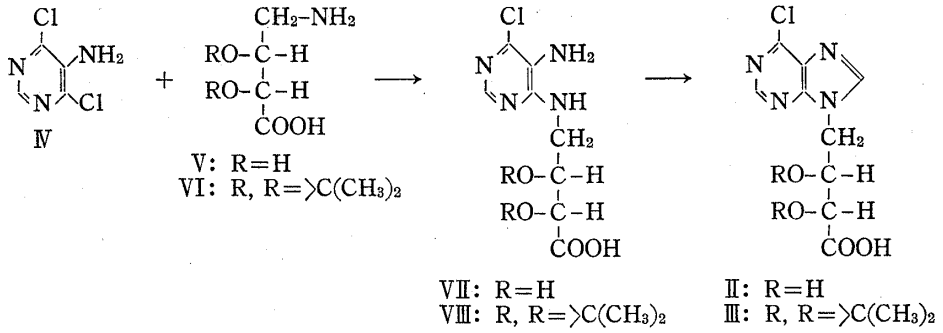


Chart 2

Our attention having been focused on the synthesis of eritadenine (I) led us first to attempt the amination of the 6-chloropurine intermediates (II and III). Treatment of II with ethanolic ammonia yielded eritadenine (I) in a good yield. Similar treatment of III gave eritadenine acetonide (IX), which was hydrolyzed with 10% acetic acid to afford eritadenine as well.

In order to prepare several compounds having structurally close relation to eritadenine, we next attempted the reaction of II with suitable amines in a similar manner. Thus, the mother compound (II) was refluxed with 70% aqueous ethylamine, 40% aqueous dimethylamine and ethanolic benzylamine to give the corresponding 6-ethylamino (X), -dimethylamino (XI) and -benzylamino (XII) compounds, respectively. The purinyl derivatives with the hydroxyamino, ethoxyamino, and benzyloxyamino groups at the 6-position (XIII, XIV, and XV) were also prepared from II by similar substitution reactions with hydroxylamine, ethoxyamine and benzyloxyamine in ethanol.

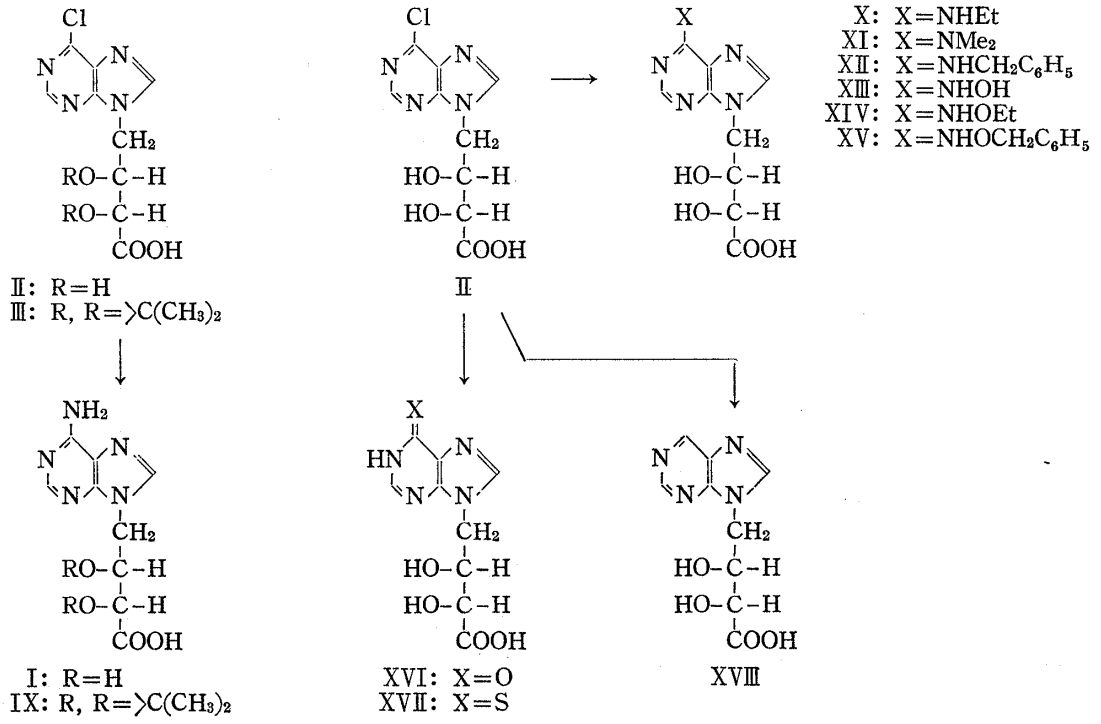


Chart 3 Chart 4

Among a number of 6-substituted purines, hypoxanthine is especially of interest to us because of its metabolical interrelation to adenine. When II was refluxed with formic acid, the 6-chloro group was hydrolyzed to form the desired hypoxanthine compound (XVI), which

was alternatively prepared from eritadenine by diazotization with sodium nitrite in acetic acid. As an analog of this hypoxanthine derivative (XVI), the 6-mercaptopurine compound (XVII) was also prepared by treatment of II with thiourea in boiling ethanol. Concomitantly, 6-hydrogen compound (XVIII) was finally prepared by catalytic reduction of II on palladium charcoal.

For the preparation of 2,6-di-substituted purine derivatives, a feasible approach seemed to be the adoption of a route involving an imidazole ring closure step. This approach required appropriately substituted pyrimidine precursors, which were easily prepared after the procedures in the literature. First of all, we attempted to prepare the compound (XIX) possessing guanine which is the biologically most important base as well as adenine. 2-Amino-6-chloro-5-nitro-4(H)pyrimidinone (XXIII)[7] and the amino-acid (VI) were condensed, and the resulting product (XXVI) was then subjected to hydrogenation on palladium charcoal in formic acid, followed by treatment with 1N sodium hydroxide, to afford the desired guanine derivative (XIX). The purinyl compounds containing isoguanine, 2-methylthio-adenine and xanthine (XX, XXI, and XXII) were also prepared as other samples of the 2,6-di-substituted analogs. The isoguanine (XX) and 2-methylthioadenine (XXI) derivatives were synthesized respectively from 4-amino-2,6-dichloro-5-nitropyrimidine (XXIV)[8] and 4-amino-6-chloro-2-methylthio-5-nitropyrimidine (XXV)[9] in a similar manner. The xanthine analog (XXII) was derived from XIX by diazotization with sodium nitrite in acetic acid.

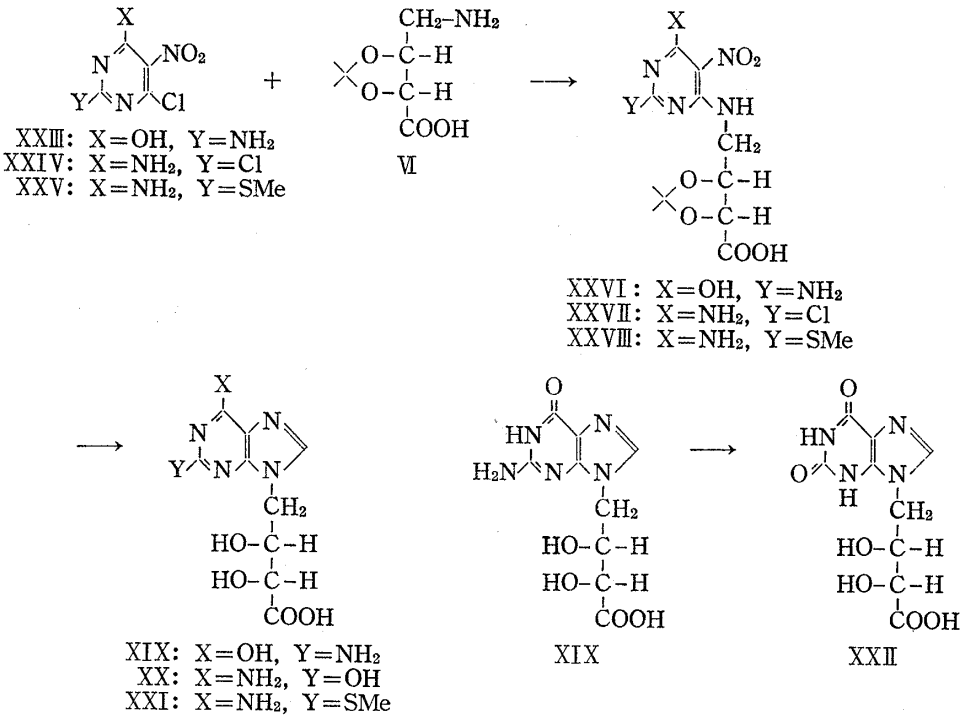


Chart 5

In an attempt to prepare 6,8-di-substituted purinyl analogs, cyclization of the 5-amino-pyrimidine compound (XXIX) derived as an intermediate for the synthesis of eritadenine as described earlier[4] was examined. The intermediate (XXIX) was heated with urea and the crude product so formed was hydrolyzed with 10% acetic acid to result in the formation

7) A. Stuart and H.C.S. Wood, *J. Chem. Soc.*, **1963**, 4186.
8) R.K. Robins, K.L. Dille, and B.E. Christensen, *J. Org. Chem.*, **19**, 930 (1954).
9) Prepared by partial amination of 4,6-dichloro-2-methylthio-5-nitro-pyrimidine.[10]
10) D.J. Brown, *J. Chem. Soc.*, **1965**, 3770.

of the desired cyclization product (XXX). For the preparation of the 6-amino-8-mercaptopurine compound (XXXI), XXIX was treated with carbon disulfide in the presence of sodium ethoxide. This treatment followed by hydrolysis with 10% acetic acid gave XXXI and its isomeric compound (XXXII) in a ratio of 5:1. The structures of these compounds were confirmed by desulfurization, respectively. The compound (XXXIII) was alternatively prepared by treatment of XXIX with ethyl orthoformate-hydrochloric acid.

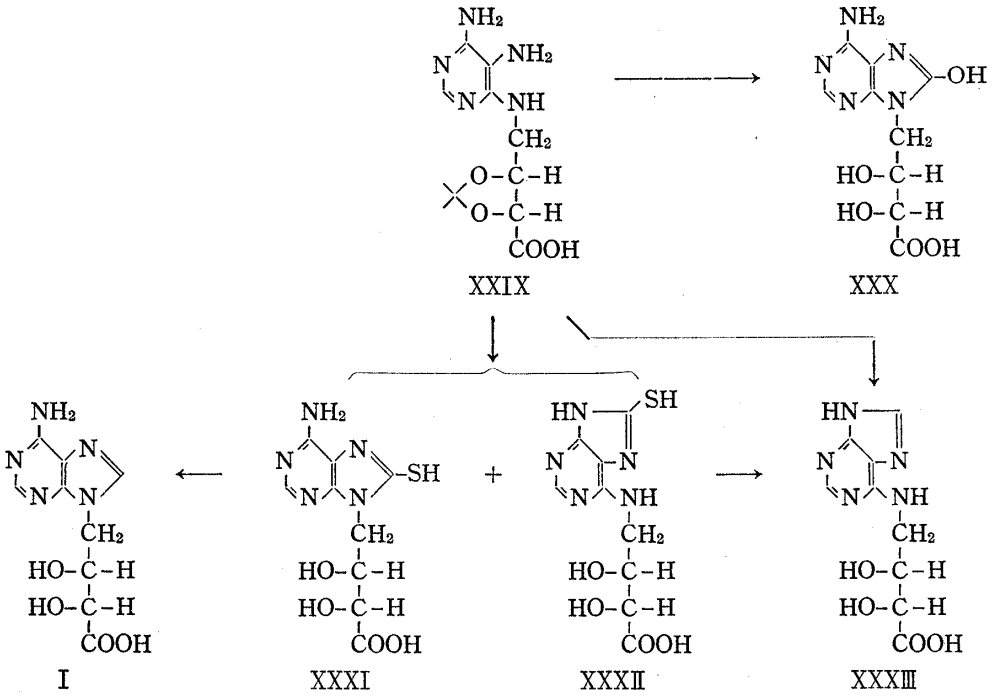


Chart 6

Hypocholesterolemic activity of the compounds prepared above were tested in rats by oral administration and compared with that of eritadenine.[11] Among the 6-substituted analogs, the ethylamino (X), hydroxyamino (XIII), ethoxyamino (XIV), and benzyloxyamino (XV) compounds were found to possess high biological potency. It is of interest that XIII, XIV, and XV exhibited activity equivalent to that of eritadenine, although X was slightly less active. The dimethylamino (XI) and benzylamino (XII) derivatives also retained activity with some decrease. Thus, basic substituents on the 6-position may be essential as evidenced by considerably reduced activity of the 6-hydroxy (XVI), -mercapto (XVII), -chloro (II), and -hydrogen (XVIII) compounds.

All of the di-substituted compounds were found to be considerably less potent. It is somewhat surprising that the introduction of the hydroxyl, mercapto, and methylthio groups at the 2- or 8-position (XX, XXI, XXX, and XXXI) exerts undesirable effect on activity despite of the presence of the 6-amino group.

In this class of compounds, stereochemistry of the side chain may be also an important factor for hypocholesterolemic activity. Thus, D-*threo*- and L-*threo*-eritadenine, the syntheses of which were preliminarily reported,[12] were found to be less active. Studies on structural alteration from this stereochemical aspect will be discussed in the following paper in this series.

11) We are indebted to Dr. H. Kikuchi and Dr. A. Tensho, Iyakushigen Institute for Medicinal Research, for these bioassays. The detailed biological data will be presented in the future.
12) M. Hashimoto, Y. Saito, H. Seki and T. Kamiya, *Tetrahedron Letters*, **1970**, 1359.

Experimental

Melting points were determined in open capillary tubes with a Thomas–Hoover melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded for all new compounds on a Hitachi Type EPI-2 spectrophotometer in agreement with their assigned structure. Ultraviolet (UV) spectra were measured on a Hitachi Type EPS-3T spectrophotometer. All evaporations were performed on rotary evaporators. Thin-layer chromatography (TLC) analysis was carried out on Eastman chromatogram sheet (6060 silica gel).

4-(5-Amino-6-chloro-4-pyrimidylamino)-4-deoxy-D-erythronic Acid (VII)——A mixture of 8.20 g of IV,[6] 6.76 g of V and 10.12 g of triethylamine in 200 ml of H_2O was refluxed for 24 hr. After being cooled, the reaction mixture was washed with AcOEt and then concentrated to about 70 ml. This concentrated solution was adjusted to about pH 3 with dil. HCl, and the resulting crystals were collected and washed with H_2O. Yield, 10.35 g. A portion of the product was recrystallized from H_2O to give analytically pure crystals, mp 201° (decomp.). UV $\lambda_{max}^{H_2O}$ mμ (ε): 264 (8300), 293 (9000). *Anal.* Calcd. for $C_8H_{11}O_4N_4Cl$: C, 36.58; H, 4.22; N, 21.33. Found: C, 36.38; H, 4.08; N, 21.54.

4-(6-Chloro-9H-purin-9-yl)-4-deoxy-D-erythronic Acid (II)——To a suspension of 6.76 g of VII in 200 ml of ethyl orthoformate was added 3 ml of conc. HCl. The mixture was stirred at room temperature for 24 hr and then evaporated. The residue was triturated with AcOEt to give a crystalline solid, which was recrystallized from iso-PrOH, mp 220° (decomp.). Yield, 6.10 g. UV $\lambda_{max}^{H_2O}$ mμ (ε): 267 (9400). *Anal.* Calcd. for $C_9H_9O_4N_4Cl$: C, 39.64; H, 3.33; N, 20.55. Found: C, 39.50; H, 3.42; N, 20.51.

4-(5-Amino-6-chloro-4-pyrimidylamino)-4-deoxy-2,3-O-isopropylidene-D-erythronic Acid (VIII)——To a solution of NaOMe prepared from 0.13 g of Na in 45 ml of absolute MeOH were added 1.06 g of VI,[6] 0.98 g of IV ,and 0.61 g of triethylamine. The mixture was refluxed for 15 hr and then concentrated to dryness. The residue was dissolved in H_2O and the resulting solution was washed with AcOEt. The aqueous layer was made acidic with dil. HCl and extracted with AcOEt. The extract was dried and evaporated to leave crystals. Yield, 0.60 g. Recrystalization from EtOH–AcOEt gave an analytically pure sample, mp 177° (decomp.). UV λ_{max}^{MeOH} mμ (ε): 268 (8800), 297 (9700). *Anal.* Calcd. for $C_{11}H_{15}O_4N_4Cl$: C, 43.64, H, 5.00; N, 18.51. Found: C, 43.64; H, 4.70; N, 18.35.

4-(6-Chloro-9H-purin-9-yl)-4-deoxy-2,3-O-isopropylidene-D-erythronic Acid (III)——To a suspension of 0.81 g of VIII in 25 ml of ethyl orthoformate was added 0.4 ml of conc. HCl. The mixture was stirred at room temperature for 3 days and then concentrated to dryness. The oily residue was triturated with iso-Pr_2O to give a solid. Yield, 0.64 g. An analytical sample was prepared by recrystallization from iso-Pr_2O, mp 150° (decomp.). UV $\lambda_{max}^{H_2O}$ mμ (ε): 266 (9300). *Anal.* Calcd. for $C_{12}H_{13}O_4N_4Cl$: C, 46.08; H, 4.19; N, 17.92. Found: C, 46.10; H, 4.15; N, 17.85.

4-(6-Amino-9H-purin-9-yl)-4-deoxy-D-erythronic Acid (I)——a) A solution of 0.50 g of II in 30 ml of EtOH saturated with NH_3 was heated at 120° in a sealed tube for 21 hr. The reaction mixture was concentrated to dryness and then H_2O was added to the residue. Acidification (pH 3) of the resulting solution with dil. HCl gave a crude crystalline ppt, which was recrystallized from H_2O to yield 0.36 g of pure eritadenine (I), mp 278° (decomp.). The IR and UV spectra were identical with those of the natural product.

b) A 0.15 g sample of IX was refluxed with 5 ml of 10% AcOH for 30 min and then evaporated. The residue was recrystallized from H_2O to yield 0.10 g of eritadenine (I), mp 278° (decomp.). Identification was made by IR and UV spectral comparison.

c) A solution of 90 mg of XXXI in 5 ml of H_2O containing 30 mg of $NaHCO_3$ was refluxed with 0.3 g of Raney Ni for 1 hr. After removal of the catalyst by filtration, the filtrate was concentrated and the resulting residue was dissolved in H_2O. Acidification (pH 3) of the solution with dil. HCl, followed by recrystallization of the precipitated solid gave 40 mg of eritadenine (I), mp 279° (decomp.). Identification was made by IR and UV spectral comparison.

4-(6-Amino-9H-purin-9-yl)-4-deoxy-2,3-O-isopropylidene-D-erythronic Acid (IX)——Treatment of 0.50 g of III in the same procedure described in the preparation of eritadenine (I) from II gave 0.32 g of the product (IX). A pure sample was prepared by recrystallization from EtOH, mp 214° (decomp.). The IR and UV spectra were identical with those of the cyclization product (IX) of XXIX with formamidine acetate.[4]

4-(6-Ethylamino-9H-purin-9-yl)-4-deoxy-D-erythronic Acid (X)——A solution of 0.75 g of II in 7.5 ml of 70% aqueous ethylamine was refluxed for 2 hr and then evaporated to dryness. The residue was dissolved in H_2O and the resulting solution was acidified to about pH 3 with dil. HCl to deposit a crystalline mass. Yield, 0.40 g. Recrystallization from EtOH–H_2O yielded analytically pure crystals, mp 242—243° (decomp.). UV $\lambda_{max}^{H_2O}$ mμ (ε): 269 (17000). *Anal.* Calcd. for $C_{11}H_{15}O_4N_5$: C, 46.97; H, 5.38; N, 24.90. Found: C, 46.92; H, 5.46; N, 24.86.

4-(6-Dimethylamino-9H-purin-9-yl)-4-deoxy-D-erythronic Acid (XI)——A solution of 0.50 g of II in 10 ml of 40% aqueous dimethylamine was refluxed for 2 hr and then evaporated to dryness. The residue was, after being converted to the HCl salt by EtOH–HCl, recrystallized from EtOH, mp 228—229° (de-

comp.). Yield, 0.27 g. UV $\lambda_{max}^{H_2O}$ mμ (ε): 278 (17900). *Anal.* Calcd. for $C_{11}H_{15}O_4N_5$ HCl: C, 41.58; H, 5.08; N, 22.05. Found: C, 41.52; H, 5.10; N, 21.84.

4-(6-Benzylamino-9H-purin-9-yl)-4-deoxy-D-erythronic Acid (XII)——A mixture of 1.36 g of II and 1.61 g of benzylamine in 30 ml of EtOH was refluxed for 8 hr. The solvent was removed and H_2O was added to the residue. The resulting solution was washed with AcOEt and then adjusted to about pH 3 with dil. HCl. The precipitated crystals were collected and washed with H_2O. Yield, 1.10 g. A portion of the crude product was recrystallized from H_2O to give a pure sample, mp 206—209° (decomp.). UV $\lambda_{max}^{H_2O}$ mμ (ε): 271 (20400). *Anal.* Calcd. for $C_{16}H_{17}O_4N_5$: C, 55.97; H, 4.99; N, 20.40. Found: C, 56.14; H, 4.83; N, 20.56.

4-(6-Hydroxyamino-9H-purin-9-yl)-4-deoxy-D-erythronic Acid (XIII)——A solution of 1.48 g of II in 45 ml of EtOH containing 0.77 g of hydroxylamine was refluxed for 6 hr. After removal of the solvent, the residue was dissolved in H_2O and adjusted to about pH 3 with dil. HCl. The separated crystalline mass was filtered and recrystallized from 70% EtOH, mp 206° (decomp.). Yield, 0.92 g. UV $\lambda_{max}^{H_2O}$ mμ (ε): 267 (13800). *Anal.* Calcd. for $C_9H_{11}O_5N_5$: C, 40.15; H, 4.12; N, 26.02. Found: C, 39.97; H, 4.14; N, 25.76.

4-(6-Ethoxyamino-9H-purin-9-yl)-4-deoxy-D-erythronic Acid (XIV)——A mixture of 1.00 g of II and 1.20 g of ethoxyamine in 12 ml of 80% EtOH was heated to 80° in a sealed tube for 4 hr. Removal of the solvent left an oily material, which was crystallized by trituration with iso-PrOH. Recrystallization from EtOH gave 0.76 g of XIV, mp 208—209° (decomp.). UV $\lambda_{max}^{H_2O}$ mμ (ε): 269 (15100). *Anal.* Calcd. for $C_{11}H_{15}O_5N_5$: C, 44.44; H, 5.09; N, 23.56. Found: C, 44.14; H, 5.01; N, 23.47.

4-(6-Benzyloxyamino-9H-purin-9-yl)-4-deoxy-D-erythronic Acid (XV)——A mixture of 1.00 g of II and 1.80 g of benzyloxyamine in 10 ml of EtOH was refluxed for 3 hr and then concentrated to dryness. The residue was dissolved in H_2O and made basic (pH 9) with dil. NaOH. After being washed with ether, the solution was concentrated to leave a crystalline mass, which was recrystallized from EtOH to give 0.55 g of the Na salt of XV, mp 205° (decomp.). UV $\lambda_{max}^{H_2O}$ mμ (ε): 271 (15600). *Anal.* Calcd. for $C_{16}H_{16}O_5N_5Na$: C, 50.41; H, 4.23; N, 18.37. Found: C, 50.15; H, 4.49; N, 18.14.

4-(6-Hydroxy-9H-purine-9-yl)-4-deoxy-D-erythronic Acid (XVI)——a) A solution of 0.60 g of II in 10 ml of HCO_2H was refluxed for 1 hr and then concentrated to dryness. The residue was dissolved in MeOH and triturated with acetone. The precipitated solid was filtered and washed with acetone. Yield, 0.30 g. An analytical sample was prepared by recrystallization of the Na salt from EtOH–H_2O, mp 215° (decomp.). UV $\lambda_{max}^{H_2O}$ mμ (ε): 251 (10700); $\lambda_{max}^{0.1N\ NaOH}$ mμ (ε): 255 (11700); $\lambda_{max}^{0.1N\ HCl}$ mμ (ε): 251 (9900). *Anal.* Calcd. for $C_9H_9O_5N_4Na$: C, 39.13; H, 3.29; N, 20.29. Found: C, 38.88; H, 3.25; N, 20.41.

b) To an ice-cooled solution of 1.00 g of eritadenine (I) in 50 ml of 20% HCO_2H was added dropwise a soln of 4.00 g of $NaNO_2$ in 15 ml of H_2O and then the mixture was stirred at room temperature. After 24 hr, the reaction mixture was concentrated to dryness, H_2O was added to the residue, and the resulting solution was put on a column of 400 ml of Amberlite IRA-400 (OH^- form). The column was washed with H_2O and then eluted with 2% HCO_2H. The eluate was evaporated to leave an oily material, which was, after being converted to the Na salt, recrystallized from EtOH–H_2O to yield 0.40 g. Identification was made by IR and UV spectral comparison.

4-(6-Mercapto-9H-purin-9-yl)-4-deoxy-D-erythronic Acid (XVII)——A mixture of 1.89 g of II and 0.53 g of thiourea in 70 ml of EtOH was refluxed for 3 hr, whereafter 15 ml of 1N NaOH was added to the reaction mixture and the resulting mixture was then refluxed for an additional hr. After evaporation of the solvent, the residue was dissolved in H_2O and acidified with dil. HCl. The precipitated crystals were collected and recrystallized from H_2O to give a pure sample, mp 240° (decomp.). Yield, 0.86 g. UV $\lambda_{max}^{H_2O}$ mμ (ε): 227 (9400), 322 (25900); $\lambda_{max}^{0.1N\ NaOH}$ mμ (ε): 233 (14300), 311 (22400); $\lambda_{max}^{0.1N\ HCl}$ mμ (ε): 225 (10100), 324 (22200). *Anal.* Calcd. for $C_9H_{10}O_4N_4S$: C, 39.99; H, 3.71; N, 20.73. Found: C, 40.05; H, 3.94; N, 21.03.

4-(9H-Purin-9-yl)-4-deoxy-D-erythronic Acid (XVIII)——A solution of 1.00 g of II in 15 ml of H_2O containing 0.4 ml of conc. NH_4OH was hydrogenated on 5% Pd-C in the usual way. After the catalyst was filtered off, the filtrate was concentrated to dryness, and the residue was dissolved in H_2O and acidified with dil. HCl. The separated crystals were collected and recrystallized from MeOH–H_2O, mp 230° (decomp.). Yield, 0.50 g. UV $\lambda_{max}^{H_2O}$ mμ (ε): 265 (7800); $\lambda_{max}^{0.1N\ NaOH}$ mμ (ε): 265 (7400); $\lambda_{max}^{0.1N\ HCl}$ mμ (ε): 264 (5600). *Anal.* Calcd. for $C_9H_{10}O_4N_4$: C, 45.38; H, 4.23; N, 23.52. Found: 45.37; H, 4.46; N, 23.52.

4-(2-Amino-6-hydroxy-5-nitro-4-pyrimidylamino)-4-deoxy-2,3-O-isopropylidene-D-erythronic Acid (XXVI)——A mixture of 2.77 g of XXIII,[7] 2.80 g of VI and 2.93 g of triethylamine in 500 ml of MeOH was stirred at room temperature for 24 hr. The mixture was concentrated to dryness, whereafter the residue was dissolved in H_2O and the resulting solution was acidified with dil. HCl. The separated solid was filtered and washed with H_2O, mp 231—233° (decomp.). Yield, 3.60 g. UV λ_{max}^{EtOH} mμ (ε): 213 (23600), 237 (16700), 283 (5600), 330 (12200). *Anal.* Calcd. for $C_{11}H_{15}O_7N_5\ H_2O$: C, 38.04; H, 4.94; N, 20.17. Found: C, 38.16; H, 4.92; N, 19.93.

4-(2-Amino-6-hydroxy-9H-purin-9-yl)-4-deoxy-D-erythronic Acid (XIX)——A solution of 5.10 g of XXVI in 120 ml of HCO_2H was hydrogenated on 5% Pd-C in the usual manner. The catalyst was filtered off and the filtrate was concentrated to dryness. The residue was refluxed with 90 ml of 1N NaOH for 30 min, and then the solution was concentrated to about 30 ml and acidified with dil. HCl. The separated solid was recrystallized from H_2O to give 1.54 g of pure crystals, mp 223° (decomp.). UV $\lambda_{max}^{H_2O}$ mμ (ε):

253 (12400), 270 (inflection); $\lambda_{max}^{0.1N\ NaOH}$ mμ (ε): 270 (10600), 260 (inflection); $\lambda_{max}^{0.1N\ HCl}$ mμ (ε): 255 (12000), 280 (7900). *Anal.* Calcd. for $C_9H_{11}O_5N_5$: C, 40.15; H, 4.12; N, 26.02. Found: C, 39.93; H, 4.21; N, 26.12.

4-(2,6-Dihydroxy-9H-purin-9-yl)-4-deoxy-D-erythronic Acid (XXII)——To an ice-cooled solution of 1.00 g of XIX in 60 ml of 20% AcOH was added portionwise 1.00 g of $NaNO_2$ and the mixture was then stirred at room temperature. After 24 hr, an additional 1.00 g of $NaNO_2$ was added to the reaction mixture and the resulting mixture was stirred for 24 hr. After removal of the solvent, the residue was dissolved in H_2O and put on a column containing 100 ml of Amberlite IRA-400 (OH^- form). The column was washed with H_2O and then eluted with 10% HCO_2H. The eluate was concentrated and the resulting crystalline mass was recrystallized from H_2O to give 0.73 g of pure crystals, mp 204—206° (decomp.). UV $\lambda_{max}^{H_2O}$ mμ (ε): 235 (7900), 264 (9800); $\lambda_{max}^{0.1N\ NaOH}$ mμ (ε): 248 (8900), 278 (9300); $\lambda_{max}^{0.1N\ HCl}$ mμ (ε): 239 (6700), 262 (9400). *Anal.* Calcd. for $C_9H_{10}O_6N_4$: C, 40.00; H, 3.73; N, 20.74. Found: C, 39.62; H, 3.79; N, 20.47.

4-(6-Amino-2-chloro-5-nitro-4-pyrimidylamino)-4-deoxy-2,3-O-isopropylidene-D-erythronic Acid (XXVII)——Condensation of 2.09 g of XXIV[8] and 1.93 g of VI in the presence of 2.03 g of triethylamine in 100 ml of MeOH was carried out in a similar manner as described for the preparation of XXVI. Yield, 2.94 g. Recrystallization from iso-PrOH gave an analytically pure sample, mp 170° (decomp.). UV $\lambda_{max}^{H_2O}$ mμ (ε): 231 (inflection), 344 (10100). *Anal.* Calcd. for $C_{11}H_{14}O_6N_5Cl$: C, 37.88; H, 4.04; N, 20.14. Found: C, 37.90; H, 4.06; N, 20.07.

4-(6-Amino-2-hydroxy-9H-purin-9-yl)-4-deoxy-D-erythronic Acid (XX)——A solution of 1.00 g of XXVII in 30 ml of MeOH containing 0.30 g of triethylamine was hydrogenated on Raney Ni in the usual manner. After the catalyst was filtered off, the filtrate was concentrated to dryness and the resulting residue was then refluxed with 10 ml of HCO_2H for 3 hr. The reaction mixture was concentrated to leave an amorphous material, to which was added 1N NaOH (10 ml) and the resulting solution was refluxed for 1 hr. After being cooled, the mixture was acidified with dil. HCl to separate a crystalline solid. Recrystallization from H_2O yielded 0.31 g of pure crystals, mp 300° (decomp.). UV $\lambda_{max}^{H_2O}$ mμ (ε): 246 (6400), 290 (11200); $\lambda_{max}^{0.1N\ NaOH}$ mμ (ε): 248 (6500), 286 (11200); $\lambda_{max}^{0.1N\ HCl}$ mμ (ε): 235 (5600), 283 (13600). *Anal.* Calcd. for $C_9H_{11}O_5N_5 \cdot \frac{1}{2}H_2O$: C, 38.85; H, 4.35; N, 25.17. Found: C, 38.74; H, 4.07; N, 24.97.

4-Amino-6-chloro-2-methylthio-5-nitropyrimidine (XXV)——To a cooled solution of 12.7 g of 4,6-dichloro-2-methylthio-5-nitropyrimidine[10] in 130 ml of ether was added dropwise 16 ml of 6% methanolic NH_3 during 50 min. After stirring at room temperature for 2.5 hr, the reaction mixture was filtered and evaporated. The resulting solid was washed with hot petroleum ether and recrystallized from benzene to yield 6.48 g of XXV, mp 179—180°. UV λ_{max}^{MeOH} mμ (ε): 222 (18000), 267 (5800), 355 (6600). *Anal.* Calcd. for $C_5H_5O_2N_4SCl$: C, 32.35; H, 2.71; N, 30.09. Found: C, 32.15; H, 2.46; N, 29.80.

4-(6-Amino-2-methylthio-5-nitro-4-pyrimidylamino)-4-deoxy-2,3-O-isopropylidene-D-erythronic Acid (XXVIII)——Condensation of 1.14 g of XXV and 1.00 g of VI in the presence of 1.05 g of triethylamine in 50 ml of MeOH was carried out in a similar manner as described for the preparation of XXVI. Recrystallization from EtOH gave 1.69 g of XXVIII, mp 205—206° (decomp.). UV λ_{max}^{MeOH} mμ (ε): 240 (shoulder), 290 (5600), 346 (17600). *Anal.* Calcd. for $C_{12}H_{17}O_6N_5S$: C, 40.10; H, 4.77; N, 19.49. Found: C, 39.96; H, 4.79; N, 19.23.

4-(6-Amino-2-methylthio-9H-purin-9-yl)-4-deoxy-D-erythronic Acid (XXI)——To a solution of 1.00 g of XXVIII in 20 ml of 90% HCO_2H was added 0.70 g of Zn dust and the mixture was heated under reflux for 2.5 hr. The reaction mixture was filtered and the filtrate was evaporated to dryness. The resulting residue was dissolved in H_2O and then H_2S was passed into the solution. After removal of the precipitate by filtration, the filtrate was concentrated to give a solid, which was collected and washed with EtOH. This crude product was dissolved in 10 ml of 1N NaOH and refluxed for 2.5 hr. After cooling, the solution was acidified with dil. HCl to separate a crystalline mass. Recrystallization from H_2O yielded 0.54 g of XXI, mp 246° (decomp.). UV $\lambda_{max}^{H_2O}$ mμ (ε): 235 (23100), 277 (15000); $\lambda_{max}^{0.1N\ NaOH}$ mμ (ε): 234 (21500), 275 (13900); $\lambda_{max}^{0.1N\ HCl}$ mμ (ε): 220 (18000), 270 (15000). *Anal.* Calcd. for $C_{10}H_{13}O_4N_5S$: C, 40.13; H, 4.38; N, 23.40. Found: C, 40.10; H, 4.28; N, 23.12.

4-(6-Amino-8-hydroxy-9H-purin-9-yl)-4-deoxy-D-erythronic Acid (XXX)——A mixture of 0.50 g of XXIX and 0.41 g of urea was heated at 170—180° for 25 min. The reaction mixture was then treated with boiling 10% AcOH (20 ml) for 30 min, and concentrated to dryness. To the residue was added H_2O and the resulting solution was stood in a refrigerator to deposit crystals, which were collected and recrystallized from H_2O, mp 252—253° (decomp.). Yield, 0.15 g. UV $\lambda_{max}^{H_2O}$ mμ (ε): 272 (12500). *Anal.* Calcd. for $C_9H_{11}O_5N_5 \cdot \frac{1}{2}H_2O$: C, 38.85; H, 4.35; N, 25.17. Found: C, 38.64; H, 4.22; N, 25.46.

4-(6-Amino-8-mercapto-9H-purin-9-yl)-4-deoxy-D-erythronic Acid (XXXI) and 4-(8-Mercapto-9H-purin-6-ylamino)-4-deoxy-D-erythronic Acid (XXXII)——To a solution of 0.96 g of XXIX in 20 ml of absolute EtOH containing NaOEt prepared from 0.18 g of Na was added 1 ml of CS_2 and the mixture was refluxed for 4 hr. After evaporation of the solvent, H_2O was added to the residue and the resulting solution was acidified with dil. HCl to separate crystals, which were collected and washed with MeOH to yield 0.65 g of the 2,3-O-isopropylidene derivative of XXXI. The filtrate and MeOH washing were combined and evaporated to dryness. The residue was dissolved in H_2O and stood in a refrigerator overnight to separate crystals, which were collected and washed to give 0.18 g of the 2,3-O-isopropylidene derivative of XXXII. These two products were treated with boiling 20% AcOH (20 ml) for 2 hr, respectively. The former, after

removal of the solvent and recrystallization of the residue from H_2O, gave 0.47 g of XXXI, mp 265—268° (decomp.). UV $\lambda_{max}^{H_2O}$ mμ (ε): 240 (19000), 288 (inflection), 306 (27300). *Anal.* Calcd. for $C_9H_{11}O_4N_5S$: C, 37.89; H, 3.89; N, 24.55; S, 11.24. Found: C, 37.58; H, 3.91; N, 24.87; S, 11.17. The latter, after a similar treatment, gave 0.09 g of XXXII, mp 240° (decomp.). UV $\lambda_{max}^{H_2O}$ mμ (ε): 240 (20000), 300 (30000), 308 (29800). *Anal.* Calcd. for $C_9H_{11}O_4N_5S$: C, 37.89; H, 3.89; N, 24.55; S, 11.24. Found: C, 38.01; H, 3.83; N, 24.83; S, 10.90.

4-(9H-Purin-6-ylamino)-4-deoxy-D-erythronic Acid (XXXIII)——a) To a suspension of 0.50 g of XXIX in 25 ml of ethyl orthoformate was added 6 drops of conc. HCl and the mixture was stirred at room temperature for 40 hr. Removal of the volatile reagents gave a pale yellow residue, which was washed with MeOH–acetone to give 0.32 g of the 2,3-isopropylidene derivative of XXXIII. This product was treated with boiling 20% AcOH (10 ml) for 30 min and then concentrated. The resulting residue was recrystallized from H_2O to yield a colorless fine crystals, mp 180° (decomp.). Yield, 0.18 g. UV $\lambda_{max}^{H_2O}$ mμ (ε): 270 (15600); $\lambda_{max}^{0.1N\ NaOH}$ mμ (ε): 274 (15600), 282 (shoulder); $\lambda_{max}^{0.1N\ HCl}$ mμ (ε): 275 (15300). *Anal.* Calcd. for $C_9H_{11}O_4N_5 \cdot \frac{1}{2}H_2O$: C, 41.23; H, 4.62; N, 26.71. Found: C, 41.45; H, 4.72; N, 26.87.

b) To a solution of 28 mg of XXXII in 3 ml of H_2O containing 9 mg of $NaHCO_3$ was added Raney Ni (0.2 g) and the mixture was heated under reflux for 1 hr. After removal of the catalyst by filtration, the filtrate was concentrated and the resulting residue was dissolved in H_2O. Acidification (pH 3) of the solution with dil. HCl and filtration of the precipitated solid gave 9 mg of XXXIII. Identification was made by IR and UV spectral comparison.

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