Tetrahedron. Vol 28, pp 899 to 906. Pergamon Press 1972. Printed in Great Britain

HYPOCHOLESTEROLEMIC ALKALOIDS OF *LENTINUS EDODES* (BERK.) SING.—I

STRUCTURE AND SYNTHESIS OF ERITADENINE

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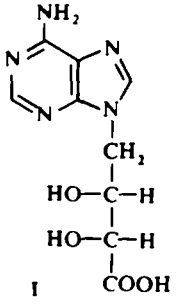
(*Received in Japan* 2 *September* 1971; *Received in the UK for publication* 15 *November* 1971)

Abstract—The absolute stereo-structure of the hypocholesterolemic alkaloid eritadenine(I), isolated from Lentinus edodes (Berk.) Sing., was established by chemical determination. The total synthesis of the optically active form confirmed the proposed structure.

IN 1966, Kaneda and Tokuda[1] found that Shiitake, *Lentinus edodes* (Berk.) Sing., which has been prized for food from ancient times in Japan because of a delicious flavor and a sweet fragrance of its own*[1], shows high hypocholesterolemic activity in rats. Subsequently, Rokujo[4] and Chibata[5] have independently succeeded in isolating an active component, designated as eritadenine,† from Shiitake. In cooperative research with Rokujo,‡ we have determined the structure of eritadenine (I) on the basis of UV and NMR studies and confirmed it by the total synthesis as reported.[6] The present paper is devoted to a more detailed report of the structure and synthesis of eritadenine, also describing subsequent investigations on other synthetic routes.

Eritadenine was isolated as a major component from Shiitake§ by modifying the



I

* The ingredients of the taste and the smell have been characterized as guanosine-5′-monophosphate by Nakajima *et al.*[2] and lenthionine by Morita *et al.*,[3] respectively.

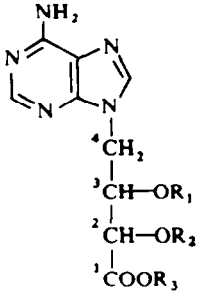
† Although the two provisional names "lentysine" and "lentinacin" had been used, both groups agreed to use the name "eritadenine" for this compound.

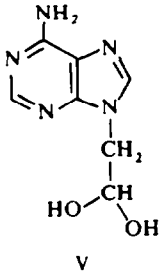
‡ They have taken charge of the investigation from a biological approach in this cooperation. Their preliminary studies revealed that eritadenine shows a marked cholesterol-lowing effect in rats.[4]

§ Our extensive analysis of the eritadenine fraction revealed the presence of two other substances, deoxyeritadenine and 4-(6-amino-9H-purin-9-yl)-propionic acid, the structures and syntheses of which have reported in a preliminary communication.[7]

procedure originally developed by Rokujo *et al.** The analytical data confirmed the molecular formula, $C_9H_{11}O_4N_5$, which was also supported by mass spectral data of derivatives, methyl ester(II) (molecular ion : *m/e* 267) and diacetate methyl ester(III) (molecular ion: *m/e* 351). As reported previously,[6] eritadenine displayed the UV absorptions: $\lambda_{max}^{H_2O}$ 261 mμ (ε, 14,300), $\lambda_{max}^{0.1N\ NaOH}$ 261 mμ (ε, 14,300) and $\lambda_{max}^{0.1N\ HCl}$ 260 mμ (ε, 14,000), suggesting the presence of 9-substituted adenine nucleus. The signals at τ 1·85 (1H, singlet, aromatic proton), 1·99 (1H, singlet, aromatic proton) and 2·98 (2H, broad singlet, NH_2) in the NMR spectrum provided a strong support for the assigned partial structure.

The IR spectrum exhibited a broad absorption at 3,500 ~ 2,200 cm^{-1} and a strong peak at 1698 cm^{-1}, assignable to hydroxyl and carboxyl groups. The presence of these groups were confirmed by esterification of eritadenine with CH_2N_2 and successive acetylation of the resulting methyl ester (II) with Ac_2O–pyridine to the diacetate methyl ester (III). The orientation of these groups in the side chain was determined on the basis of the NMR spectral data of III. The 1H doublet at τ 4·87 (J = 5 Hz) and the 1H quartet at τ 4·45 (J = 5 ~ 6 Hz) are assignable to the protons at acetoxy-bearing C_2 and C_3, respectively, while the 2H doublet at τ 5·50 corresponds to the C_4-methylene protons, indicative of attachment to C_3. From these NMR spectral data, it follows that eritadenine has the structure I'. The proof for the vicinal relationship of the two OH groups was also provided by the fact that oxidation of eritadenine





V

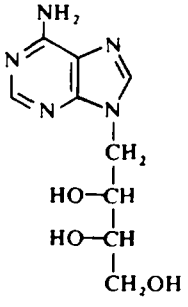
I' $R_1 = R_2 = R_3 = H$
II $R_1 = R_2 = H, R_3 = CH_3$
III $R_1 = R_2 = COCH_3, R_3 = CH_3$
IV $R_1, R_2 = C(CH_3)_2, R_3 = CH_3$

with $NaIO_4$ gave aldehyde hydrate V, the structural assignment of which was based upon the following NMR spectral data. By addition of D_2O to d_6-DMSO solution, the 2H doublet at τ 3·86 (J = 6 Hz) attributed to the OH protons disappeared and the 1H multiplet at τ 4·87 due to the hydrated aldehyde proton became a triplet (J = 5 Hz).

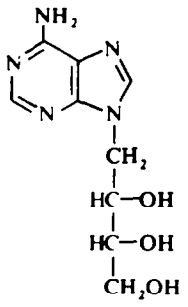
Information on the stereochemistry of the two OH groups was derived from the following evidence. In the NMR spectrum of the acetonide methyl ester (IV) which was obtained by treatment of II with acetone-$POCl_3$, the C_4-methylene signal, in contrast to that of II, shifted from τ 5·6 ~ 6·1 to τ 5·08. This significant deshielding apparently indicates that the C_4-methylene group is oriented *cis* with respect to the

* The original procedure of Rokujo is unpublished. This improved modification was made by working with them.

methoxycarbonyl group. Thus the configuration of the OH functions of eritadenine was deduced to be *erythro*. This deduction was conclusively confirmed as follows. The methyl ester (II) was reduced with $NaBH_4$ in i-PrOH to give the triol (VI), which was found to be the optical antipode of VII with the *erythro* configuration (2-(S), 3-(S)), already synthesized by Ikehara *et al.*[8] Furthermore, from this fact it was inevitably concluded that the absolute configuration of eritadenine is 2-(R), 3-(R).

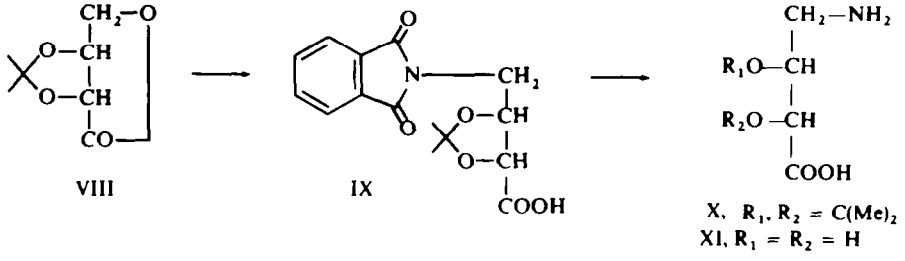


VI



VII

Now having thus established the absolute stereo-structure of eritadenine as I, we turned our attention to the synthesis of the optical active compound for definitive structural confirmation, and also for the acquisition of a large quantity of the compound for biological tests. As the starting material, we chose 2,3-O-isopropylidene-D-erythronolactone (VIII) carrying the required configuration, derivable conveniently from isoascorbic acid through D-erythronolactone.[9] Reaction of VIII with potassium phthalimide in DMF gave adduct IX in a good yield. Treatment of IX with hydrazine hydrate yielded smoothly 4-amino-4-deoxy-2,3-O-isopropylidene-D-erythronic acid (X). Although X had been already prepared by Hanessian[10] from VIII through reaction with NaN_3 followed by catalytic reduction, our approach provides a more convenient synthetic method for obtaining X. In addition, by hydrolysis of IX with 6N HCl,



VIII IX

X, $R_1, R_2 = C(Me)_2$
XI, $R_1 = R_2 = H$

CHART 1

4-amino-4-deoxy-D-erythronic acid (XI), which might serve as an intermediate for our goal as well, was also easily obtained in a high yield. X reacted with 4-amino-6-chloro-5-nitropyrimidine (XII) in the presence of Et_3N in MeOH to give condensation product XIII in 94% yield. Subjected to catalytic reduction on Pd-C in HCO_2H, XIII suffered reductive formylation with removal of the acetonide protective group

to give the 5-formylamino compound (XVII) in a yield of 92%. For a purpose presented below, isolation of the intermediate 5-amino compound (XIV) was attempted by catalytic hydrogenation of XIII on Raney Ni in dil NaOH, resulting in a 90% yield of the desired XIV, from which XVII was also obtained by treatment with

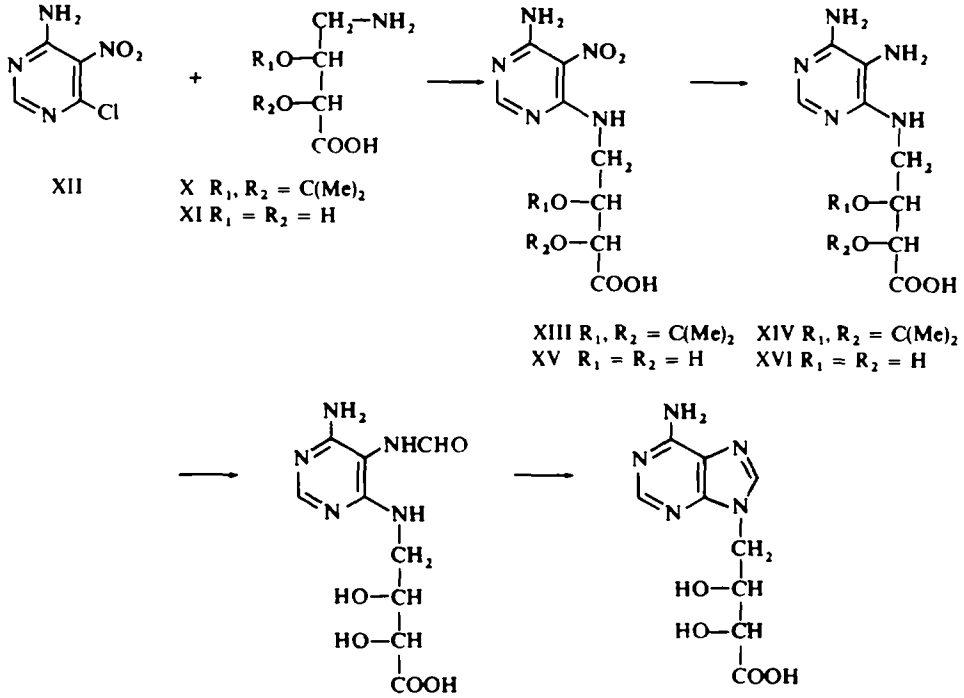
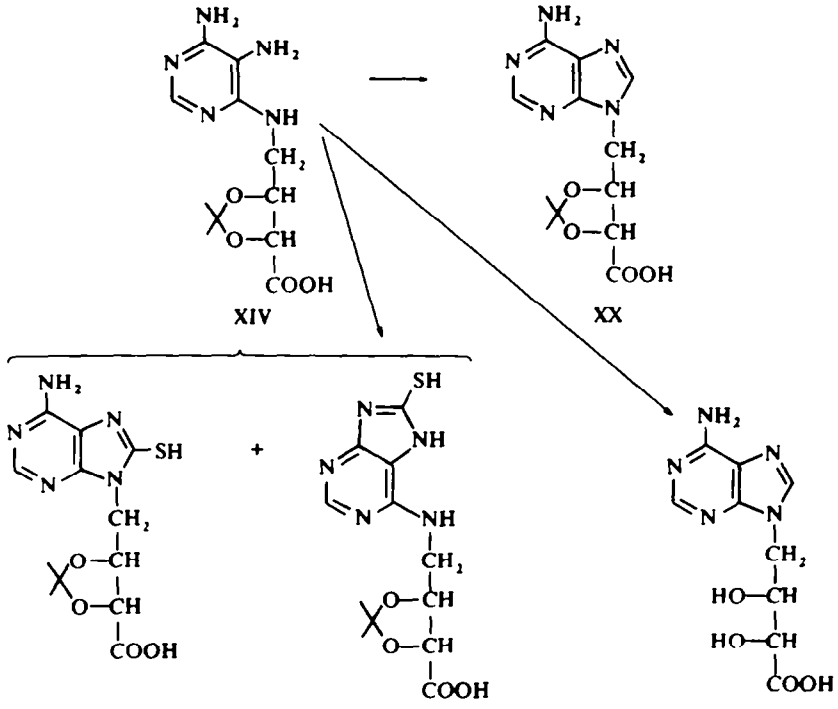


CHART 2

HCO_2H. The same product (XVII) was alternatively prepared from XI by the same sequence of reactions through the 5-nitro (XV) and 5-amino (XVI) compounds as shown in Chart 2. On final treatment with 1N NaOH, XVII was successfully cyclized in the desired direction, yielding exclusively eritadenine (I) in a yield of 90%. This synthetic product proved to be identical with naturally occurring eritadenine by m.p., optical rotation and IR spectral comparison.

The imidazole ring closure step has been found to be a bottleneck in purine synthesis because of the high reaction temperatures required or elaborate kinetine type by-products. In connection with the above effective procedure, we were interested in the comparison with some other cyclization methods using NH_2CHO or CS_2. Cyclization with NH_2CHO was first carried out on XIV to furnish an insufficient yield of the desired product, eritadenine. Similar treatment of XIV with HCO_2H—NH_2CHO led also to approximately the same result. When XIV was refluxed with CS_2 in the presence of EtONa, two products, XVIII and XIX, were generated in a ratio of 5:1, details of which will be presented in the following paper. Cyclization of XIV with formamidine acetate was also attempted, resulting less satisfactorily

in a 50% yield of eritadenine acetonide (XX), which was hydrolyzed with 10% AcOH to give eritadenine. As a result of these experiments, the above method using HCO_2H-NaOH was found eventually to be the most suitable for obtaining eritadenine.



XIV XX XVIII XIX I

CHART 3

EXPERIMENTAL

M.ps were determined with a Thomas-Hoover melting point apparatus in unsealed capirally tubes and are uncorrected. IR and UV spectra were recorded on a Hitachi Type EPI-S2 spectrophotometer and a Hitachi Type EPS-3T spectrophotometer, respectively. NMR spectra were determined on a Varian A-60 spectrometer in d_6-DMSO with TMS as an internal standard. Mass spectra were taken on a Hitachi RMU-60 spectrophotometer. Optical rotations were measured with a JASCO Model ORD/UV-5 optical rotatory dispersion recorder. TLC analysis was carried out on Eastman chromagram sheet (6060 silica gel).

Isolation of eritadenine (I) *from* Lentinus edoes (*Berk.*) *Sing.* The methanolic extract prepared from 5 kg of the dried mushroom was dissolved in H_2O and adsorbed on a column containing 4 l of Amberlite IR-120 ion-exchange resin (H^+ form). After washing with H_2O, the resin column was eluted with 2 l of 2% NH_4OH and concentrated to dryness. The residue was dissolved in H_2O and adsorbed on a column containing 2 l of Amberlite IRA-400 ion-exchange resin (OH^- form). The column was washed with H_2O and AcOH-NH_4OH buffer (pH 5·0), and eluted with 0·5 N AcOH. Fractions showing UV maximum absorption at 260 mμ were combined and evaporated to give a crude crystalline solid. Recrystallization from 10% AcOH yielded 2·20 g of eritadenine as colourless needles, m.p. 279° (dec), $[\alpha]_D$ +49·6° (c = 1·0, 0·1 N NaOH) and + 15·7° (c = 2·0, N HCl), IR ν_{max}^{Nujol}: 3500-2200 and 1698 (broad) cm^{-1}, NMR: τ 1·85 (1H.s, aromatic H), 1·99 (1 H, s, aromatic H), 2·98 (2 H, broad s, $-NH_2$) and 5·6 ~ 6·1 (4 H, m, $<C\underline{H}_2$,

$\rangle$C<u>H</u>—OH, C<u>H</u>—OH), UV: $\lambda_{max}^{H_2O}$ 261 mμ (ε, 14,300), $\lambda_{max}^{0.1N\,NaOH}$ 261 mμ (ε, 14,300) and $\lambda_{max}^{0.1N\,HCl}$ 260 mμ (ε, 14,000), (Found: C, 42·90; H, 4·36; N, 27·70. $C_9H_{11}O_4N_5$ requires: C, 42·69; H, 4·38; N, 27·67%).

Eritadenine methyl ester (II). A suspension of 100 mg of eritadenine (I) in 15 ml of THF was treated with excess ethereal CH_2N_2 and stirred for 20 hr at room temp. The solvent was removed and the residue crystallized from MeOH. Recrystallization from MeOH–H_2O gave 82 mg of II as colourless needles, m.p. 231° (dec), IR ν_{max}^{nujol}: 1730 and 1220 cm^{-1}, NMR: τ 5·6 ~ 6·1 (4 H, m, $\rangle$C<u>H</u>$_2$, $\rangle$C<u>H</u>—OH, $\rangle$C<u>H</u>—OH) and 6·38 (3 H, s, —COOC<u>H</u>$_3$). Mass spectrum (80 eV, source temp 250°): 267 (M^+), 250, 218, 208, 190, 179, 178, 149, 148, 136, 135 (base peak). (Found: C, 44·82; H, 4·80; N, 26·01. $C_{10}H_{13}O_4N_5$ requires: C, 44·94; H, 4·90; N, 26·21%).

Eritadenine diacetate methyl ester (III). To a suspension of 100 mg of the methyl ester (II) in 2 ml of pyridine was added 1 ml of Ac_2O and the mixture stirred for 5 hr at room temp. The resulting soln was poured onto crushed ice and extracted with $CHCl_3$. The extract was washed with H_2O and dried over $MgSO_4$. After removal of the solvent, the residue was recrystallized from MeOH to give 83 mg of III as colourless scales, m.p. 225° (dec), IR ν_{max}^{nujol}: 1765, 1740 and 1230 cm^{-1}, NMR: τ 4·45 (1 H, broad q, J = 5–6 Hz, $\rangle$C<u>H</u>—OAc), 4·87 (1 H, d, J = 5 Hz, $\rangle$C<u>H</u>—OAc), 5·50 (2 H, d, J = 6 Hz, $\rangle$C<u>H</u>$_2$), 6·34 (3 H, s, —COOC<u>H</u>$_3$), 7·86 (3 H, s, —OCOC<u>H</u>$_3$) and 8·08 (3 H, s, —OCOC<u>H</u>$_3$). Mass spectrum (80 eV, source temp 250°): 351 (M^+), 308, 292 (base peak), 266, 250, 218, 190, 178· 149, 148, 136, 135. (Found: C, 47·69; H, 4·80; N, 19·78. $C_{14}H_{17}O_6N_5$ requires: C, 47·86; H, 4·88; N, 19·94%).

Eritadenine acetonide methyl ester (IV). A 200 mg sample of the methyl ester (II) was added to 20 ml of acetone containing 500 mg of $POCl_3$, followed by stirring for 2 hr at room temp. The resulting soln was poured into an ice cold soln of $NaHCO_3$ aq and, after evaporation of acetone, was extracted with $CHCl_3$. The extract was dried over $MgSO_4$ and evaporated to dryness. The resulting crude crystalline solid was recrystallized from acetone–ether to give 140 mg of IV as colourless scales, m.p. 181°, IR ν_{max}^{nujol}: 1750, 1200 and 1095 cm^{-1}, NMR: τ 5·08 (2 H, m, $\rangle$C<u>H</u>$_2$), 5·65–5·95 (2 H, m, $\rangle$C<u>H</u>—O—, $\rangle$C<u>H</u>—O—), 6·77 (3 H, s, —COOC<u>H</u>$_3$), 8·46 (3 H, s, C—C<u>H</u>$_3$) and 8·72 (3 H, s, C—C<u>H</u>$_3$). (Found: C, 51·04; H, 5·63; N, 22·51. $C_{13}H_{17}O_4N_5$ requires: C, 50·81; H, 5·58; N, 22·79%).

Triol (VI). To a suspension of 200 mg of the methyl ester (II) in 30 ml of i-PrOH was added 200 mg of $NaBH_4$ and the mixture was refluxed for 20 hr. After removal of solvent, the residue was dissolved in H_2O and adsorbed on a column containing 15 ml of Dowex 50W (H^+ form). The column was washed with H_2O and eluted with 4% NH_4OH. The eluent was concentrated to give a crystalline solid, which was recrystallized from EtOH–H_2O to afford 110 mg of VI as colourless prisms, m.p. 219–220°, $[\alpha]_D$ +29·7° (c = 0·4, N HCl). (Found: C, 44·80; H, 5·48; N, 29·04. $C_9H_{13}O_3N_5$ requires: C, 45·18; H, 5·48; N, 29·28%). IR spectrum (nujol) is identical with that of VII.

Aldehyde hydrate (V). To a soln of 100 mg of eritadenine in 4 ml of H_2O containing 35 mg of $NaHCO_3$ was added 90 mg of $NaIO_4$ under ice bath cooling. After stirring for 3 hr at the same temp, crystals separated and were collected and recrystallized from H_2O to give 48 mg of V as colourless prisms, m.p. > 300°, IR ν_{max}^{nujol}: 3500–2300, 1040 and 1090 cm^{-1}, NMR: τ 3·86 (2 H, broad d, J = 6 Hz, —CH(O<u>H</u>)$_2$), 4·87 (1 H, m, —C<u>H</u>(OH)$_2$) and 5·94 (2 H, d, J = 5 Hz, $\rangle$C<u>H</u>$_2$). By addition of D_2O the signal at τ 3·86 was disappeared and the signal at τ 4·87 was changed to a triplet (J = 5 Hz). (Found: C, 42·83; H, 4·55; N, 36·15. $C_7H_9O_2N_5$ requires: C, 43·07; H, 4·65; N, 35·89%).

4-Phthalimido-4-deoxy-2,3-O-isopropylidene-D-erythronic acid (IX). A mixture of 30·0 g of the lactone acetonide (VIII) and 35·5 g of potassium phthalimide in 250 ml of DMF was stirred under reflux for 5 hr. After evaporation of the solvent, the residue was dissolved in H_2O and acidified with dil HCl to separate a crystalline solid, which was filtered and washed with H_2O, yield 46·7 g (80%). An analytical sample was prepared by recrystallization from i-PrOH, m.p. 196° (dec). (Found: C, 58·89; H, 4·80; N, 4·66. $C_{15}H_{15}O_6N$ requires: C, 59·01; H, 4·95; N, 4·59%).

4-Amino-4-deoxy-2,3-O-isopropylidene-D-erythronic acid (X). To a suspension of 30·5 g of the phthalimido adduct (IX) in 300 ml of 50% aqueous EtOH was added 12·5 g of 100% hydrazine hydrate. After refluxing for 1 hr, the resulting soln was cooled in an ice bath and acidified with 20% AcOH to separate the crystals of phthalhydrazide, which were filtered off and the filtrate was concentrated to about 50 ml. Addition of acetone to the concentrated soln precipitated a crystalline solid, which was filtered and washed with acetone, yield 13·5 g (70%). An analytical sample was prepared by recrystallization from EtOH–H_2O,

m.p. 189° (dec), $[\alpha]_D$ +85·0° (c = 1·1, 60% acetone). (Found: C, 43·35; H, 7·99; N, 7·41. $C_7H_{13}O_4N.H_2O$ requires: C, 43·51; H, 7·83; N, 7·25%).

4-*Amino*-4-*deoxy*-D-*erythronic acid* (XI). A 30·5 g sample of the phthalimido adduct (IX) was hydrolyzed with 300 ml of 6N HCl by refluxing for 5 hr. The resulting soln was cooled in an ice bath to separate the crystals of phthalic acid, which were filtered off. The filtrate was concentrated to dryness and the residue was dissolved in H_2O, followed by neutralization with conc NH_4OH. The crystalline ppt obtained was filtered and washed with MeOH, yield 12·2 g (86%). An analytically pure sample was obtained by recrystallization from MeOH-H_2O, m.p. 224° (dec), $[\alpha]_D$ +33·2° (c = 1·3, H_2O). (Found: C, 35·55; H, 6·61; N, 10·31. $C_4H_9O_4N$ requires: C, 35·55; H, 6·71; N, 10·37%).

4-(6-*Amino*-5-*nitro*-4-*pyrimidylamino*)-4-*deoxy*-2,3-O-*isopropylidene*-D-*erythronic acid* (XIII). A mixture of 0·97 g of the amino acid (X), 0·87 g of the pyrimidine precursor (XII) and 1·01 g of Et_3N in 50 ml of MeOH was stirred for 13 hr at room temp. After removal of solvent, the residual solid was dissolved in H_2O and acidified with dil HCl to separate a crystalline solid which was filtered and washed with H_2O, yield 1·47 g (94%). Recrystallization from MeOH gave an analytically pure sample, m.p. 228° (dec), UV λ_{max}^{EtOH}: 230 sh (17,700) and 339 mμ (ε, 10,100). (Found: C, 42·24; H, 4·73; N, 22·47. $C_{11}H_{15}O_6N_5$ requires: C, 42·17; H, 4·83; N, 22·36%).

4-(6-*Amino*-5-*nitro*-4-*pyrimidylamino*)-4-*deoxy*-D-*erythronic acid* (XV). Under the same conditions used in the preceding case, condensation of 5·95 g of the amino acid (XI) with 7·70 g of the pyrimidine precursor (XII) in the presence of 11·15 g of Et_3N in 180 ml of 80% aqueous MeOH was carried out and 11·08 g (92%) of XV obtained. An analytical sample was prepared by recrystallization from 20% AcOH, m.p. 236–237° (dec), UV $\lambda_{max}^{H_2O}$: 230 (16,600) and 345 mμ (ε, 9700). (Found: C, 35·04; H, 3·93; N, 25·78. $C_8H_{11}O_6N_5$ requires: C, 35·17; H, 4·06; N, 25·64%).

4-(5,6-*Diamino*-4-*pyrimidylamino*)-3-*deoxy*-2,3-O-*isopropylidene*-D-*erythronic acid* (XIV). A 0·78 g sample of the nitro compound (XIII) was dissolved in 13 ml of H_2O containing 0·12 g of NaOH and hydrogenated over Raney Ni in the usual manner. After the catalyst was filtered off, the filtrate was acidified with dil HCl to give a precipitate of crystalline solid, which was filtered and washed with H_2O, yield 0·72 g (90%). An analytical sample was prepared by recrystallization from H_2O, m.p. 223° (dec), UV λ_{max}^{EtOH}: 218 (26,600) and 280 mμ (ε, 10,800). (Found: C, 41·47; H, 6·77; N, 21·60. $C_{11}H_{17}O_4N_5.2H_2O$ requires: C, 41·37; H, 6·63; N, 21·93%).

4-(5,6-*Diamino*-4-*pyrimidylamino*)-4-*deoxy*-D-*erythronic acid* (XVI). As for XIV, 5·46 g of the 5-nitro compound (XV) dissolved in 170 ml of H_2O containing 0·44 g of NaOH was hydrogenated over Raney Ni to give 4·66 g (96%) of XVI. Recrystallization from H_2O gave an analytically pure sample, m.p. 244–245° (dec), UV $\lambda_{max}^{H_2O}$: 221 (19,700) and 285 mμ (ε, 10,600). (Found: C, 39·50; H, 5·19; N, 29·04. $C_8H_{13}O_4N_5$ requires: C, 39·50; H, 5·39; N, 28·80%).

4-(6-*Amino*-5-*formylamino*-4-*pyrimidylamino*)-4-*deoxy*-D-*erythronic acid* (XVII): (i) A soln of 0·50 g of the 5-amino compound (XIV) in 15 ml of 90% HCO_2H was refluxed for 1 hr. After removal of HCO_2H, the residue was dissolved in H_2O, followed by evaporation to dryness in order to remove the remaining HCO_2H. The resulting residue was dissolved in a small amount of H_2O and left overnight in a refrigerator. The precipitating crystals were collected and washed with H_2O, yield 0·52 g (96%). m.p. 190° (dec), UV $\lambda_{max}^{H_2O}$: 222 (35,100) and 265 mμ (ε, 7100). (Found: C, 39·73; H, 4·82; N, 25·81. $C_9H_{13}O_5N_5$ requires: C, 39·85; H, 4·83; N, 25·82%).

(ii) The same treatment of 1·22 g of the 5-amino compound (XVI) (as for XIV) gave 1·24 g (91%) of the 5-formylamino compound (XVII).

(iii) A 0·62 g sample of XIII was dissolved in 15 ml of 90% HCO_2H and hydrogenated over Pd-C in the usual manner. After the catalyst was filtered, the filtrate was refluxed for 1 hr and worked up as for XIV to yield 0.49 g (92%) of XVII.

4-(6-*Amino*-9H-*purin*-9-*yl*)-4-*deoxy*-D-*erythronic acid* (*eritadenine*) (I). (i) A soln of 0·50 g of the 5-formylamino compound (XVII) in 5 ml of 1N NaOH was refluxed for 15 min. After cooling, the soln was acidified with dil HCl to separate a crystalline solid, which was filtered and washed with H_2O, yield 0·42 g (90%). Recrystallization from 10% AcOH gave an analytically pure sample, m.p. 279° (dec), $[\alpha]_D$ +51·1° (c = 1·3, 0·1 N NaOH) and +14·8° (c = 2·0, N HCl). (Found: C, 42·48; H, 4·22; N, 27·53. $C_9H_{11}O_4N_5$ requires: C, 42·69; H, 4·38; N, 27·67%). The IR and UV spectra were identical with those of naturally occurring eritadenine.

(ii) A mixture of 0·50 g of the 5-amino compound (XIV) and 2 ml of NH_2CHO was heated at 160° for 20 min. After cooling, acetone was added to the resulting soln, and the precipitated solid was filtered and recrystallized from H_2O to give 0·03 g of eritadenine, identified with the natural product by IR comparison.

(iii) A 0·50 g sample of the 5-amino compound (XIV) was added to a mixture of 1 ml of NH_2CHO and 1 ml of 98% HCO_2H and the mixture refluxed for 30 min. After cooling, EtOH was added to the resulting soln, and the precipitate filtered and recrystallized from H_2O to give 0·04 g of eritadenine; identification by IR.

(iv) A mixture of 0·10 g of eritadenine acetonide (XX) and 5 ml of 10% AcOH was refluxed for 30 min and then cooled in an ice bath to separate crystals, which were collected and washed with H_2O to give 58 mg of eritadenine; identification by IR.

4-(6-*Amino*-9H-*purin*-9-*yl*)-4-*deoxy*-2,3-O-*isopropylidene*-D-*erythronic acid* (*eritadenine acetonide*) (XX). To a soln of 0·50 g of the 5-amino compound (XIV) in 10 ml of methyl cellosolve was added 0·25 g of formamidine acetate and the mixture refluxed for 20 min. After evaporation of solvent, the residue was dissolved in H_2O and acidified with dil HCl. The resulting crystalline ppt was filtered and recrystallized from EtOH–H_2O to give 0·23 g of eritadenine acetonide (XX), m.p. 216° (dec), UV: $\lambda_{max}^{H_2O}$ 261 mμ (ε, 14,200), $\lambda_{max}^{0.1N\,NaOH}$ 261 mμ (ε, 14,300), $\lambda_{max}^{0.1N\,HCl}$ 260 mμ (ε, 13,500). (Found: C, 47·61; H, 5·61; N, 22·96. $C_{12}H_{15}O_4N_5 \cdot \frac{1}{2}H_2O$ requires: C, 47·67; H, 5·33; N, 23·17%).

Acknowledgement—The authors are grateful to Professor Y. Inubushi of Kyoto University for his helpful criticism of the manuscript.

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