Hypocholesterolemic Alkaloids of Lentinus edodes (Berk.) Sing. II. A Novel Synthesis of Eritadenine

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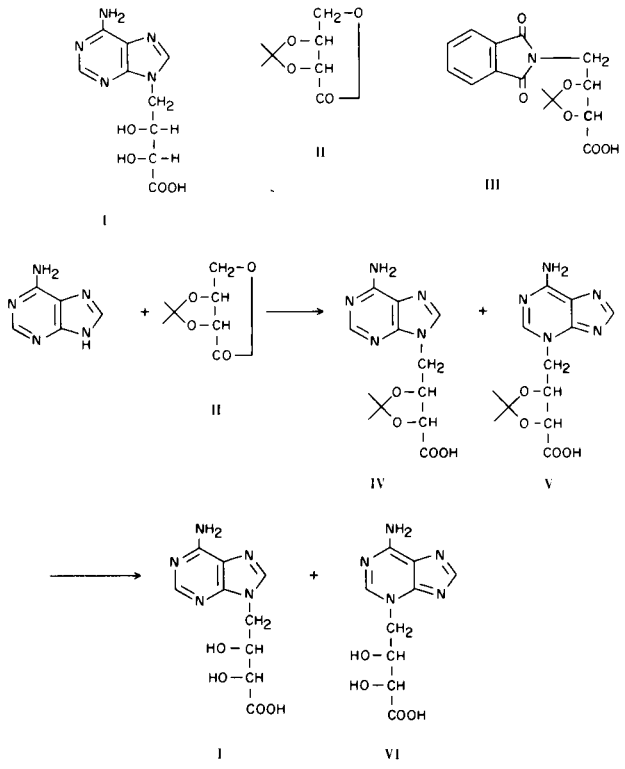
A novel and convenient method for the synthesis of eritadenine (I) was established. Direct condensation of adenine and 2,3-*O*-isopropylidene-D-erythronolactone (II) under basic condition, followed by removal of the protecting group, afforded eritadenine (I) in fairly good yield, with accompany of a small amount of the N_3-isomer (VI). A similar reaction of adenine 1-oxide gave exclusively eritadenine oxide (VII), which was catalytically reduced to give eritadenine (I). This procedure also provided pyrimidinyl derivatives (VIII, IX and X) corresponding to cytosine, uracil, and thymine.

Eritadenine (I), occupying a unique position as an alkaloid with its structural or biogenetical resemblance to nucleosides, is a new hypocholesterolemic agent isolated from Lentinus edodes (Berk.) Sing. Its structure has been established by chemical determination and total synthesis as described in the preceding paper (1). The high biological activity of eritadenine in rats has prompted us to explore a more convenient method for its synthesis, because the usual route (2a-c) to the N_9-substituted adenine, involving an imidazole ring closure step, seemed to us to be rather circuitous for acquisition of a large quantity of eritadenine. As reported in a preliminary communication (3), we have now established a novel and convenient synthesis of eritadenine by direct condensation of adenine and 2,3-*O*-isopropylidene-D-erythronolactone (II) under basic condition. In this paper we wish to give the full experimental details of our study (4). Our interest in a biological evaluation of various homologs might be also answered by the utilization of this method through which some compounds containing other ring systems in place of adenine could be readily prepared.

Our new approach rested on the basis of the fact that, by allowing potassium phthalimide to react with 2,3-*O*-isopropylidene-D-erythronolactone (II) (5) in boiling dimethylformamide, the phthalimido adduct (III) was obtained in a high yield as reported previously (1). As was that case, the adenide anion, in which the negative charge might be considered to be predominantly located at N_9 (6), could be expected to serve as a nucleophile and attack the CH_2-O bond of the lactone ring, leading to formation of eritadenine acetonide (IV). Thus, we now examined the reaction of the sodium salt of adenine, prepared from adenine and sodium hydride in dimethylformamide (7d), with the lactone (II) by heating the reaction mixture to reflux of dimethylformamide. The result indicated that the expected reaction took place and the desired product (IV) existed in the reaction mixture. This product was hydrolyzed with 10% acetic acid without isolation and

SCHEME I



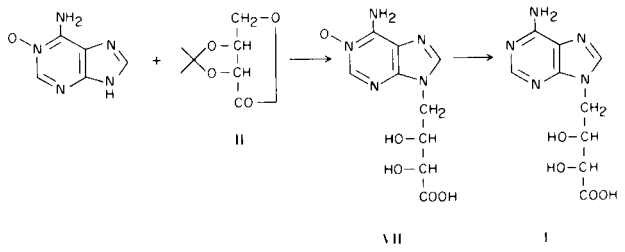
there was secured a 37% of eritadenine (I), identity of which was made by melting point, optical rotation, uv and ir spectral comparison with the naturally occurring product. In addition, this direct coupling reaction was also found to form a minor product, which was isolated as the hydrolyzed product (VI) as well and characterized to be the N_3-isomer by the uv spectral data (8). The separation of eritadenine (I) and the isomer (VI) was accomplished by adsorption on an ion-exchange resin column (Amberlite IRA-400, OH^- form) followed by elution with dilute acetic acid.

In most of the previously reported alkylations of adenine with halides, the distribution of *N*-isomers had been observed. Under neutral condition adenine is alkylated at N_3 with small amounts of N_1- and N_7-products (10a-d), while the basic catalyzed alkylation reaction usually gives a mixture of N_3- and N_9-isomers in which the N_9-isomer is the major product (7b,c). Our result for the above condensation reaction is in agreement with these latter observations. The formation of the N_3-isomer under basic condition probably occurs on the free adenine which might be in a small amount due to equilibrium with the adenide anion.

With our preliminary trial succeeded as expected, our effort was then directed to search the optimal reaction condition. After a wide variety of bases and solvents have been examined under various conditions, this condensation followed by the hydrolysis was eventually accomplished with a 45% average yield of eritadenine (11). The yield was realized when adenine was reacted with 1.3 mole equivalent of the lactone (II) in the presence of 1 mole equivalent of sodium carbonate in boiling dimethylformamide for 20 hours, although this procedure always brought about 6 ~ 8% yield of the undesired isomer (VI). In order to minimize the isomer distribution, some studies were made by changing reaction conditions but this trial was unfruitful. However, this practical obstacle was overcome by fractional crystallization of the mixture after being converted to the sodium salts.

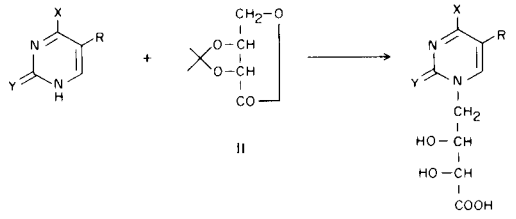
An attempt to avoid the formation of the by-product (VI) was also carried out by using adenine 1-oxide (12) as a starting material, in view of the reported observation that, on alkylation of the oxide with benzyl bromide, only 9-benzyladenine 1-oxide was obtained (13). Reaction of adenine 1-oxide with the lactone (II) under a similar condition to that described above, followed by hydrolysis of the resulting product with dilute acetic acid, was found to give exclusively eritadenine oxide (VII), which was catalytically reduced on Raney nickel to yield eritadenine (I). This route, however, was rather less favorable because of an insufficient total yield (33%), which was attributable to a sparing solubility of adenine 1-oxide.

SCHEME II



II VII I

SCHEME III



II

VIII, X NH_2, Y O, R H
IX, X OH, Y O, R H
X, X OH, Y O, R CH_3

From our interest in an extention of the present reaction as well as a biological evaluation of compounds related to eritadenine, we further examined its application to some pyrimidine bases for the preparation of analogous pyrimidine derivatives. Thus, condensations of cytosine, uracil and thymine with the lactone (II) were respectively carried out under similar conditions and hydrolyses of the resulting products with dilute acetic acid resulted in 10 ~ 40% yields (14) of the desired products (VIII, IX and X). It is noteworthy that no N_3-substituted isomer was detected during the course of these condensation reactions. The specificity of our reaction is in interesting contrast to the reported alkylation of these pyrimidine bases with alkyl halides, which is usually more complicated by the fact that a mixture of N_1- and N_3-alkyl derivatives were obtained (15a,b). While no evidence bearing on this difference is available at present, a plausible explanation of the observed result in our reaction may be advanced on the basis of the steric concept. The attack of the N_3 anion of the pyrimidine bases to the CH_2-O bond of the lactone (II) may be sterically hindered by the substituents of both reactants.

The above sequence involving the direct coupling method for the synthesis of the N_1-substituted pyrimidine derivatives also represents a distinct convenience over routes involving ring closure steps because of the greater simplicity, even though the yield of the products was relatively low. Thus, the present procedure provides a general applicability for preparing analogous compounds.

EXPERIMENTAL

All evaporations were performed on rotatory evaporators *in vacuo*. Melting points were determined in open capillary tubes with a Thomas-Hoover melting point apparatus and are uncorrected. Ir and uv spectra were recorded on a Hitachi Type EPI-S2 spectrophotometer and a Hitachi Type EPS-3T spectrophotometer, respectively. 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).

4-(6-Amino-9*H*-purin-9-yl)-4-deoxy-D-erythronic Acid (Eritadenine) (I) and 4-(6-Amino-3*H*-purin-3-yl)-4-deoxy-D-erythronic Acid (VI).

(i) To a suspension of sodium adenide, prepared from 2.70 g. (0.02 mole) of adenine and 0.96 g. (0.02 mole) of sodium hydride (7d), in 45 ml. of anhydrous dimethylformamide was added 3.17 g. (0.03 mole) of the lactone (II) and the mixture was stirred at reflux for 8 hours. After evaporation of the solvent, the residual solid was dissolved in water, acidified with dilute hydrochloric acid and concentrated to separate a crystalline solid, which was filtered and washed with water. This solid was then added to 20 ml. of 10% acetic acid and refluxed for 30 minutes. After the solution was concentrated, the resulting crystalline solid was dissolved in dilute ammonium hydroxide and put on an Amberlite IRA-400 (OH^- form) resin column (100 ml.). After washing with water until the eluate was neutral, elution was carried out with 0.05 *N* acetic acid and fractions showing uv maximum absorption at 260 mμ were combined and evaporated to give the N_3-isomer (VI) as a crude crystalline solid. Recrystallization from 10% aqueous pyridine gave 0.30 g. (6%) of VI, m.p. 294-296° dec.; $[\alpha]_D$+91.6° (c = 1.4, 0.1 *N* sodium hydroxide); uv: λ max (*p*H 7) 213 (17,500) and 276 mμ (ε, 14,800), λ max (0.1 *N* hydrochloric acid) 219 (11,200) and 276 mμ (ε, 17,600), λ max (0.1 *N* sodium hydroxide) 276 mμ (ε, 13,200); λ min (*p*H 7) 245 mμ (ε, 3,800), λ min (0.1 *N* hydrochloric acid) 237 mμ (ε, 2,900) and λ min (0.1 *N* sodium hydroxide) 246 mμ (ε, 3,300).

Anal. Calcd. for $C_9H_{11}O_4N_5$: C, 42.69; H, 4.38; N, 27.67. Found: C, 42.45; H, 4.48; N, 27.39.

Upon further elution with 0.1 *N* acetic acid and evaporation of fractions having the same uv absorption, a crystalline solid was obtained. Recrystallization from 10% acetic acid gave 1.90 g. (37%) of eritadenine (I), m.p. 278-279° dec.; $[\alpha]_D$+51.4° (c = 1.6, 0.1 *N* sodium hydroxide). This was identified with the natural product by tlc, optical rotation, uv and ir spectral comparison.

Anal. Calcd. for $C_9H_{11}O_4N_5$: C, 42.69; H, 4.38; N, 27.67. Found: C, 42.48; H, 4.33; N, 27.39.

(ii) A mixture of 8.10 g. (0.06 mole) of adenine, 12.30 g. (0.078 mole) of the lactone (II) and 6.36 g. (0.06 mole) of sodium carbonate in 180 ml. of dimethylformamide was refluxed with stirring for 20 hours. According to the operation described above, the reaction mixture was worked up to give 9.90 g. of a crude mixture of eritadenine (I) and its isomer (VI).

Separation of I and VI.

(a) The half (4.95 g.) of the above mixture was worked up using 300 ml. of Amberlite IRA-400 (OH^- form) in the same way as described above to provide 3.42 g. (45%) of eritadenine (I) and 0.55 g. (7%) of the isomer (VI).

(b) The remaining 4.95 g. of the mixture was dissolved in boiling 10% aqueous pyridine and then cooled to separate the crystals of the isomer (VI), which were filtered off (0.39 g., 5%) and the filtrate was evaporated to dryness. The residue was dissolved in dilute sodium hydroxide and again evaporated to dryness. The resulting crude sodium salt of eritadenine (I) was recrystallized from 50% ethanol to yield 3.30 g. (40%) of the pure eritadenine sodium salt, m.p. 275° dec.

Anal. Calcd. for $C_9H_{10}O_4N_5Na$: C, 39.28; H, 3.99; N, 25.45; Na, 8.36. Found: C, 39.12; H, 3.70; N, 25.57; Na, 8.40.

Eritadenine Oxide (VII).

A mixture of 6.04 g. (0.04 mole) of adenine 1-oxide (12) 8.84 g. (0.056 mole) of the lactone (II) and 4.24 g. (0.04 mole) of sodium carbonate in 200 ml. of dimethyl sulfoxide was stirred at 150° for 5 hours. After evaporation of the solvent, the residue was dissolved in water and acidified with dilute hydrochloric acid, whereupon a crystalline solid precipitated out. This solid was collected by filtration and then added to 200 ml. of 10% acetic acid. After the suspension was refluxed for 1 hour, the resulting solution was filtered in order to remove impurities and the filtrate was then cooled in an ice bath. The precipitated crystals were collected and washed with water to give 4.20 g. (39%) of eritadenine oxide (VII). An analytical pure sample was prepared by recrystallization from 10% acetic acid, m.p. 277-280° dec.; uv: λ max (water) 233 (45,000), 263 (8,500) and 292 mμ (ε, 2,600), λ max (0.1 *N* hydrochloric acid) 213 (30,300) and 260 mμ (ε, 12,500), λ max (0.1 *N* sodium hydroxide) 232 (24,300), 271 (9,300) and 307 mμ (ε, 4,500).

Anal. Calcd. for $C_9H_{11}O_5N_5$: C, 40.15; H, 4.12; N, 26.06. Found: C, 40.00; H, 4.12; N, 26.30.

Eritadenine (I) from Eritadenine Oxide (VII).

A solution of 0.54 g. of eritadenine oxide (VII) in 20 ml. of 0.1 *N* sodium hydroxide was hydrogenated on Raney nickel in the usual manner. After the catalyst was filtered off, the filtrate was neutralized with 20 ml. of 0.1 *N* hydrochloric acid and concentrated to about 10 ml. The separated crystalline solid was filtered and washed with water to give 0.43 g. (84%) of eritadenine (I), m.p. 276-278° dec., which was identified with the natural product by tlc, uv and ir spectral comparison.

4-(4-Amino-1*H*-pyrimidin-2-on-1-yl)-4-deoxy-D-erythronic Acid (VIII).

A mixture of 2.22 g. (0.02 mole) of anhydrous cytosine, 4.74 g. (0.03 mole) of the lactone (II) and 2.12 g. (0.02 mole) of sodium carbonate in 60 ml. of dimethylformamide was stirred at reflux for 20 hours. The resulting solution was evaporated to dryness, and the residue was dissolved in 100 ml. of 20% acetic acid and refluxed for 1 hour. After the solution was evaporated, the residue was dissolved in water and put on a column containing 100 ml. of Amberlite IRA-400 (OH^- form). The column was washed with water and then eluted with 0.05 *N* acetic acid. Evaporation of the eluate gave a crystalline material which was recrystallized from water to give 0.30 g. of the cytosine derivative (VIII), m.p. 285° dec.; uv: λ max (water) 279 mμ (ε, 10,400), λ max (0.1 *N* hydrochloric acid) 283 mμ (ε, 12,700), λ max (0.1 *N* sodium hydroxide) 275 mμ (ε, 8,500).

Anal. Calcd. for $C_8H_{11}O_5N_3$: C, 41.92; H, 4.84; N, 18.34. Found: C, 41.60; H, 4.91; N, 18.16.

4-(1*H*,3*H*-Pyrimidin-2,4-dion-1-yl)-4-deoxy-D-erythronic Acid (IX).

A mixture of 2.24 g. (0.02 mole) of uracil, 4.74 g. (0.03 mole) of the lactone (II) and 3.20 g. (0.03 mole) of sodium carbonate in 50 ml. of dimethylformamide was refluxed with stirring for 22 hours. After removal of the solvent, the residue

was dissolved in 100 ml. of 20% acetic acid and refluxed for 1 hour, then evaporated to dryness. The residue was dissolved in water and put on a column containing 100 ml. of Amberlite IRA-400 (OH^- form) ion-exchange resin. After washing with water followed by 0.1 *N* acetic acid, elution was carried out with 0.5 *N* formic acid. Evaporation of the eluate gave a crude crystalline product, which was recrystallized from water to provide 0.65 g. of the uracil derivative (IX), m.p. 240-241° dec.; uv: λ max (water) 267 mμ (ε, 10,300), λ max (0.1 *N* hydrochloric acid) 266 mμ (ε, 10,200), λ max (0.1 *N* sodium hydroxide) 265 mμ (ε, 8,400).

Anal. Calcd. for $C_8H_{10}O_6N_2$: C, 41.74; H, 4.38; N, 12.17. Found: C, 41.69; H, 4.65; N, 12.05.

4-(5-Methyl-1*H*,3*H*-pyrimidin-2,4-dion-1-yl)-4-deoxy-D-erythronic Acid (X).

A mixture of 2.56 g. (0.02 mole) of thymine, 4.74 g. (0.03 mole) of the lactone (II) and 3.18 g. (0.03 mole) of sodium carbonate in 60 ml. of dimethylformamide was refluxed with stirring for 15 hours. The solution was evaporated, and the residue was dissolved in 100 ml. of 20% acetic acid and refluxed for 1 hour. After evaporation of the solvent, the residue was dissolved in water and put on the ion-exchange resin column of 100 ml. of Amberlite IRA-400 (OH^- form). After being washed with water followed by 0.1 *N* acetic acid, the column was eluted with 0.5 *N* formic acid. After the eluate was evaporated, the residue was dissolved in 20% sodium hydroxide and triturated with ethanol. The crude sodium salt of the thymine derivative (X) so formed was recrystallized from aqueous ethanol to afford 1.00 g., m.p. 233-236° dec.; uv: λ max (water) 272 mμ (ε, 10,000), λ max (0.1 *N* hydrochloric acid) 272 mμ (ε, 9,800), λ max (0.1 *N* sodium hydroxide) 271 mμ (ε, 7,600).

Anal. Calcd. for $C_9H_{10}O_6N_2Na_2 \cdot H_2O$: C, 35.30; H, 3.95; N, 9.15. Found: C, 35.43; H, 4.02; N, 8.94.

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