

Nucleoside Analogs. I. A Synthesis of 1,3-Dihydroxy-2-(6-substituted-9-purinyl)cyclohexane

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Biologically active 1,3-dihydroxy-2-(6-substituted-9-purinyl)cyclohexanes (VI-X) were prepared by a reaction between *trans*-2-amino-1,3-cyclohexanediol (I) and 4,6-dichloro-5-aminopyrimidine (II), followed by cyclization and nucleophilic substitution.

Compounds VI and VII showed a weak inhibitory effect against *Xanthomonas oryzae*, a pathogenic bacteria of leaf blight of rice plant.

Nucleoside antibiotics have been found to possess significant antimicrobial and antitumor activities (1-6). Most of the nucleoside antibiotics have adenine as a purine base and an uncommon sugar as a carbohydrate moiety. Therefore, it can be expected that nucleoside analogs which have adenine and cyclitols as their components could exhibit biological activity.

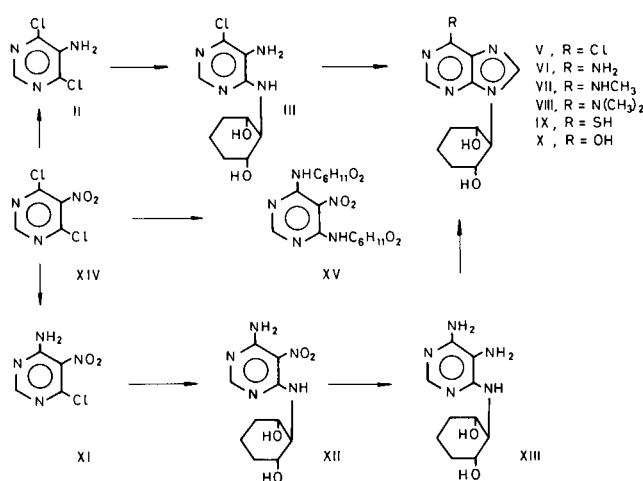
Very recently Schaeffer and Vince (7) described the synthesis of 6-amino-9-(1-hydroxy-2-hydroxymethyl-4-cyclohexyl)purine by a reaction between 1-amino-3-hydroxymethyl-4-hydroxycyclohexane and 5-amino-4,6-dichloropyrimidine, followed by cyclization and amination.

Since many inosamines have been prepared in our laboratory (8-10), the method of Schaeffer and his co-worker (7) encouraged us to attempt to prepare 6-substituted-9-(polyhydroxycyclohexyl)purines.

In the present article, beginning with *trans*-2-amino-1,3-cyclohexanediol and chloropyrimidine, a synthesis of 1,3-dihydroxy-2-(6-substituted-9-purinyl)cyclohexanes is described.

When a mixture of *trans*-2-amino-1,3-cyclohexanediol (I) (II) and 5-amino-4,6-dichloropyrimidine (II) (12) was heated in 1-butanol under reflux for 48 hours, 1,3-dihydroxy-2-(5-amino-4-chloro-6-pyrimidinylamino)cyclohexane (III) was obtained in a yield of 53%. Treatment of III with ethyl orthoformate afforded 1,3-*O*-ethoxymethylidene-2-(6-chloro-9-purinyl)cyclohexane (IV) which was readily converted to 1,3-dihydroxy-2-(6-chloro-9-purinyl)cyclohexane (V) by heating in ethanol. Compound V exhibited the proper ultraviolet absorption spectra for 6-chloropurine (13).

When V was heated with liquid ammonia in an autoclave at 60° for 18 hours, 1,3-dihydroxy-2-(6-amino-9-purinyl)cyclohexane (VI) was obtained in 68% yield.



This compound exhibited the ultraviolet absorption characteristic of adenine at 263 μ (14).

Furthermore, when V was treated with other nucleophilic reagents such as 40% aqueous methylamine, 20% aqueous dimethylamine, thiourea and sodium hydroxide, the corresponding 6-substituted-purines (VII-X) were obtained.

An alternate synthetic route to VI was accomplished by the following sequence of reactions. 4-Amino-5-nitro-6-chloropyrimidine (XI) (15) was condensed with I under reaction condition analogous to that described above, to give 1,3-dihydroxy-2-(5-nitro-4-amino-6-pyrimidinylamino)cyclohexane (XII) in 90% yield. Reduction of the nitro group in XII was carried out by the modified method of Robins *et al.* (14) in which XII was treated with zinc powder in boiling water affording 1,3-dihydroxy-2-(4,5-diamino-6-pyrimidinylamino)cyclohexane (XIII) in 62% yield. Ring closure of XIII was carried out with ethyl

orthoformate or formamide to give VI in 46% or 53% yield, respectively.

When 4,6-dichloro-5-nitropyrimidine (XIV) was heated with I in boiling 2-methoxyethanol, 4,6-di(1,3-dihydroxy-2-cyclohexylamino)-5-nitropyrimidine (XV) was obtained in good yield, but the desired compound, 1,3-dihydroxy-2-(4-chloro-5-nitro-6-pyrimidinylamino)cyclohexane could not be obtained.

The biological activity of compounds VI-X was tested by spraying a test solution containing a sample of compound (1,000 mcg/ml) on leaves of rice plant previously inoculated with *Xanthomonas oryzae*, a pathogenic bacteria of leaf blight of rice plant. Among these compounds VI and VII showed a weak inhibitory effect against the microorganism, but their activities were weaker than those of Chloramphenicol (1,000 mcg/ml) and phenazine 5-oxide (100 mcg/ml) which were used as control compounds.

EXPERIMENTAL

Melting points were determined on a Mitamura Riken micro hot stage and uncorrected. The melting points marked with an asterisk were measured in a liquid bath and corrected. The infrared spectra (potassium bromide discs) were determined with a Hitachi EPI-2 spectrophotometer. The ultraviolet absorption spectra were determined in water with a Hitachi EPS-2 spectrophotometer.

trans-2-Amino-1,3-cyclohexanediol (I).

Cyclization of glutaraldehyde with nitromethane in the presence of sodium bicarbonate followed by catalytic hydrogenation afforded the product (11).

4,6-Dichloro-5-aminopyrimidine (II).

This compound was prepared by the method of D. J. Brown (12).

1,3-Dihydroxy-2-(5-amino-4-chloro-6-pyrimidinylamino)cyclohexane (III).

A mixture of 2.46 g. (15 mmoles) of II, 3.85 g. (29 mmoles) of I and 50 ml. of 1-butanol was heated with 3.0 g. of triethylamine. After being refluxed for 48 hours under a slow stream of nitrogen, the reaction mixture was evaporated *in vacuo*. The residue was dissolved in 35 ml. of hot water and then cooled to room temperature. A 2.86 g.-portion of the product was collected by filtration. The crude product was dissolved in hot water and decolorized with charcoal. The solution was again evaporated and the residue was recrystallized from hot water to give 2.04 g. (52.6% yield) of pale yellow crystals, m.p. 244-245.5°.

Anal. Calcd. for $C_{10}H_{15}ClN_4O_2$: C, 46.42; H, 5.84; N, 21.66. Found: C, 46.66; H, 5.97; N, 21.43.

1,3-Dihydroxy-2-(6-chloro-9-purinylo)cyclohexane (V).

A mixture of 1.0 g. of III and 28 ml. of ethyl orthoformate was heated for 48 hours under reflux and then 1.0 ml. of acetic anhydride was added to the solution. The mixture was heated for an additional 3 hours, and the solution was evaporated under reduced pressure. The residue was dissolved in 60 ml. of chloroform and the solution was passed through a short column of activated alumina. The solution was evaporated and the residue

was crystallized from *n*-hexane to give 0.41 g. (22.2% yield) of crystals, m.p. 72-73°. This compound was identified as 1,3-O-ethoxymethylidene-2-(6-chloro-9-purinylo)cyclohexane (IV).

Anal. Calcd. for $C_{14}H_{17}ClN_4O_3HC(OC_2H_5)_3$: C, 53.28; H, 6.97; N, 11.84. Found: C, 53.87; H, 7.33; N, 11.95.

The crystals of IV were dissolved in hot ethanol and evaporated under reduced pressure several times, followed by recrystallization from boiling water to give pale yellow needles, m.p. 234-237°. Additional product was obtained from the *n*-hexane mother liquor by an analogous procedure. The total yield was 50.2% (0.52 g.). UV λ max (pH 1) 266; (pH 7) 267; (pH 13) 259 μ .

Anal. Calcd. for $C_{11}H_{13}ClN_4O_2$: C, 49.17; H, 4.88; N, 20.85. Found: C, 49.21; H, 5.20; N, 20.51.

1,3-Dihydroxy-2-(6-amino-9-purinylo)cyclohexane (VI).

Method A.

A mixture of 538 mg. of V and 20 ml. of liquid ammonia was heated in an autoclave at 60° for 18 hours. Then an excess amount of ammonia was removed and the residual solid was recrystallized from 150 ml. of boiling water to give colorless crystals (235 mg.) of m.p. 285° (sublimation).

The mother liquor was evaporated under reduced pressure to give 101 mg. of the second crop of the product. The total yield was 67.5% (336 mg.). UV λ max (pH 1) 262; (pH 7) 263; (pH 13) 263 μ .

Anal. Calcd. for $C_{11}H_{15}N_5O_2$: C, 53.00; H, 6.07; N, 28.10. Found: C, 52.71; H, 6.37; N, 27.72.

Method B.

A 197 mg.-portion of XIII was heated in 13 g. of ethyl orthoformate under reflux for 40 hours and heating was then continued for an additional 3 hours with 1 ml. of acetic anhydride. The mixture was evaporated *in vacuo* and the residue was recrystallized from boiling water to give 94 mg. (45.8% yield) of the product, which was identified to be the compound produced by Method A by comparison of IR and UV spectra.

Method C.

A 1.00-g. portion of XIII was heated in 10 ml. of formamide under reflux for two and a half hours. After being cooled to room temperature, crystals formed which were collected by filtration, washed with ice-cold water and dried *in vacuo*. The product, 557 mg. (53.4% yield) was identified as VI by IR and UV spectra.

1,3-Dihydroxy-2-(6-methylamino-9-purinylo)cyclohexane (VII).

A mixture of 269 mg. of V, 14 ml. of 40% aqueous methylamine and 10 ml. of ethanol was heated under reflux for 3 hours. Evaporation of the mixture *in vacuo* produced a crystalline residue. The product was washed with 20 ml. of cold water to give 221 mg. (84.0% yield) of crystals, m.p. 256° (sublimation). A portion of the product was recrystallized from boiling water to give fine needles, m.p. 256° (sublimation). UV λ (pH 1) 264; (pH 7) 268; (pH 13) 268 μ .

Anal. Calcd. for $C_{12}H_{17}N_5O_2$: C, 54.74; H, 6.51; N, 26.60. Found: C, 54.40; H, 6.69; N, 26.21.

1,3-Dihydroxy-2-(6-dimethylamino-9-purinylo)cyclohexane (VIII).

A mixture of 235 mg. of V and 15 ml. of 20% aqueous dimethylamine was heated under reflux for 3 hours. Upon cooling to room temperature, crystals formed. The product was collected by filtration and washed with cold water; yield, 143 mg. (59.2%), m.p.* 248-248.5°.

The crude product was recrystallized from boiling water to

give an analytically pure sample which showed the same melting point. UV λ max (pH 1) 270; (pH 7) 278; (pH 13) 278 m μ .

Anal. Calcd. for C₁₃H₁₉N₅O₂: C, 56.30; H, 6.91; N, 25.26. Found: C, 55.77; H, 7.27; N, 24.98.

1,3-Dihydroxy-2-(6-mercapto-9-purinylo)cyclohexane (IX).

A mixture of 269 mg. of V and 77 mg. of thiourea was heated in 7 ml. of 1-butanol under reflux for 1 hour. The mixture was cooled in a refrigerator overnight and the product was collected by filtration. The crude product was recrystallized from boiling water to give 90 mg. (33.8% yield) of fibrous crystals, m.p. 300-310° (sublimation). UV λ max (pH 1) 350; (pH 7) 340; (pH 13) 312 m μ .

Anal. Calcd. for C₁₁H₁₄N₄O₂S: C, 49.62; H, 5.30; N, 21.04; S, 12.02. Found: C, 49.35; H, 5.49; N, 21.00; S, 11.62.

1,3-Dihydroxy-2-(6-hydroxy-9-purinylo)cyclohexane (X).

To a mixture of 156 mg. of V and 10 ml. of water was added 2 ml. of 1 N sodium hydroxide and the mixture was heated under reflux for 4 hours. The reaction mixture was cooled in an ice bath and the pH of the solution was adjusted to 4 by the addition of glacial acetic acid. The crystals which formed were collected by filtration and dried. Yield, 64 mg. (44.3%), m.p. 343° (sublimation). UV λ max (pH 1) 252; (pH 7) 251; (pH 13) 256 m μ .

Anal. Calcd. for C₁₁H₁₄N₄O₃: C, 52.79; H, 5.64; N, 22.39. Found: C, 52.60; H, 6.01; N, 21.94.

4-Amino-5-nitro-6-chloropyrimidine (XI).

This compound was prepared by the method of Boon and coworkers (15).

1,3-Dihydroxy-2-(5-nitro-4-amino-6-pyrimidinylamino)cyclohexane (XII).

A mixture of 1.55 g. (10 mmoles) of XI, 2.64 g. (20 mmoles) of I, 2.0 g. of triethylamine and 40 ml. of 2-methoxyethanol was heated to boiling under a slow stream of nitrogen for 2 hours. Then the mixture was cooled to room temperature and the precipitate which formed was collected by filtration to give 2.16 g. (90.4% yield) of a crude product, m.p. 260° (sublimation). Recrystallization of the crude product from 2-methoxyethanol afforded crystals which gave the same melting point.

Anal. Calcd. for C₁₀H₁₅N₅O₄: C, 44.60; H, 5.62; N, 26.01. Found: C, 44.85; H, 5.84; N, 25.54.

1,3-Dihydroxy-2-(4,5-diamino-6-pyrimidinylamino)cyclohexane (XIII).

A 1.5 g.-portion of XII was added to 50 ml. of boiling water in which 10 g. of zinc powder was suspended. After XII had completely dissolved, the hot solution was filtered, and the filtrate was evaporated under reduced pressure. The residue was recrystallized from ethanol to give 822 mg. of yellow needles (61.7% yield), m.p. 229-232°.

The crude product was dissolved in methanol and 4 N sulfuric acid was added to the solution to pH 3. The sulfate crystallized from the solution and was collected by filtration. Recrystal-

lization from methanol gave a sample for analysis, m.p. 150-152°.

Anal. Calcd. for C₁₀H₁₇N₅O₂·H₂SO₄·H₂O: C, 33.76; H, 5.96; N, 19.71. Found: C, 33.75; H, 6.16; N, 19.46.

4,6-Di(1,3-dehydroxy-2-cyclohexylamino)-5-nitropyrimidine (XV).

A mixture of 0.97 g. of 4,6-dichloro-5-nitropyrimidine (XIV) (15), 1.0 g. of I and 40 ml. of 2-methoxyethanol was heated under reflux with 1.3 ml. of triethylamine for 2 hours. The reaction solution, upon cooling to room temperature, gave crystals which were collected by filtration and washed with water. The crude product was recrystallized from boiling water to give 0.75 g. (51.4% yield) of fine crystals, m.p. 278-280° dec.

Anal. Calcd. for C₁₆H₂₅N₅O₆: C, 50.12; H, 6.57; N, 18.27. Found: C, 50.26; H, 6.62; N, 18.14.

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