Enzyme Inhibitors II

Synthesis of *trans*-3-(6-Substituted-9-purinyl)cyclohexanols as Adenosine Deaminase Inhibitors

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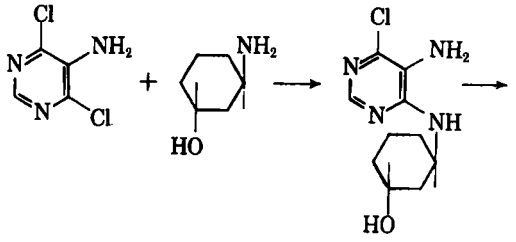
The syntheses of *trans*- and *cis*-3-[9-(6-chloropurinyl)] cyclohexanols (IV and XI) have been accomplished by condensation of 5-amino-4,6-dichloropyrimidine with *trans*- and *cis*-3-aminocyclohexanol followed by ring closure of the resultant pyrimidine to the desired purines. Several 6-substituted analogs of IV and XI were prepared by nucleophilic displacement of the 6-chloro group. Evaluation of the resultant isosteric nucleosides as inhibitors of adenosine deaminase revealed that those compounds with a 6-amino group were inhibitory and that the 3′-hydroxyl group, whether *trans* or *cis*, is not involved in binding to the enzyme.

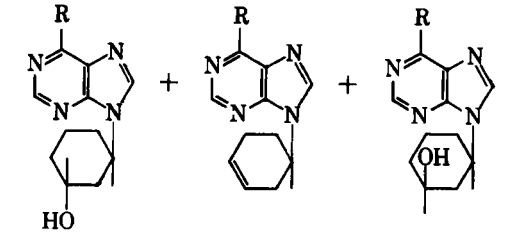
PURINE NUCLEOSIDES are utilized as substrates by a variety of enzymes, but few studies have been made to determine which of the atoms and functional groups of the nucleoside are important for binding to the enzyme. Recently, a number of nucleoside antibiotics has been discovered which are capable of inhibiting certain enzymatic reactions. For example, psicofuranine has been shown to be an inhibitor of the enzyme, xanthosine-5′-phosphate aminase (1, 2). In addition, the enzyme, adenosine phosphorylase, has been shown to be inhibited by the antibiotic, tubercidin (3). Both of these antibiotics are 6-aminopurines in which an unusual sugar is present at the 9-position.

The authors have been interested in the chemistry and biochemistry of purine nucleosides but have been concerned with the ease by which the ribose moiety can be removed hydrolytically and enzymatically from the purine nucleus. Consequently, we have been interested in preparing compounds which resemble nucleosides sterically but which are presumably stable to hydrolytic or enzymatic cleavage. These compounds, the isosteric nucleosides, are purines which are substituted at the 9-position by a cycloaliphatic group, which in turn may be hydroxylated so that it resembles the carbohydrate moiety of a nucleoside (4, 5). We have previously described the results of the enzymatic evaluation using adenosine deaminase with those isosteric nucleosides which were substituted at the 9-position of the purine nucleus with cyclopentyl, *cis*- and *trans*-2-hydroxycyclopentyl and *cis*- and *trans*-2-hydroxycyclohexyl groups (6). The present paper describes the synthesis and evaluation of *trans*-3-(6-substituted-9-purinyl)-cyclohexanols as inhibitors of adenosine deaminase.

Chemistry.—The method which was selected for the synthesis of the desired purines is a modification of the elegant method of Montgomery and Temple (7). This method involves the condensation of 5-amino-4,6-dichloropyrimidine (I) with an appropriate amine, followed by ring closure of the condensation product to give the appropriate purine. This method is particularly valuable when it is



I II III



IV, R=Cl
V, R=NH_2
VI, R=NHMe
VII, R=NMe_2
VIII, R=$NHNH_2$
IX, R=SH

X, R=Cl

XI, R=Cl
XII, R=NH_2

desired to synthesize 6-chloro-9-substituted purines uncontaminated by the corresponding 6-chloro-7-substituted isomer, which would result by alkylation of 6-chloropurine. Thus, catalytic hydrogenation of *m*-acetamidophenol with a rhodium-on-alumina catalyst gave as the major product *trans*-3-acetamidocyclohexanol, which was separated from the *cis* isomer by fractional crystallization (8, 9).

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Saponification of the *trans* isomer gave the desired *trans*-3-aminocyclohexanol (II) (9), which on condensation with I in the presence of triethylamine gave the substituted pyrimidine (III) in good yield. Attempted cyclization of III with diethoxymethyl acetate resulted in considerable decomposition, even though this appears to be the reagent of choice for related purine syntheses (7). Finally, when III was allowed to react with ethyl orthoformate and the crude reaction product chromatographed on neutral alumina, a 35% yield of IV was obtained along with a 20% yield of the dehydrated product X. The identity of X was established by its conversion into 9-cyclohexylpurine (10). Furthermore, since 6-chloro-9-(2-cyclohexenyl) purine is a known compound (4) and is different from X, the double bond in X must be at the 3-position of the cyclohexenyl ring. In a subsequent preparation of IV, in which a crude sample of II had been employed in addition to the two products previously isolated (IV and X), a third product (XI) was obtained which, on the basis of its elemental analysis, was isomeric with IV. Consequently, it became necessary to establish the stereochemistry of the two products (IV and XI) since they were to be used to evaluate the mode of binding to adenosine deaminase. The assignment of stereochemistry was done by both I.R. and NMR analyses. It was observed that the infrared spectrum of XI exhibited strong absorption at 1060 cm.$^{-1}$, whereas IV was essentially free of absorption in this region. It is known that compounds with an equatorial hydroxyl absorb near 1050 cm.$^{-1}$, whereas compounds with axial hydroxyl exhibit little or no absorption in that region (11). It is reasonable to assume that the *cis* purine would have its substituents diequatorial; whereas in the *trans* compound the bulky purine nucleus would occupy the equatorial position which would force the hydroxyl group to occupy the axial position. Therefore, the purine which exhibits absorption at 1060 cm.$^{-1}$ (XI) is *cis*, and the compound which is free of absorption in that region (IV) is *trans*. An identical assignment of configuration was made on the basis of the NMR spectra. Several investigators have found that the NMR signal of the hydrogen on the carbon atom bearing the hydroxyl group in a cyclohexanol exhibits a half-width of 7 cps. if the hydrogen is equatorial, but exhibits a half-width of 22 cps. if the hydrogen is axial (12–14) and that the signal from an equatorial hydrogen is approximately 0.5 p.p.m. downfield from the axial hydrogen (15). In our investigation, one compound (IV) exhibited a mutiplet at δ4.51 with a half-width of 8 cps. for the hydrogen on the carbon atom bearing the hydroxyl group (C_3'), whereas the other compound (XI) exhibited a multiplet at δ3.92 with a half-width of 23 cps. for the corresponding hydrogen. Clearly this establishes that the hydrogen at C_3' in IV is equatorial, whereas the hydrogen at C_3' in XI is axial. In addition, in both compounds the NMR signal for the hydrogen at C_1' was a multiplet with a half-width of 22 cps. These results establish that in both IV and XI the hydrogen at C_1' is axial (12–14); consequently, the purine nucleus must occupy the equatorial position. These results establish that in IV the hydroxyl and purine groups are *trans*, whereas in XI these groups are *cis*.

The key intermediates, IV and XI, were individually converted into their corresponding 6-substituted derivatives by modification of the known procedures; the experimental details of these transformations are described under *Experimental*.

EXPERIMENTAL[1]

trans-3-(5-Amino-6-chloro-4-pyrimidinylamino)cyclohexanol (III).—A solution of 5.05 Gm. (31.0 mmoles) of 5-amino-4,6-dichloropyrimidine, 3.58 Gm. (31.1 mmoles) of *trans*-3-aminocyclohexanol,[2] and 3.10 Gm. (31.1 mmoles) of triethylamine in 46 ml. of *n*-butyl alcohol was heated under reflux for 6.5 hours, then the volatile materials were removed *in vacuo*. The residue was triturated with 20 ml. of water, and the resultant solid was collected by filtration. Recrystallization of the crude product from aqueous methanol gave 5.8 Gm. (77%) of the desired product, m.p. 228–230°. For analysis, a small sample was recrystallized from aqueous methanol, m.p. 229–231°.

Anal.[3]—Calcd. for $C_{10}H_{15}ClN_4O$: C, 49.50; H, 6.23; N, 23.08. Found: C, 49.39; H, 6.41; N, 23.01.

Cyclization of trans-3-(5-Amino-6-chloro-4-pyrimidinylamino)cyclohexanol with Triethyl Orthoformate.—A mixture of 2.0 Gm. (8.2 mmoles) of III and 25 ml. of triethyl orthoformate was heated under reflux for 48 hours, then the volatile materials were removed *in vacuo*. The residue was allowed to react at 0° with 25 ml. of a 20% solution of ammonia in methanol for 20 hours. Concentration of the mixture gave a viscous residue which could not be crystallized. Consequently, it was chromatographed on neutral alumina (Grade I, 40 Gm.). Elution of the column with 100 ml. of benzene gave a fraction which crystallized from ethyl acetate; yield, 0.42 Gm. (20%), m.p. 152–155°. Two recrystallizations of the crude material from ethyl acetate gave the analytical sample, which has been identified as 6-chloro-9-(3-cyclohexenyl)-purine (X), m.p. 162°; $\lambda_{max.}^{pH1}$ 265 (ϵ, 7,820); $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3150 (C=C); 1590 and 1560 (C=C and C=N).

Anal.—Calcd. for $C_{11}H_{11}ClN_4$: C, 56.29; H, 4.73; N, 23.87. Found: C, 56.17; H, 4.80; N, 23.80.

Elution of the alumina column with an additional 100 ml. of benzene followed by 75 ml. of chloroform gave a small amount of resinous substance which was discarded. Subsequent elution of the column with chloroform (300 ml.) and evaporation of the eluate gave a residue which, on trituration with ethyl acetate, gave a colorless crystalline solid; yield, 0.70 Gm. (35%), m.p. 150–155°. Several recrystallizations of the crude product from benzene gave the analytical sample of *trans*-3-(6-chloro-9-purinyl)cyclohexanol (IV), m.p. 159–160°; $\lambda_{max.}^{pH1}$ 264 (ϵ, 9,760); $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3350(OH); 1590 and 1560 (C=C and C=N).

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[1] The infrared spectra were determined on a Perkin-Elmer model 137 spectrophotometer; the ultraviolet spectra and enzyme rates were determined on a Perkin-Elmer model 4000 A spectrophotometer. The melting points were determined on a Kofler Heizbank and are corrected.

[2] This compound was prepared by the catalytic hydrogenation of *m*-acetamidophenol essentially by the procedure described in the literature (9), except that a 5% rhodium-on-alumina catalyst was employed.

[3] Galbraith Microanalytical Laboratories, Knoxville, Tenn.

Anal.—Calcd. for $C_{11}H_{13}ClN_4O$: C, 52.28; H, 5.18; N, 22.17. Found: C, 52.46; H, 5.30; N, 21.93.

In a larger experiment which employed crude II, a third compound was eluted in a 10% yield from the alumina column after IV and X were removed. Recrystallization of this crude product gave *cis*-3-(6-chloro-9-purinyl)cyclohexanol (XI), m.p. 190°; $\lambda_{max.}^{pHl}$ 265 (ϵ, 12,400); $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3350 (OH); 1590 and 1560 (C=C and C=N); 1060 (C—O—H).

Anal.—Calcd. for $C_{11}H_{13}ClNO$: C, 52.28; H, 5.18; N, 22.17. Found: C, 52.39; H, 5.29; N, 22.32.

trans-3-(6-Amino-9-purinyl)cyclohexanol (V).—A solution of 247 mg. (0.98 mmole) of IV in 5 ml. of liquid ammonia was heated in a stainless steel bomb at 55° for 16 hours. The ammonia was allowed to evaporate at room temperature, and the residue was extracted with chloroform. Evaporation of the chloroform solution gave 224 mg. (98%) of crystalline solid, m.p. 238°. Recrystallization of the crude product from aqueous ethanol, then from absolute ethanol, gave the analytical material, m.p. 241–243°; $\lambda_{max.}^{pHl}$ 259 (ϵ, 15,550); $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3400 (OH and NH_2); 1655 (NH_2); 1590 and 1550 (C=C and C=N).

Anal.—Calcd. for $C_{11}H_{15}N_5O$: C, 56.63; H, 6.48; N, 30.03. Found: C, 56.57; H, 6.60; N, 30.08.

trans-3-(6-Methylamino-9-purinyl)cyclohexanol (VI).—A solution of 200 mg. (0.79 mmole) of IV in 5 ml. of ethanol and 5 ml. of 40% aqueous methylamine was heated under reflux for 2 hours, then evaporated to dryness *in vacuo*. The residue was extracted with chloroform; the insoluble methylamine hydrochloride, m.p. 228°, was removed by filtration. The residue, obtained after evaporation of the filtrate, on crystallization from benzene gave 165 mg. (84.6%) of the product, m.p. 173–175°. Recrystallization of the crude product from chloroform–benzene mixture gave pure VI, m.p. 175°; $\lambda_{max.}^{pHl}$ 264 (ϵ, 15,300); $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3300 (OH and NH); 1650 (NH); 1575 and 1530 (C=C and C=N).

Anal.—Calcd. for $C_{12}H_{17}N_5O$: C, 58.27; H, 6.92; N, 27.58. Found: C, 58.12; H, 7.04; N, 27.45.

trans-3-(6-Dimethylamino-9-purinyl)cyclohexanol (VII).—A solution of 150 mg. (0.62 mmole) of IV in 5 ml. of ethanol and an equal volume of aqueous 25% dimethylamine was heated under reflux for 2 hours, and the mixture was evaporated to dryness *in vacuo*. An aqueous solution (5 ml.) of the residue was stirred with 2 Gm. of Dowex 1-X2 resin in CO_3 form for 2 hours. The resin was removed by filtration, and the aqueous filtrate was evaporated *in vacuo* to give a solid residue which, after several crystallizations from benzene, gave pure VII, m.p. 156°; yield, 78 mg. (48%); $\lambda_{max.}^{pHl}$ 269 (ϵ, 15,660); $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3300 (OH); 1590 and 1550 (C=C and C=N).

Anal.—Calcd. for $C_{13}H_{19}N_5O$: C, 59.75; H, 7.33; N, 26.80. Found: C, 59.65; H, 7.35; N, 26.90.

trans-3-(6-Hydrazino-9-purinyl)cyclohexanol (VIII).—To 1.5 ml. of hydrazine (95%) was added over a period of 2 minutes 0.17 Gm. (0.67 mmole) of IV. Initially, the mixture was cooled in an ice-bath, then stirred at room temperature for 2 hours. The residue, obtained after removing the volatile materials *in vacuo*, was repeatedly digested with small volumes of chloroform to extract the desired product from the insoluble hydrazine hydrochloride. The combined chloroform extracts on evaporation yielded 100 mg. (60.2%) of the crude product, which after two crystallizations from a methanol–ethyl acetate mixture gave pure VIII, m.p. 175° dec. $\lambda_{max.}^{pHl}$ 263 (ϵ, 12,500); $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3300 (OH); 1625 (NH_2); 1575 and 1525 (C=C and C=N).

Anal.—Calcd for $C_{11}H_{16}N_6O$: C, 52.26; H, 6.58; N, 33.25. Found: C, 52.50; H, 6.64; N, 33.37.

trans-3-(6-Mercapto-9-purinyl)cyclohexanol (IX).—A solution of 252 mg. (1.00 mmole) of IV and 76.1 mg. (1.00 mmole) of thiourea in 5 ml. of *n*-propyl alcohol was heated under reflux for 1 hour. The product precipitated from the reaction mixture after a short time of refluxing. After cooling, the solid was collected by filtration, washed with cold *n*-propyl alcohol, and dried, m.p. 340–350°; yield, 230 mg. (95%). A portion of the product was crystallized from a large amount of a cellosolve and methanol mixture to give pure IX, m.p. 345–350°; $\lambda_{max.}^{pHl}$ 227 (ϵ, 8,750) and 325 (ϵ, 17,500); $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3450 (OH); 1595 and 1530 (C=C and C=N).

Anal.—Calcd for $C_{11}H_{14}N_4OS$: C, 52.78; H, 5.64; N, 22.4. Found: C, 52.46; H, 5.50; N, 22.47.

cis-3-(6-Amino-9-purinyl)-cyclohexanol (XII).—A solution of 200 mg. (0.79 mmole) of XI in 10 ml. of methanol saturated with ammonia was heated in a stainless steel bomb at 55° for 20 hours. The volatile materials were removed *in vacuo*, and the crystalline residue was then extracted with hot chloroform (5 × 10 ml.). The solid residue, obtained on evaporating the chloroform solution, was crystallized from methanol to yield 100 mg. (54%) of the desired product, m.p. 215–218°. Two recrystallizations from methanol gave the analytically pure *cis*-3-(6-amino-9-purinyl)-cyclohexanol, m.p. 222°; $\lambda_{max.}^{pHl}$ 258.5 (ϵ, 16,050); $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3375, 3225 (OH and NH_2); 1655 (NH_2); 1600 and 1560 (C=C and C=N).

Anal.—Calcd for $C_{11}H_{15}N_6O$: C, 56.63; H, 6.48; N, 30.03. Found: C, 56.70; H, 6.56; N, 29.83.

9-Cyclohexylpurine.—A small sample of X was hydrogenated with a palladium-on-charcoal catalyst in ethanol in the presence of magnesium oxide. The product which was obtained, m.p. 92°, was identical to 9-cyclohexyl-purine, which has previously been reported in the literature (10).

Reagents and Assay Procedure

Adenosine and adenosine deaminase were purchased from the Sigma Chemical Co. The general method of assay employed is described in Colowick and Kaplan (16) and involves measuring the rate of disappearance of the absorption band of adenosine at 265 mμ. All reactions were run in 0.05 *M* phosphate buffer at pH 7.6 at 25°. The stock solutions of all reagents were prepared in 0.05 *M* phosphate buffer at pH 7.6. For the assay, the cell contained a total volume of 3.1 ml., which was 0.066 m*M* with respect to adenosine. In those cases where inhibition was studied, varying amounts of the buffer were replaced by the appropriate volume of a solution of the inhibitor in buffer. A sufficient amount of enzyme was used, so that the initial rate of reaction gave a change of approximately 0.8–1.0 absorbance units per minute.

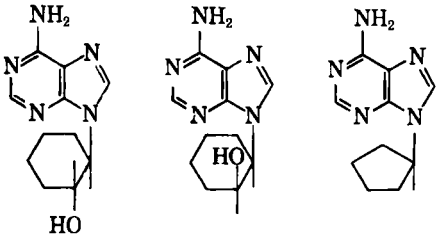
TABLE I.—PARTIAL INHIBITION OF ADENOSINE DEAMINASE BY ISOSTERIC NUCLEOSIDES

Compd.	Concn., m*M*[a]	*Vo/V*	% Inhibition
V	0.12	1.14	13
XIII	0.12	1.64	39
XII	0.12	1.14	13
XIV	0.12	1.28	22
XV	0.12	1.16	14

[a] The concentration of adenosine in all experiments was 0.066 m*M*.

RESULTS

When the isosteric nucleosides were tested as inhibitors of adenosine deaminase, two of the compounds were inhibitory (V and XII); the remaining compounds, when tested at concentrations three to four times that of the substrate, were essentially noninhibitory. A plot of *Vo/V versus I*, where *Vo* = initial velocity of the enzymic reaction in the absence of inhibitor, *V* = initial velocity of the enzymic reaction in the presence of inhibitor, and *I* = concentration of inhibitor (17) for V and XII, revealed a nonlinear relationship of *Vo/V* to the concentration of inhibitor. Consequently, V and XII were not evaluated by this technique; rather the percentage inhibition of a 0.12 m*M* solution of inhibitor was compared to the percentage inhibition of a 0.12 m*M* solution of a close structural analog. Thus, V and XII were compared to *trans*-2-(6-amino-9-purinyl) cyclohexanol (XIII), *cis*-2-(6-amino-9-purinyl) cyclohexanol (XIV), and (6-amino-9-purinyl)-cyclopentane (XV). The results of such a comparison are given in Table I.



XIII XIV XV

DISCUSSION

In some earlier work on isosteric nucleosides as inhibitors of adenosine deaminase, in which the purine nucleus was substituted at the 9-position by cyclopentyl, *cis*- and *trans*-2-hydroxycyclopentyl, and *cis*- and *trans*-2-hydroxycyclohexyl groups, those compounds with a 6-amino group were the most effective inhibitors of the enzymatic reaction (6). In addition, the 2′-hydroxyl group of the isosteric nucleosides makes a contribution to binding since the 2′-hydroxy compounds were more effective inhibitors than the cyclopentyl compound. The isosteric nucleosides with a *trans*-2′-hydroxyl group were slightly more effective inhibitors than those compounds with a *cis*-2′-hydroxyl group (6).

In the present work, we have also observed that inhibition of adenosine deaminase occurred only with those compounds that contained an amino group at the 6-position of the purine nucleus (V and XII). It should be noted, however, that V and XII are considerably less potent as inhibitors of adenosine deaminase than XIII and XIV (see Table I). A comparison of the data shows that the compound with the *trans*-2′-hydroxyl group (XIII) is a better inhibitor than the compound with the *cis*-2′-hydroxyl group (XIV) and that both are better than the compounds with the *cis*- and *trans*-3′-hydroxyl groups (V and XII). Furthermore, it can be seen that V and XII are essentially identical to the 9-cyclopentyl compound (XV) in their inhibitory ability. Therefore, these results establish that there is little or no contribution of the 3′-hydroxyl group to the binding of isosteric nucleosides to the enzyme. If one assumes that the inhibitor binds at the same place on the enzyme to which the substrate binds, then the 3′-hydroxyl group of adenosine offers little to the binding of substrate to the enzyme.

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