Enzyme Inhibitors III

Syntheses of *cis*-(6-Substituted-9-purinyl)cycloalkylcarbinols as Adenosine Deaminase Inhibitors

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The syntheses of *cis*-4-(6-chloro-9-purinyl)cyclohexylcarbinol (VI) and *cis*-3-(6-chloro-9-purinyl)cyclopentylcarbinol (XVII) have been accomplished by the condensation of 5-amino-4,6-dichloropyrimidine with the appropriate amino alcohol, followed by ring closure of the resultant substituted pyrimidine to give the desired purines. Nucleophilic displacement of the 6-chloro group in VI and XVII gave the following 6-substituted derivatives: (*a*) hydroxy, (*b*) mercapto, (*c*) amino, (*d*) methylamino, and (*e*) dimethylamino. Evaluation of these substrate analogs as inhibitors of adenosine deaminase revealed that those compounds with an amino or methylamino group at the 6-position of the purine nucleus were inhibitory and that the hydroxymethyl group on the cyclopentyl or cyclohexyl nucleus makes only a small contribution to binding of the inhibitor to the enzyme.

THE UTILIZATION of chemicals for the treatment of disease has been the goal of much research since ancient times. During the past 20 years, tremendous progress has been made in our understanding of cellular chemistry and biochemistry. For example, in the area of purine metabolism the biosyntheses and many cellular transformations of purines, their nucleosides, and nucleotides have been elucidated (1). In cancer chemotherapy it is apparent that one of the areas of fruitful investigation is the inhibition of one or more stages of purine biosynthesis or purine transformations.

Many important enzymes utilize purine nucleosides or nucleotides as their normal substrates, but the mode of binding of these compounds to the various enzymes has not been studied extensively. Because of the interest in determining which atoms and functional groups of purine nucleosides and nucleotides are important in binding to various enzymes, the authors have begun a study of the inhibition of certain enzymes by a variety of substrate analogs. As the first enzyme for such an investigation, adenosine deaminase has been selected. Previous studies on the inhibition of adenosine deaminase by purine nucleoside analogs gave information concerning the importance to binding by the 2′- and the 3′-hydroxyl group of the ribose moiety and the types of groups which are necessary at the 6-position of the purine nucleus for binding to the enzyme (2, 3). This paper describes the synthesis and enzymatic evaluation of *cis*-3-(6-substituted-9-purinyl)cyclopentylcarbinols and *cis*-4-(6-substituted-9-purinyl)cyclohexylcarbinols as inhibitors of adenosine deaminase.

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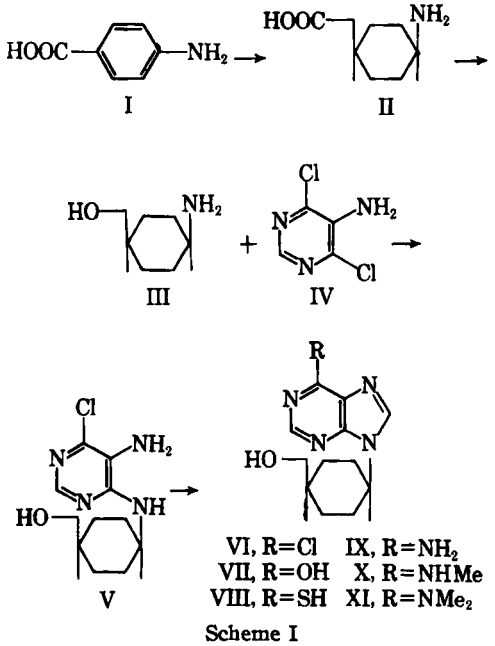
Chemistry

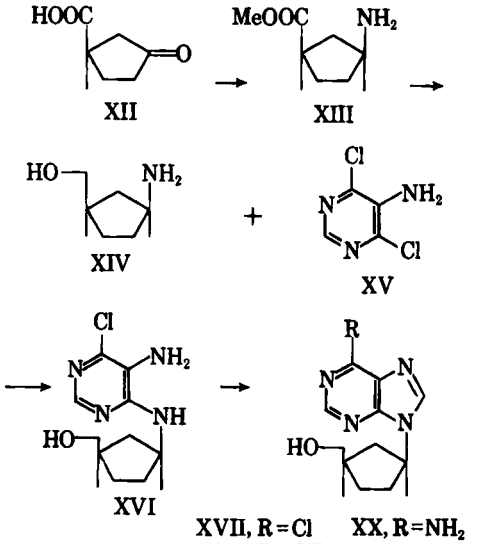
The general synthetic procedure for the synthesis of the desired compounds is a modification of the method of Montgomery and Temple (4) and involves the preparation of a 6-chloro-9-substituted purine, which can be converted into a variety of 6-substituted analogs by reaction with appropriate nucleophilic reagents. The synthesis of the 6-chloro-9-substituted purines involved the preparation of an appropriate hydroxymethylcycloalkylamine which, on condensation with 5-amino-4,6-dichloropyrimidine followed by ring closure, generated the desired purine. For the synthesis of the hydroxymethylcyclohexylpurines (Scheme I), *p*-aminobenzoic acid (I) was reduced by catalytic hydrogenation to *cis*-4-carboxycyclohexylamine (II) by a modification of the literature procedure (5, 6). Conversion of II into its ethyl ester, followed by reduction with lith-



Scheme I

ium aluminum hydride, gave the desired *cis*-4-hydroxymethylcyclohexylamine (III). Condensation of III with 5-amino-4,6-dichloropyrimidine (IV) resulted in the formation of V which, on reaction with triethyl orthoformate and acetic anhydride, gave *cis*-4-(6-chloro-9-purinyl)cyclohexylcarbinol (VI) in moderate yield; the 6-hydroxy analog (VII) was also formed during the cyclization of V. Nucleophilic displacement of the 6-chloro group of VI by thiourea, ammonia, methylamine, and dimethylamine gave the corresponding 6-substituted compounds (VIII–XI) in good yields.

The synthesis of the hydroxymethylcyclopentylpurines is outlined in Scheme II. 3-Oxocyclopentanecarboxylic acid (XII) (7) was converted to its oxime which, on catalytic hydrogenation, gave *cis*-3-carboxycyclopentylamine (8). Esterification of the acid gave the corresponding methyl ester (XIII). Conversion of XIII to XIV was accomplished by reduction with lithium aluminum hydride. Condensation of *cis*-3-hydroxymethylcyclopentylamine (XIV) with XV gave in good yield the desired pyrimidine (XVI), which underwent ring closure with

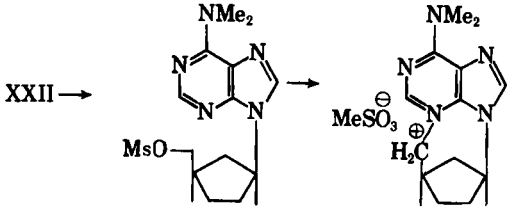


XVII, R=Cl XX, R=NH_2
XVIII, R=OH XXI, R=NHMe
XIX, R=SH XXII, R=NMe_2

Scheme II

triethyl orthoformate and acetic anhydride to generate the key intermediate (XVII). This intermediate was converted to its 6-substituted derivatives by modification of known procedures; the details are described under *Experimental*.

Concerning the stereochemistry of the hydroxymethylcyclopentylpurines (XVII–XXII), it is believed that the substituents on the cyclopentyl nucleus are *cis*. Our assignment of the *cis* configuration is based on the following observations. When the 6-dimethylamino derivative (XXII) was allowed to react with methanesulfonyl chloride in pyridine solution, a product was obtained which possessed the characteristic spectral properties of the methanesulfonate (XXIII). When XXIII was heated under reflux in an acetone or chloroform solution, a new isomeric product was isolated which, in contrast to XXIII, was soluble in water and insoluble in acetone or chloroform. In addition, the ultraviolet spectrum of XXIV exhibited a maximum at 291 mμ, whereas XXII and XXIII exhibited maxima at 270 and 272 mμ, respectively, in acidic solution. The saltlike character of the product and the shift in the ultraviolet maximum can be readily explained on the basis of the quaternary salt (XXIV). In the



XXIII XXIV

nucleoside area, Clark, *et al.* were the first to prepare cyclo-nucleosides and stated that only those ribonucleosides with a β-configuration (*i.e.*, the purine and the hydroxymethyl groups are *cis*) are capable of forming the quaternary salt (9). Later, Baker and Joseph prepared a similar quaternary salt using an appropriately blocked derivative of puromycin (10). Based on these results, it is logical to assign a *cis* relationship of the hydroxymethyl group and the purine nucleus in the cyclopentyl analogs XVII–XXII.

EXPERIMENTAL[1]

cis-4-Hydroxymethylcyclohexylamine (III).—A solution of 976 mg. (4.69 mmoles) of *cis*-4-ethoxycarbonylcyclohexylamine hydrochloride (11) in 3 ml. of water was stirred at room temperature with 10 ml. of a saturated solution of sodium bicarbonate for 30 minutes. The mixture was extracted with chloroform (5 × 10 ml.); the combined extracts were dried over sodium sulfate and concentrated *in vacuo*. The crude *cis*-4-ethoxycarbonylcyclohexylamine (650 mg., 3.82 mmoles) was dissolved in 10 ml. of ether and slowly added to a suspension of 220 mg. (5.8 mmoles) of lithium aluminium hydride in 20 ml. of ether at 0°. The mixture was stirred for 1 hour at 0°, then heated under reflux for 1 additional hour. The excess lithium aluminium hydride was destroyed with ether saturated with water (20 ml.) and then with water (4–5 drops). The ether layer was decanted, and the solid inorganic residue was triturated with chloroform (2 × 15 ml.) and filtered. The combined organic extracts were dried over sodium sulfate, concentrated *in vacuo*, and gave on distillation 317 mg. (58%) of III, b.p. 84–85°/0.5 mm. $\bar{\nu}$ in $cm.^{-1}$: 3400–3300 (OH and NH_2).

Anal.[2]—Calcd. for $C_7H_{15}NO$: C, 65.07; H, 11.70; N, 10.84. Found: C, 64.83; H, 11.58; N, 10.65.

The hydrochloride of III, prepared in the usual manner, was obtained as a white crystalline compound in a 78% yield, m.p. 164–166°.

Anal.—Calcd. for $C_7H_{16}ClNO$: C, 50.80; H, 9.67; Cl, 21.40; N, 8.45. Found: C, 50.55; H, 9.81; Cl, 21.27; N, 8.40.

[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] The analyses reported in this paper were performed by Galbraith Microanalytical Laboratories, Knoxville, Tenn.

cis - 4 - (5 - Amino - 6 - chloro - 4 - pyrimidinylamino)cyclohexylcarbinol (V).—A solution of 9.03 Gm. (70 mmoles) of III, 10.90 Gm. (66.5 mmoles) of 5-amino-4,6-dichloropyrimidine (IV), and 9.0 Gm. (89 mmoles) of triethylamine in 120 ml. of *n*-butanol was heated under reflux for 16 hours; then the volatile materials were removed *in vacuo*. The residue on crystallization from water, gave a yellowish solid (V); yield, 8.36 Gm. (48.4%). One recrystallization of the crude product from water gave the analytical sample, m.p. 201°. $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-3}$): pH 1, 305 (12.8); pH 7, 290 (8.83), 262 (8.69); pH 13, 290 (9.47), 262 (9.32). $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3420 (OH), 3290 (NH_2).

Anal.—Calcd. for $C_{11}H_{17}ClN_4O$: C, 51.46; H, 6.68; Cl, 13.82; N, 21.82. Found: C, 51.56; H, 6.69; Cl, 14.01; N, 21.74.

cis-4-(6-Chloro-9-purinyl)cyclohexylcarbinol (VI).—A solution of V (1.005 Gm., 3.91 mmoles) in 30 ml. of triethyl orthoformate was refluxed for 48 hours. Acetic anhydride (0.8 ml.) was then added to the solution, and the mixture was refluxed for an additional 3 hours. The volatile materials were removed *in vacuo;* the residue (1.18 Gm.) was treated with methanolic ammonia (20%, 15 ml.) at 0° overnight, after which time the volatile materials were removed *in vacuo* at 25°. The residue was dissolved in chloroform (30 ml.), washed with water (3 × 5 ml.), dried with sodium sulfate, and concentrated to dryness. The crude product (VI, 998 mg.) was crystallized from chloroform-hexane; yield, 507 mg. (48.5%), m.p. 160°. Recrystallization of the crude VI from chloroform-hexane gave the analytical sample, m.p. 166°. $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-3}$) pH 1, 264 (9.78); pH 7, 264 (9.96); pH 13, 266 (9.78). $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3400 (OH); 1590, 1560 (C=C and C=N).

Anal.—Calcd. for $C_{12}H_{15}ClN_4O$: C, 54.03; H, 5.67; Cl, 13.29; N, 21.01. Found: C, 54.25; H, 5.46; Cl, 13.42; N, 21.17.

The aqueous washings obtained after the methanolic ammonia treatment were concentrated to dryness. The residue (170 mg.) was dissolved in aqueous sodium hydroxide (0.1 *N*, 10 ml.), decolorized with charcoal, and acidified to pH 3 with glacial acetic acid. On cooling, a crystalline material was obtained, collected by filtration, and washed with cold water. Recrystallization of the crude material from water gave the analytical sample of VII, m.p. 326–328°. $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-3}$): pH 1, 250 (11.65); pH 7, 251 (10.87); pH 13, 254 (12.50). $\bar{\nu}$ in cm.$^{-1}$: 3400 (OH), 1680 (C=O); 1590, 1550 (C=C and C=N).

Anal.—Calcd. for $C_{12}H_{16}N_4O_2$: C, 58.04; H, 6.49; N, 22.56. Found: C, 58.11; H, 6.39; N, 22.33.

cis - 4 - (6 - Mercapto - 9 - purinyl)cyclohexylcarbinol (VIII).—A solution of 153 mg. (0.576 mmoles) of VI and 40 mg. (0.526 mmoles) of thiourea in 4 ml. of *n*-propanol was heated under reflux for 1 hour, then cooled in an ice bath. The solid which precipitated was collected by filtration, washed with cold *n*-propanol (3 ml.), and dried; yield, 134 mg. (96%), m.p. 320°. Two crystallizations of the crude material from aqueous methyl cellosolve gave the analytical sample (VIII), m.p. 327–327.5° dec. $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-3}$): pH 1, 227 (7.98), 325 (20.0); pH 13, 232 (13.7), 311 (22.3). $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3370 (OH); 2800–2000 (acidic hydrogen); 1585 and 1525 (C=C and C=N).

Anal.—Calcd. for $C_{12}H_{16}N_4OS$: C, 54.50; H, 6.10; N, 21.18; S, 12.13. Found: C, 54.23; H, 6.18; N, 20.99; S, 12.27.

cis-4-(6-Amino-9-purinyl)cyclohexylcarbinol (IX).—A mixture of 286 mg. (1.08 mmoles) of VI and 3 ml. of liquid ammonia was heated in a stainless steel bomb at 55° for 24 hours. The ammonia was allowed to evaporate, and the residual solid (IX) was recrystallized from aqueous ethanol; yield, 178 mg. (66.9%), m.p. 177°. $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-3}$): pH 1, 260 (14.5); pH 7, 261 (14.8); pH 13, 261 (14.8). $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3450, 3350 (OH and NH); 1690 (NH); 1605 and 1560 (C=C and C=N).

Anal.—Calcd. for $C_{12}H_{17}N_5O \cdot H_2O$: C, 54.32; H, 7.22; N, 26.40. Found: C, 54.47; H, 7.24; N, 26.27.

cis - 4 - (6 - Methylamino - 9 - purinyl)cyclohexylcarbinol (X).—To 204 mg. (0.76 mmole) of VI in 3.5 ml. of ethanol was added 10 ml. of 40% methylamine in water; the reaction mixture was refluxed for 3 hours. The reaction mixture was evaporated to dryness *in vacuo*, and the residual solid was extracted with hot benzene (3 × 10 ml.). The benzene extracts, on cooling, gave a crystalline product which was collected by filtration. One recrystallization of the crude material from benzene gave the pure product (X), m.p. 212°; yield, 40.5 mg. (20.4%). $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3300 (OH and NH): 1620 (C=C and C=N). $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-3}$): pH 1, 265 (15.9); pH 7, 267 (15.5); pH 13, 269 (15.3).

Anal.—Calcd. for $C_{13}H_{19}N_5O$: C, 59.75; H, 7.33; N, 26.80. Found: C, 60.01; H, 7.47; N, 26.69.

cis - 4 - (6 - Dimethylamino - 9 - purinyl)cyclohexylcarbinol (XI).—A solution of 123 mg. (0.462 mmole) of VI in 2.5 ml. of ethanol and 2.5 ml. of aqueous dimethylamine (25%) was heated under reflux for 1 hour. The volatile materials were removed *in vacuo*, and the residue was recrystallized twice from benzene and hexane to give the analytical sample (XI); yield, 90 mg. (70.8%), m.p. 144°. $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-3}$): pH 1, 269 (18.9); pH 7, 277 (19.6); pH 13, 277 (19.6). $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3350 (OH); 1590, 1550 (C=C and C=N).

Anal.—Calcd. for $C_{14}H_{21}N_5O$: C, 61.09; H, 7.68; N, 25.43. Found: C, 61.27; H, 7.75; N, 25.43.

3-Methoxycarbonylcyclopentylamine (XIII).—Methane sulfonic acid (1.0 ml., 11.0 mmoles) was slowly added to a suspension of 1.24 Gm. (9.6 mmoles) of 3-carboxycyclopentylamine (8) in absolute methanol (50 ml.). The mixture was refluxed for 12 hours, then stirred at 0° with 1.5 Gm. of solid sodium carbonate for 30 minutes. The solvent was evaporated at 25–30°. The residue was triturated with chloroform (3 × 30 ml.); the chloroform extract was filtered through a Celite pad, and the solvent was evaporated *in vacuo*. The residual crude product (XIII) was purified by distillation; yield, 1.06 Gm. (76.8%), b.p. 73–74°/2.5 mm. $\bar{\nu}$ in cm.$^{-1}$ (film): 3300 (NH); 1720 (C=O); 1640 (NH).

Anal.—Calcd. for $C_7H_{13}NO_2$: C, 58.73; H, 9.15; N, 9.78. Found: C, 58.69; H, 9.39; N, 9.61.

3-Hydroxymethylcyclopentylamine (XIV).—A solution of XIII (790 mg., 5.52 mmoles) in ether (40 ml.) was added dropwise at 0° to a suspension of lithium aluminum hydride (650 mg., 17.1 mmoles) in ether (40 ml.). After stirring the mixture for 1 hour at room temperature, it was refluxed for 1 more hour. The excess of lithium aluminum hydride was

decomposed with ether saturated with water, then with water (2.5 ml.). The ethereal layer was decanted, and the insoluble residue was triturated with chloroform (3 × 20 ml.). The combined ethereal and chloroform extracts were dried with sodium sulfate and concentrated *in vacuo*. The crude product (XIV) was purified by distillation; yield, 465 mg. (73%), b.p. 90–91°/2.5 mm.

Anal.—Calcd. for $C_6H_{13}NO$: C, 62.43; H, 11.35; N, 12.14. Found: C, 62.38; H, 11.50; N, 11.97.

cis - 3 - (5 - Amino - 6 - chloro - 4 - pyrimidinylamino)cyclopentylcarbinol (XVI).—A solution of 4,6-dichloro-5-aminopyrimidine (149 mg., 0.908 mmole), 3-hydroxymethylcyclopentylamine (XIV, 105 mg., 0.913 mmole), and triethylamine 144 mg., 1.42 mmoles) in *n*-butanol (5 ml.) was refluxed for 6.5 hours. The volatile materials were removed *in vacuo*, and the residue (353 mg.) was extracted with benzene (5 × 10 ml.). Concentration of the benzene extract gave a gum (164 mg.) which was crystallized from benzene. The crude product (66 mg., 33%, m.p. 154°), after recrystallization from benzene, gave the analytical sample (XVI), m.p. 162°. $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-3}$); pH 1, 305 (13.1); pH 7, 290 (10.1), 262 (9.70); pH 13, 290 (10.1).

Anal.—Calcd. for $C_{10}H_{15}ClN_4O$: C, 49.38; H, 6.13; Cl, 14.61; N, 23.09. Found: C, 49.28; H, 6.34; Cl, 14.33; N, 22.85.

cis - 3 - (6 - Chloro - 9 - purinyl)cyclopentylcarbinol (XVII).—A solution of XVI (163 mg., 0.672 mmole) in triethyl orthoformate (10 ml.) was refluxed for 68 hours. Acetic anhydride (0.2 ml.) was then added to the solution, and the mixture was heated under reflux for an additional 3 hours. The volatile materials were removed *in vacuo;* the residue (185 mg.) was treated with methanolic ammonia (20%, 3 ml.) at 0° overnight. The volatile materials were removed *in vacuo* at 25°; the residual gum was dissolved in chloroform (20 ml.) and filtered through a short alumina column. The filtrate was concentrated, and the residue was crystallized from chloroform-hexane; yield, 84 mg. (50%), m.p. 104°. Recrystallization of the crude product from chloroform-hexane gave the analytical sample (XVII), m.p. 112°. $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-3}$): pH 1, 264 (9.38); pH 7, 265 (9.28); pH 13, 266 (9.07). $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3360 (OH); 1580, 1545 (C=C and C=N).

Anal.—Calcd. for $C_{11}H_{13}ClN_4O$: C, 52.37; H, 5.14; Cl, 14.03; N, 22.69. Found: C, 52.16; H, 5.36; Cl, 14.31; N, 22.44.

cis - 3 - (6 - Hydroxy - 9 - purinyl)cyclopentylcarbinol (XVIII).—To a mixture of 258 mg. (1.02 mmoles) of XVII in 12 ml. of water was added 2 ml. of 1.1 *N* sodium hydroxide; the mixture was heated under reflux for 4 hours, after which time the reaction mixture was decolorized with charcoal, and the filtrate was acidified at 0° with acetic acid. The white precipitate (120 mg.) was collected and recrystallized from water, m.p. 274–276°; yield of XVIII, 71 mg. (29.7%). $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3440 (OH); 2800–2300 (acidic hydrogen); 1680 (C=O); 1590, 1540 (C=C and C=N). $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-3}$): pH 1, 251 (12.4); pH 7, 251 (14.7); pH 13, 255 (15.5).

Anal.—Calcd. for $C_{11}H_{14}N_4O_2$: C, 56.40; H, 6.02; N, 23.92. Found: C, 56.38; H, 5.86; N, 23.78.

cis - 3 - (6 - Mercapto - 9 - purinyl)cyclopentylcarbinol (XIX).—To a suspension of 263 mg. (1.04 mmoles) of XVII in 10 ml. of *n*-propyl alcohol was added 81.8 mg. (1.07 mmoles) of thiourea. The mixture was heated under reflux for 2 hours, then cooled in an ice-bath. The solid was collected by filtration, washed with 3 ml. of cold *n*-propyl alcohol, and dissolved in 8 ml. of 5% sodium hydroxide. The solution was filtered, chilled in an ice-bath, and acidified to pH 5 with 1.3 ml. of glacial acetic acid. The white product was collected by filtration and dried. The crude material, after recrystallization from methanol, gave the pure product (XIX), m.p. 295–300°; yield, 74.8 mg. (28.8%). $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3500 (OH); 2700–2300 (acidic hydrogen); 1600 (C=C and C=N). $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-3}$): pH 1, 325 (18.3); pH 7, 323 (25.0); pH 13, 310 (19.7).

Anal.—Calcd. for $C_{11}H_{14}N_4OS$: C, 52.78; H, 5.64; N, 22.38. Found: C, 52.54; H, 5.79; N, 22.20.

cis - 3 - (6 - Amino - 9 - purinyl)cyclopentylcarbinol (XX).—To 277 mg. (1.10 mmoles) of XVII in a stainless steel bomb was added 10 ml. of liquid ammonia, and the reaction mixture was heated at 60° for 17 hours. On evaporation of the ammonia, there was obtained a yellow semisolid mass, which was dissolved in ethanol. Addition of acetone caused the precipitation of ammonium chloride, which was removed by filtration. The filtrate was evaporated to dryness *in vacuo* and gave a white residue (189 mg.). One recrystallization of the crude material from chloroform and hexane gave the pure product (XX), m.p. 129–131°; yield, 85.5 mg. (33.4%). $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3400 (OH); 3230 (NH); 1670 (NH); 1600, 1570 (C=C and C=N). $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-3}$): pH 1, 261 (8.96); pH 7, 261 (9.91); pH 13, 261 (9.67).

Anal.—Calcd. for $C_{11}H_{15}N_5O$: C, 56.63; H, 6.48; N, 30.03. Found: C, 56.37; H, 6.46; N, 30.22.

cis - 3 - (6 - Methylamino - 9 - purinyl)cyclopentylcarbinol (XXI).—A mixture of 203 mg. (0.800 mmole) of XVII, 10 ml. of 40% methylamine in water, and 10 ml. of ethanol was heated in a stainless steel bomb at 85° for 2.5 hours. Concentration of the reaction mixture gave a residue which was extracted with a 1:1 mixture of benzene and chloroform (3 × 3 ml.). Evaporation of the extracts gave a crude XXI which could not be crystallized as its free base. The hydrochloride of XXI was prepared by adding concentrated hydrochloric acid to a methanolic solution of crude XXI and evaporating the mixture *in vacuo*. Recrystallization of the residual solid from methanol and ether, then from methanol and ethyl acetate gave 85 mg. (37.5%) of the hydrochloride of XXI, m.p. 189.5–192°. $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3300 (OH); 2650, 1690 (C=N—H$^+$); 1610 (C=C and C=N). $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-3}$): pH 1, 265 (12.9); pH 7, 269 (12.9); pH 13, 268 (11.9).

Anal.—Calcd. for $C_{12}H_{18}ClN_5O$: C, 50.79; H, 6.39; Cl, 12.49; N, 24.69. Found: C, 50.54; H, 6.26; Cl, 12.45; N, 24.65.

cis - 3 - (6 - Dimethylamino - 9 - purinyl)cyclopentylcarbinol (XXII).—A mixture of 212 mg. (0.838 mmole) of XVII, 11 ml. of 25% dimethylamine in water, and 10 ml. of ethanol was refluxed for 20 hours, after which time the volatile materials were removed *in vacuo*. The residue was extracted with a 1:1 mixture of benzene and chloroform (3 × 30 ml.). Evaporation of the organic extract gave the crude XXII which could not be crystallized as the

free base. Thus, the crude XXII was converted to its hydrochloride by adding concentrated hydrochloric acid to a methanol solution of XXII, followed by evaporation *in vacuo*. The residual solid was recrystallized from methanol and ethyl acetate and gave 144 mg. (53.3%) of the hydrochloride of XXII, m.p. 195–198.5°. $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3400 (OH); 2750 and 1670 (C=$\overset{+}{\text{N}}$—H); 1590 (C=C and C=N). $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-3}$): pH 1, 270 (17.4); pH 7, 277 (18.1); pH 13, 277 (17.9).

Anal.—Calcd. for $C_{13}H_{20}ClN_5O$: C, 52.43; H, 6.77; N, 23.52. Found: C, 52.20; H, 6.64; N, 23.37.

Conversion of XXII to Its Quaternary Salt by Means of Its Mesylate Ester.—To a solution of 965 mg. (3.24 mmoles) of XXII in 6 ml. of pyridine cooled in an ice-bath was added 0.8 ml. of methanesulfonyl chloride with stirring. After 18 hours at 3°, the reaction mixture was poured into 40 ml. of ice-water, and the mixture was extracted with chloroform (4 × 30 ml.). The combined organic extracts were washed with sodium bicarbonate solution, water, dried with anhydrous magnesium sulfate, filtered, and evaporated *in vacuo* to dryness to give 682 mg. (62.0%) of the crude mesylate (XXIII) as an oil. $\bar{\nu}$ in cm.$^{-1}$ (film): 1590 (C=C and C=N); 1340 and 1170 (sulfonate ester). $\lambda_{max.}^{pH\ 1}$ 272 mμ.

A solution of 309 mg. of crude XXIII in 4 ml. of acetone containing 5 drops of methanol was heated under reflux for 4 hours, then the volatile materials were removed *in vacuo*. The residue was triturated with 1 ml. of acetone, and the resulting crystalline solid was collected by filtration; yield, 200 mg. (63.6%), m.p. 202–203°. One recrystallization of the crude product from methanol and acetone gave the pure product (XXIV), m.p. 202–203°. $\bar{\nu}$ in cm.$^{-1}$ (KBr): 1620, 1595, and 1555 (C=C and C=N); 1190 (sulfonate). $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-3}$): pH 1, 290 (15.8).

Anal.—Calcd. for $C_{14}H_{21}N_5O_3S$: C, 49.53; H, 6.24; N, 20.64; S, 9.42. Found: C, 49.83; H, 6.39; N, 20.40; S, 9.15.

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 (12) 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.

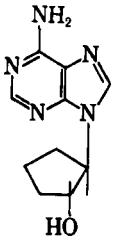
Results

Enzymatic evaluation of these groups of compounds revealed that only those compounds with an amino or methylamino group at the 6-position of the purine nucleus (IX, X, XX, and XXI) were inhibitory at concentrations two to three times that of substrate. The percentage inhibition of adenosine deaminase by a 0.12 m*M* solution of inhibitor is given in Table I. For comparison, the percentage inhibition of two closely related structural analogs has also been included in Table I. These analogs are *trans*-2-[9-(6-aminopurinyl)]cyclopentanol (XXV) and *trans* - 2 - [9 - (6-aminopurinyl)]cyclohexanol (XXVI).

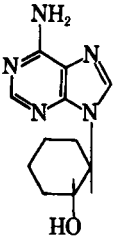
TABLE I.—PARTIAL INHIBITION OF ADENOSINE DEAMINASE BY ANALOGS OF PURINE NUCLEOSIDES

Compd.	Concn., m*M*[a]	*Vo/V*	% Inhibition
IX	0.12	1.25	20
X	0.12	1.30	23
XX	0.12	1.28	22
XXI	0.12	1.20	16
XXV	0.12	1.54	35
XXVI	0.12	1.53	35

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



XXV



XXVI

DISCUSSION

Only those purine nucleoside analogs with an amino or methylamino group at the 6-position of the purine nucleus were inhibitory. Those compounds with a chloro, hydroxy, mercapto, or dimethylamino group at the 6-position were essentially noninhibitory at concentrations two to three times that of adenosine. In previous investigations 6-aminopurine nucleoside analogs have been prepared which were substituted at the 9-position by cyclopentyl, *cis*- and *trans*-2-hydroxycyclopentyl, *cis*- and *trans*-2-hydroxycyclohexyl, and *cis*- and *trans*-3-hydroxycyclohexyl groups. These studies (2) showed that the 2′-hydroxy group of the cycloaliphatic nucleus in both the cyclopentyl and cyclohexyl analogs makes a significant contribution to binding to the enzyme; the *trans* compounds are slightly more effective than the *cis* isomers. Furthermore, the 3′-hydroxy group on the cyclohexyl nucleus made little or no contribution to binding to the enzyme since these compounds were no more effective in inhibiting the enzymatic reaction than the unsubstituted cyclopentyl analog (3).

The present study gives information concerning the contribution to binding made by the hydroxymethyl group on the substituent at the 9-position of the purine. An examination of Table I reveals that IX, X, XX, and XXI are inhibitory, but that they are less inhibitory than the corresponding compounds with a 2′-hydroxy group (XXV and XXVI). Therefore, the effect of the hydroxymethyl group on binding to the enzyme is small. Consequently, if it is assumed that XX binds to the same place on the enzyme that adenosine does, the hydroxymethyl group of adenosine makes only a small contribution to binding. The study of the bulk tolerance at the

6-position of the purine nucleus and of the steric requirements for binding to adenosine deaminase is continuing.

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Sterol Metabolism in Larvae of the Confused Flour Beetle, *Tribolium confusum*

By EDWARD E. SMISSMAN, NEIL A. JENNY, and STANLEY D. BECK

Dehydroepiandrosterone, pregnenolone, and progesterone were isolated as metabolic products during normal insect metabolism of dietary sterols in the confused flour beetle, *Tribolium confusum*. Their identity was established by elemental analysis, thin-layer chromatography, gas chromatography, ultraviolet absorption, optical rotation, and 2,4-dinitrophenylhydrazone derivatives compared to authentic samples. A proposed metabolic pathway of dietary sterols in *T. confusum* larvae is outlined.

THE INDISPENSABILITY of sterols for the growth of insects has been amply established by numerous nutritional studies on a variety of species (1–3). Higher animals are able to synthesize sterols from simple compounds such as acetate, while the lack of sterol synthesis seems to be a metabolic defect common to all insects (4).

The requirement for specific sterols varies widely among the insecta, and most larvae exhibit only moderate specificity with respect to their need for dietary sterols. Several dietary sterols of the larvae of *Tribolium confusum* are converted to cholesterol and 7-dehydrocholesterol which are the principal tissue sterols of the insect (5). The present and related metabolic studies (5–7) indicate the ability of insects to metabolize dietary sterols to the extent of altering the side chain and the degree of saturation in the B ring of the sterol nucleus. The objective of this study is to demonstrate the molecular alteration of side-chain substituted sterols during normal insect metabolism by isolation and characterization of the ketosteroid metabolic products.

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EXPERIMENTAL AND RESULTS

Since the results obtained in a previous study indicated that dietary sterols are converted into cholesterol and 7-dehydrocholesterol (6), it was decided to grow the *T. confusum* larvae on a natural diet supplemented with a dietary sterol that could be converted to cholesterol. The beetle larvae were reared from egg to larval maturity using a basic diet of 4 parts white flour, 4 parts graham wheat flour, and 1 part yeast with the additional dietary sterol, ergosterol, present in the yeast. Before isolation of lipids, the larvae were held for 24 hours on a sterol-free diet to minimize interference from sterols contained in the gut contents.

Extraction.—In previous studies of steroid isolation, many types of solvent systems have been used. Weakly polar solvents are used most commonly. Since it was difficult to determine which procedure would be best for this study, several extraction procedures were used in an attempt to separate the steroids from the numerous other constituents of the insect body. In all cases the larvae were first washed with diethyl ether and skellysolve B to remove any diet material on the surface of the insects and any steroids present in the insect epicuticle.

Skellysolve B Extraction.—After washing the larvae, 250 Gm. of larvae in 100 ml. of skellysolve B was homogenized in a Waring Blendor for 5 seconds. This homogenate of larvae was extracted with 1.5 L. of skellysolve B in a Soxhlet apparatus for 48 hours. The skellysolve B extract was concentrated, redissolved in 100 ml. of 5% alcoholic potassium hydroxide solution, and refluxed for 1 hour. The hydrolyzate was poured into 300 ml. of distilled water, and the nonsaponifiable material was extracted three times with 400 ml. of skellysolve B. The organic layer was washed several times with water and dried over anhydrous magnesium sulfate.