Enzyme Inhibitors IV

Syntheses of 6-Substituted-9-(5-deoxy-β-D-xylofuranosyl)purines and Their Evaluation as Inhibitors of Adenosine Deaminase

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A series of 6-substituted-9-(5-deoxy-β-D-xylofuranosyl)purines was prepared by the following procedure. D-Xylose was converted by an eight-step reaction sequence into 2-acetyl-3-benzoyl-5-deoxy-D-xylofuranosyl chloride (VI) which, on condensation with chloromercuri-6-chloropurine, gave 6-chloro-9-(2-acetyl-3-benzoyl-5-deoxy-β-D-xylofuranosyl)purine (VIII). Displacement of the 6-chloro group in VIII, followed by removal of the blocking groups, gave the following 6-substituted nucleosides: (*a*) amino, (*b*) methylamino, (*c*) dimethylamino, (*d*) mercapto, (*e*) methoxy, (*f*) hydrogen. Those nucleosides with a 6-amino, 6-methylamino, 6-dimethylamino, and 6-methoxy group inhibited, to a varying degree, the enzyme adenosine deaminase. In addition, the 6-amino analog was a substrate of adenosine deaminase.

During the past decade, a number of antibiotics have been isolated which show interesting biological activity since they possess varying degrees of activity against neoplastic tissues. Several of the antibiotics possessing anticancer activity are purine nucleosides that contain an unusual sugar at the 9-position of the purine nucleus and have an amino or substituted amino group at the 6-position of the purine nucleus. Several examples of the nucleoside antibiotics are psicofuranine (1), cordycepin (2),[1] and tubercidin (3)—all of which contain a 6-aminopurine moiety as the common grouping—and puromycin, a nucleoside which contains a 6-dimethylaminopurine group (4, 5). Consequently, we have been interested in synthesizing purine nucleosides which contain at the 9-position an unusual sugar, since these compounds may be potential anticancer agents. In addition, we have been studying which atoms and functional groups of nucleosides and isosteric nucleosides are important for binding to enzymes that utilize nucleosides as their substrates (6–8). This paper describes the synthesis of some 6-substituted-9-(5-deoxy-β-D-xylofuranosyl) purines and reports on the evaluation of these compounds as inhibitors of adenosine deaminase.

Chemistry

The general method of synthesis of the desired nucleosides involves the condensation of an appropriately blocked chlorosugar with chloromercuri-6-chloropurine to generate a nucleoside whose 6-chloro group could be caused to undergo displacement by a variety of reagents. For the synthesis of the required chlorosugar (VI), xylose (I) was converted into the blocked 3,5-anhydro derivative (II) by modification of known procedures (9, 10). Reaction of II with lithium aluminum hydride resulted in the formation of 1,2-isopropylidene-5-deoxy-α-D-xylofuranose (III) in an excellent yield. The 3-hydroxy group of III was protected with a benzoyl group to give IV. The isopropylidene group of IV was removed by means of a mixture of acetic anhydride and sulfuric acid in glacial acetic acid and gave an excellent yield of 1,2-di-*O*-acetyl-3-*O*-benzoyl-5-deoxy-D-xylofuranose which was separated into its α- and β-anomers (V*a* and V*b*).[2] Treatment of V*a* with anhydrous hydrogen chloride in ether solution gave the chloro derivative (VI) which, on condensation with chloromercuri-6-chloropurine (VII), formed the blocked 6-chloropurine nucleoside (VIII). Several attempts were made to remove the blocking groups of VIII so that a crystalline 6-chloropurine nucleoside could be obtained; but in all cases a noncrystalline product resulted. Therefore, the crude nucleoside was employed for the preparation of compounds IX through XIV, all of which were obtained analytically pure.

The preparation of nucleosides by the condensation of an acylated chlorosugar with chloromercuri-6-chloropurine could lead theoretically to a 7- or 9-substituted purine and could give either an α- or β-nucleoside. It is, in fact, usually true that 9-substituted purines are favored and that *trans*-nucleosides are formed. Previous studies have established that the ultraviolet maximum for a 7-methyl or a 7-ethyladenine is at a longer wavelength than for the corresponding 9-substituted derivative (11). A similar but even larger shift is observed in the corresponding 6-dimethylamino analogs, and this observation was used in the structural elucidation of puromycin (12). For example, it has been found (12)

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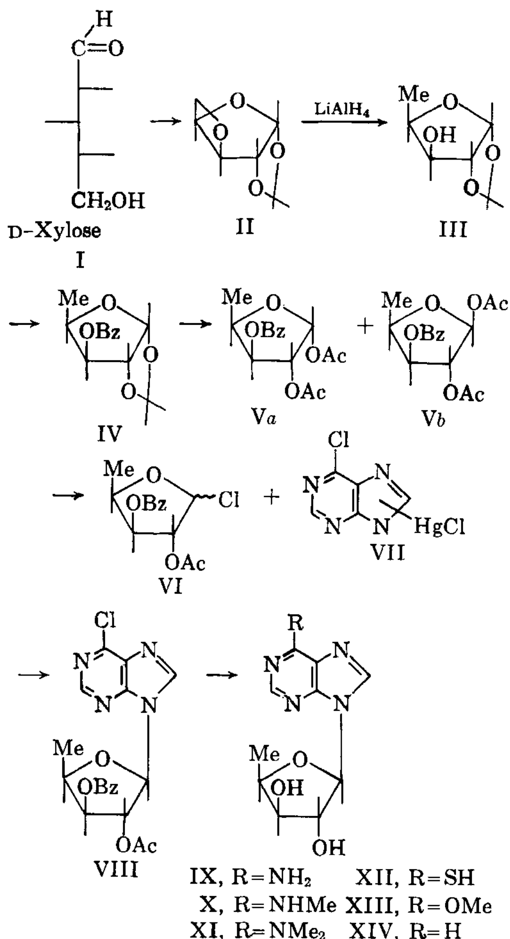
[1] See *Reference 2* for a recently revised structure.

[2] The assignment of anomeric configuration of V was based on the observation that V*a* was more dextrorotatory than V*b* (Hudson's rules) and by the fact that in the NMR spectra the C-1 proton signal in V*a* appeared at 6.5 δ as a doublet with J_{1-2} = 4.5 cps., characteristic of the C-1–C-2 *cis* coupled protons, whereas the C-1 proton in V*b* appeared at 6.18 δ as a singlet. [For leading references and related NMR assignments, see Casini, G., and Goodman, L., *J. Am. Chem. Soc.*, **86**, 1427(1964).]



that the ultraviolet maximum of 6-dimethylamino-9-ethylpurine at pH 1 is 270 mμ, whereas the maximum for the corresponding 7-ethyl analog at pH 1 is 290 mμ. An examination of the ultraviolet spectrum of the dimethylamino analog (XI) revealed a maximum at 268 mμ, clearly in a position to establish that XI is substituted at the 9-position of the purine nucleus. Finally, we have assigned tentatively a β-configuration to these nucleosides based on the generalization given by Baker (13) which involves the neighboring group participation of the 2-*O*-acetyl group of VI during the condensation with VII to generate a *trans* C_1–C_2 nucleoside—in this case a β-nucleoside.

EXPERIMENTAL[3]

1,2 - O - Isopropylidene - 5 - deoxy - α - D - xylofuranose (III).—To a stirred suspension of lithium aluminum hydride (10.5 Gm., 278 mmoles) in anhydrous ether (260 ml.) was added, over a period of 10 minutes, a solution of 23.8 Gm. (139 mmoles) of 1,2-*O*-isopropylidene-3,5-anhydro-α-D-xylofuranose (II) (10) in anhydrous ether (130 ml.). The mixture was stirred under reflux for 18 hours, then cooled in an ice bath. The excess reagent was decomposed by dropwise addition of water (25 ml.), and the insoluble solids were removed by filtration and washed with anhydrous ether (4 × 100 ml.). The filtrate and washings were combined, dried with magnesium sulfate, and filtered. The filtrate was evaporated *in vacuo* at 40° to a colorless syrup. The syrup crystallized completely on seeding; yield, 23.8 Gm. (99%), m.p. 71–72°. The analytical sample of III was prepared by sublimation of the crude product at 80°/0.5 mm., m.p. 75–76°. $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3450 (OH); 1380 (gem-dimethyl).

Anal.[4]—Calcd. for $C_8H_{14}O_4$: C, 55.17; H, 8.10. Found: C, 55.37; H, 8.06.

This product was prepared previously by a different procedure and had a melting point of 68–69° (14).

1,2 - O - Isopropylidene - 3 - O - benzoyl - 5-deoxy-α-D-xylofuranose (IV).—To a stirred solution of III (950 mg., 5.45 mmoles) in anhydrous pyridine (5 ml.), protected from moisture and cooled in an ice bath, was added dropwise, over a period of 10 minutes, a solution of benzoyl chloride (840 mg., 6.00 mmoles) in methylene chloride (3 ml.). After 18 hours at room temperature, the mixture was poured into a mixture of aqueous sodium bicarbonate (15 ml.) and ice water (30 ml.). The mixture was stirred for 30 minutes, and the organic layer was separated. The aqueous layer was extracted with methylene chloride (2 × 20 ml.). The combined organic extracts were dried with magnesium sulfate and evaporated *in vacuo* at 45° to a pale yellow syrup which contained a trace of benzoic anhydride; yield, 1.59 Gm. (105%). The crude syrup was dissolved in chloroform (10 ml.) and introduced on a column (1.5 cm. diam.) of neutral alumina (15 Gm.) in chloroform. The column was eluted with chloroform, and 200 ml. of eluate was collected. Evaporation of the eluate *in vacuo* at 40° gave IV as an almost colorless syrup; yield, 1.47 Gm. (97%). The analytical sample was obtained by drying a small amount of syrup in high vacuum at 56°. $\bar{\nu}$ in cm.$^{-1}$ (film): 1730 (C=O of benzoate); 1380 (gem-dimethyl); 1265 (C—O—C).

Anal.—Calcd. for $C_{15}H_{18}O_5$: C, 64.74; H, 6.52. Found: C, 64.57; H, 6.70.

1,2-Di-O-acetyl-3-O-benzoyl-5-deoxy-α(and β)-D-xylofuranose (Va and Vb).—To a solution of IV (1.43 Gm., 5.15 mmoles) in a mixture of glacial acetic acid (26 ml.) and acetic anhydride (3 ml.) was added slowly concentrated sulfuric acid (1.55 ml.). During the addition, the temperature was maintained between 10° and 20°. The flask was stoppered and allowed to stand at room temperature for 26 hours. The yellow mixture was poured into ice water (150 ml.) and stirred for 1 hour. The aqueous mixture was extracted with chloroform (3 × 50 ml.); the chloroform extract was washed with aqueous sodium bicarbonate (2 × 50 ml.), dried with magnesium sulfate, and filtered. The filtrate was evaporated *in vacuo* at 45° and gave a yellow syrup. Crystallization of this syrup from 5 ml. of absolute ethanol gave 1,2-di-*O*-acetyl-3-*O*-benzoyl-5-deoxy-β-D-xylofuranose (V*b*) which was collected by filtration and washed with additional absolute ethanol; yield, 720 mg. (43%), m.p. 114–116°. One recrystallization from absolute ethanol gave the analytical sample with a recovery of 78%, m.p. 118–119°, $[\alpha]_D^{20}$ + 10.8° (C, 2.30, $CHCl_3$). $\bar{\nu}$ in cm.$^{-1}$ (KBr): 1750 (acetate C=O); 1725 (benzoate C=O); 1280 (benzoate C—O—C); 1235 (acetate C—O—C).

[3] 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.

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

Anal.—Calcd. for $C_{16}H_{18}O_7$: C, 59.62; H, 5.63. Found: C, 59.83; H, 5.55.

The filtrate and washings from the crystallization of V*b* were combined and evaporated *in vacuo* at 40° to obtain 1,2-di-*O*-acetyl-3-*O*-benzoyl-5-deoxy-α-D-xylofuranose (V*a*) as a yellow syrup; yield, 790 mg. (47%). An analytical sample was obtained as a colorless syrup by evaporative distillation of 47 mg. of the crude syrup from an oil bath at 110–112°/0.05 mm. $[\alpha]_D$ + 91.6° (C, 3.00, $CHCl_3$). $\bar{\nu}$ in cm.$^{-1}$ (film): 1750 (acetate C=O); 1725 (benzoate C=O); 1272 (benzoate C—O—C); 1235 (acetate C—O—C).

Anal.—Calcd. for $C_{16}H_{18}O_7$: C, 59.62; H, 5.63. Found: C, 59.90; H, 5.92.

6 - Chloro - 9 - (2 - O - acetyl - 3 - O - benzoyl-5-deoxy-β-D-xylofuranosyl)purine (VIII).—A solution of 1,2-di-*O*-acetyl-3-*O*-benzoyl-5-deoxy-α-D-xylofuranose (V*a*) (790 mg., 2.45 mmoles) in ethereal hydrogen chloride (20 ml.) saturated at 0° and containing acetyl chloride (1 ml.) was kept at 2–3° for 63 hours, then evaporated *in vacuo*. The residue was evaporated with toluene (3 × 10 ml.) to remove traces of hydrogen chloride and acetic acid. The residual chlorosugar (VI) was dissolved in xylene (5 ml.) and added to an azeotropically dried suspension of 955 mg. (2.45 mmoles) of chloromercuri-6-chloropurine (VII) in xylene (20 ml.). The mixture was stirred under reflux for 3 hours and filtered when cool. After the filtrate was evaporated *in vacuo* at 55°, the residual yellow syrup was extracted with hot chloroform (2 × 20 ml.); the extract was washed with 30% aqueous potassiun iodide solution (2 × 10 ml.), then with water (1 × 20 ml.). The dried (magnesium sulfate) chloroform solution was evaporated *in vacuo* at 40° to a yellow syrup; yield, 920 mg. (90%). $\bar{\nu}$ in cm.$^{-1}$(film): 1750 (acetate C=O)sh; 1735 (benzoate C=O); 1600 and 1570 (C=C and C=N); 1270 (benzoate C—O—C); 1220 (acetate C—O—C).

Several attempts were made to prepare the analytical sample of VIII and of the corresponding deblocked 6-chloro analog, but only noncrystalline compounds were obtained which gave analytical results outside of the acceptable range. Consequently, VIII was used in the crude form for the synthesis of the other 6-substituted nucleosides.

6 - Amino - 9 - (5 - deoxy - β - D - xylofuranosyl)-purine (IX).—A mixture of VIII (920 mg.) and 25 ml. of methanolic ammonia (saturated at 3°) was heated in a stainless steel bomb for 8 hours at 100°. The light brown solution was evaporated *in vacuo* at 40°. The residual amber syrup was dissolved in methanol (2 ml.), and 10% methanolic picric acid (10 ml.) was added. The mixture containing the precipitated picrate was cooled in an ice bath for 1 hour, filtered, and the picrate was washed with additional cold methanol; yield, 374 mg. (35%). The picrate decomposes at 200–202° without melting. A suspension of the picrate (314 mg.) in water (75 ml.) was stirred with Dowex-1 (CO_3) with gentle warming (50°) until the supernatant liquid had become colorless. The resin was removed by filtration and washed with water. The filtrate and washings were combined and evaporated *in vacuo* at 40°. The residual syrup was dried by evaporating absolute ethanol; yield, 142 mg. (86%). The partially crystallized syrup was taken up in acetone and filtered. Two recrystallizations of the crude product from acetone gave the analytical sample, m.p. 227–228°. $\bar{\nu}$ in cm.$^{-1}$(KBr): 3400, 3150 (OH, NH_2); 1665 (NH); 1610, 1570 (C=C and C=N); 1100, 1055, 1020 (C—O—C and C—OH). $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-3}$): pH 1, 257. (14.5); pH 7.6, 259 (15.1); pH 13, 259 (15.3); $[\alpha]_D^{20}$ −66.3° (C, 1.00 in H_2O).

Anal.—Calcd. for $C_{10}H_{13}N_5O_3$: C, 47.79; H, 5.21; N, 27.87. Found: C, 47.77; H, 5.33; N, 27.92.

6 - Methylamino - 9 - (5 - deoxy - β - D - xylofuranosyl)purine (X).—A mixture of crude VIII (900 mg.), methanol (10 ml.), and 10% methanolic methylamine (9 ml.) was heated in a steel bomb at 100° for 4 hours, then the dark solution was evaporated *in vacuo* at 40°. The residual syrup was dissolved in methanol (3 ml.) and treated with 10% methanolic picric acid. On addition of water (5 ml.), a syrup separated which crystallized on standing overnight. The yellow picrate was collected by filtration and washed with methanol; yield, 362 mg. (34%). The picrate decomposes at 200–205° without melting.

To a suspension of the crude picrate (362 mg.) in water (200 ml.) at 45° was added Dowex-1 (CO_3), and the mixture was stirred until no further decolorization of the supernatant was observed. The resin was removed by filtration and washed with water (100 ml.). The combined filtrate and washings were evaporated *in vacuo* at 40°. On evaporation of a methanol solution of the residual yellow syrup (112 mg.), crystallization occurred. The residue was triturated with a small amount of methanol, and the crystals (69 mg.) were collected by filtration. An additional 17 mg. of the compound was obtained from the mother liquor. Three recrystallizations of the combined crops from methanol gave 23 mg. (12%) of the analytical sample, m.p. 135–137°. $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3360 (OH); 1620, 1575 (sh), and 1540 (sh) (C=C and C=N). $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-3}$): pH 1, 262 (16.6); pH 7.6, 265 (15.8); pH 13, 266 (16.3); $[\alpha]_D^{20}$ − 68.1° (C, 0.62 in H_2O).

Anal.—Calcd. for $C_{11}H_{15}N_5O_3$: C, 49.80; H, 5.70; N, 26.40. Found: C, 49.58; H, 5.90; N, 26.08.

6 - Dimethylamino - 9 - (5 - deoxy - β - D - xylofuranosyl)purine (XI).—A mixture of crude VIII (200 mg.) and 25% aqueous dimethylamine (7.5 ml.) in methanol (15 ml.) was heated in a steel bomb at 105° for 6 hours. The light yellow solution was evaporated *in vacuo* at 40° to dryness, and the residual yellow syrup was dried by evaporation with absolute ethanol. On trituration of the residue with acetone, dimethylamine hydrochloride separated as needles (17 mg.) which were removed by filtration and washed with acetone. The combined filtrate and washings were evaporated, dissolved in 25 ml. of water, and stirred for 1 hour with Dowex-1 (CO_3). The resin was removed by filtration and washed with water. The combined filtrates were concentrated *in vacuo* at 40° to dryness and finally dried in high vacuum to yield a yellow glass; yield, 176 mg. (85%).

A solution of the crude nucleoside (176 mg.) in 1 ml. of water saturated with ethyl acetate was intimately mixed with 2 Gm. of Celite (analytical filter aid). This was packed on top of a column (22.5 × 2.3 cm.) prepared with 25 Gm. of Celite (analytical filter aid) and 12.5 ml. of water saturated with ethyl acetate. The column was eluted with ethyl acetate saturated with water, and 5-ml. fractions were collected. Fractions 2–6, containing material absorb-

ing at 275 mμ, were combined and evaporated *in vacuo* to give a pale yellow glass; yield, 158 mg. (90% recovery).

The nucleoside was dissolved in 2 ml. of chloroform and introduced on a column (1.2 × 3.5 cm.) of neutral grade II alumina (3.5 Gm.) in chloroform. The column was washed with 50 ml. of chloroform, and the washings were discarded. Elution with 25 ml. of chloroform: methanol (9:1 v/v), and removal of the solvent gave a pale yellow glass. An acetone solution of the nucleoside was treated with charcoal and filtered through a Celite pad. The filtrate was concentrated to dryness and finally dried in high vacuum at 65° to yield a colorless glass which was analytically pure; yield, 115 mg. (56%). $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3400 (broad, OH); 1600, 1560 (sh), and 1530 (sh) (C=C and C=N). $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-3}$): pH 1, 268 (17.8); pH 7.6, 275 (18.2); pH 13, 275 (19.0); $[\alpha]_D^{20}$ −62.4° (C, 0.68 in H_2O).

Anal.—Calcd. for $C_{12}H_{17}N_5O_3$: C, 51.60; H, 6.14; N, 25.08. Found: C, 51.57; H, 6.38; N, 25.23.

6-Mercapto-9-(5-deoxy-β-D-xylofuranosyl)purine (XII).—To a solution of crude VIII (0.895 Gm.) in 30 ml. of methanol saturated with hydrogen sulfide was added 6.5 ml. of 1 *N* methanolic sodium hydrogen sulfide. The reaction mixture was refluxed for 20 minutes, during which time hydrogen sulfide was slowly passed through the solution. The hydrogen sulfide source was removed, 2 ml. of 1 *N* sodium methoxide added, and the mixture was refluxed for 2.5 hours. The solution was evaporated *in vacuo* to dryness, and the residue was partitioned between 15 ml. of water and 20 ml. of ether. The aqueous layer was washed further with ether (2 × 20 ml.), then filtered through a thin layer of Nuchar. The filtrate was cooled in an ice bath and acidified with glacial acetic acid. The resulting precipitate was collected by filtration and washed with water; yield, 148 mg. (26%). After three recrystallizations of the crude product from methanol and drying for 3 hours at 65° *in vacuo*, the nucleoside was obtained as a hemimethanolate, m.p. 195° dec. $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3450 (OH); 3000–2650 (acidic hydrogen); 1590 and 1520 (C=C and C=N).

Anal.—Calcd. for $C_{10}H_{12}N_4O_3S \cdot {}^1/_2CH_3OH$: C, 44.35; H, 4.96; N, 19.71; S, 11.28. Found: C, 43.78; H, 5.24; N, 19.88; S, 11.29.

An analytical sample, free of solvent of crystallization, was obtained after drying at 100° *in vacuo* for 24 hours, m.p. 186–189° dec. $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-3}$): pH 1, 325 (21.7); pH 7.6, 317 (22.2); pH 13, 312 (23.4); $[\alpha]_D^{20}$ − 102° (C, 0.63 in 0.1 *N* NaOH).

Anal.—Calcd. for $C_{10}H_{12}N_4O_3S$: C, 44.77; H, 4.51; N, 20.88; S, 11.95. Found: C, 44.51; H, 4.70; N, 20.68; S, 11.63.

6-Methoxy-9-(5-deoxy-β-D-xylofuranosyl)-purine (XIII).—To a solution of crude VIII (0.766 Gm.) in 35 ml. of methanol was added 5.5 ml. (5.5 moles) of 1 *N* aqueous sodium hydroxide. The mixture was stirred and heated under reflux for 1 hour and evaporated *in vacuo* to dryness. A solution of the residue in 15 ml. of water was stirred with Dowex-1 (CO_3) for 1 hour. The resin was removed by filtration and washed with water (25 ml.). The filtrate and washings were combined and stirred overnight with Amberlite IRC-50(H+). The resin was removed by filtration and washed with water. The combined filtrates were evaporated *in vacuo* at 40° to dryness. The residue was dissolved in methanol (10 ml.) and filtered through a Celite pad to remove a small amount of insoluble matter. After the filtrate was evaporated to dryness, the residue was extracted with ethyl acetate. Evaporation of the ethyl acetate extract gave a residual yellow syrup, which could not be crystallized; yield, 172 mg.

A solution of the crude nucleoside (172 mg.) was purified by partition chromatography on Celite using ethyl acetate saturated with water as the mobile phase; yield, 125 mg., m.p. 134–138°. One recrystallization of the crude product from ethyl acetate gave the analytical sample, m.p. 141°. $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3400 (broad) (OH); 1600 and 1580 (sh) (C=C and C=N). $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-3}$): pH 1, 249 (10.5); pH 7.6, 250 (11.1); pH 13, 250 (11.0); $[\alpha]_D^{20}$ − 69.3° (C, 0.69 in H_2O).

Anal.—Calcd. for $C_{11}H_{14}N_4O_4$: C, 49.62; H, 5.30; N, 21.04. Found: C, 49.33; H, 5.15; N, 21.25.

9-(5-Deoxy-β-D-xylofuranosyl)purine (XIV).—A solution of crude VIII (0.871 Gm.), in 22 ml. of methanol was hydrogenated at atmospheric pressure using a 10% palladium-on-carbon (250 mg.) catalyst and magnesium oxide (84 mg., 2.08 mmoles) as acid acceptor. The catalyst was removed by filtration through a Celite pad and washed with methanol. The combined filtrates were evaporated *in vacuo* at 40° to yield a syrup which was dissolved in 40 ml. of chloroform. The chloroform solution was washed with water (3 × 15 ml.), dried with magnesium sulfate, and evaporated *in vacuo* at 40° to give an almost colorless glass; yield, 0.531 Gm. $\bar{\nu}$ in cm.$^{-1}$ (film): 1750 (acetate C=O); 1730 (benzoate C=O); 1595 and 1585 (C=C and C=N); 1270 (benzoate C—O—C); 1230 (acetate C—O—C); 710 (monosubstituted benzene).

A solution of the crude 9-(2-*O*-acetyl-3-*O*-benzoyl-5-deoxy-β-D-xylofuranosyl)purine (0.531 Gm.) in 30 ml. of methanolic ammonia (saturated at 0°) was kept at 4° for 20 hours and evaporated *in vacuo* at 40° to a yellow syrup, from which water (4 × 5 ml.) was evaporated to remove methyl benzoate; yield, 0.428 Gm. Partition chromatography on Celite, using ethyl acetate saturated with water as the mobile phase, gave 159 mg. of crystalline material. The crystalline mass was triturated with a small amount of ethyl acetate, and the pale yellow crystals were removed by filtration; yield, 126 mg. (25.6%).

Three recrystallizations of the crude product from acetone gave the analytical sample (XIV), m.p. 201–203°. $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3300 (OH); 1600 and 1590 (C=C and C=N). $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-3}$): pH 1, 263 (5.59); pH 7.6, 263 (6.95); pH 13, 263 (7.05); $[\alpha]_D^{20}$ − 55.7° (C, 0.65 in H_2O).

Anal.—Calcd. for $C_{10}H_{12}N_4O_3$: C, 50.83; H, 5.12; N, 23.71. Found: C, 51.01; H, 5.34; N, 23.49.

Reagents and Assay Procedure

Adenosine and adenosine deaminase were purchased from the Sigma Chemical Co. The general method of assay has previously been described (15). The enzymatic reactions were performed in 0.05 *M* phosphate buffer at pH 7.6 at 25°. The substrate and all inhibitors were also 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. To study inhibition, appropriate amounts of buffer were

TABLE I.—50% INHIBITION AND PARTIAL INHIBITION OF ADENOSINE DEAMINASE BY CERTAIN 6-SUBSTITUTED-9-(5-DEOXY-β-D-XYLOFURANOSYL) PURINES

Compd.	m*M* Concn. for 50% Inhibition[a]	$[I/S]_{0.5}$	% Inhibition by 0.12 m*M* Concn. of Inhibitor[a]
IX (6-NH_2)[b]	0.21	3.2	32
X (6-NHMe)	0.020	0.31	86
XIII (6-OMe)	0.17	2.6	45
XI (6-NMe_2)	...	...	26

[a] The concentration of adenosine in all experiments was 0.066 m*M*. [b] This compound is also a substrate of adenosine deaminase with a $K_m = 6.4 \times 10^{-3}$ *M*.

excluded from the cells and replaced by an equal volume of a solution of the inhibitor in buffer. An 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. Those compounds which were inhibitory were evaluated by determining the concentration required to give 50% inhibition. The ratio of the millimolar concentration of the inhibitor to the millimolar concentration of the substrate for 50% inhibition $[I/S]_{0.5}$ can be used to compare the relative ability of a compound to inhibit the enzymatic reaction. This $[I/S]_{0.5}$ ratio can be used to compare the inhibitory property of any compound, provided that the substrate concentration is always held constant. To determine the concentration of an inhibitor required for 50% inhibition, a plot of Vo/V *versus* I is made, where Vo = initial velocity of the uninhibited enzymatic reaction, V = initial velocity of the inhibited enzymatic reaction at various inhibitor concentrations, and I = the various concentrations of inhibitor (16).

RESULTS

The initial screening of the 5-deoxyxylofuranosyl nucleosides revealed that the compounds with a 6-mercapto group (XII) and a 6-hydrogen atom (XIV) on the purine nucleus were essentially noninhibitory at concentrations twice that of the substrate. Those nucleosides with an amino (IX), methylamino (X), dimethylamino (XI), and methoxy group (XIII) at the 6-position of the purine nucleus were inhibitory. The results of the determination of the $[I/S]_{0.5}$ value for compounds IX, X, and XIII are given in Table I. The 6-dimethylamino analog (XI) was also inhibitory; but when an attempt was made to determine $[I/S]_{0.5}$ for XI, a nonlinear relationship existed when Vo/V was plotted against the concentration of the inhibitor. In addition, XI was an inhibitor weaker than IX, X, or XIII; consequently, it was impossible to add sufficient quantities of XI to the test solution to cause 50% inhibition of the enzymatic reaction because both the inhibitor and substrate exhibit a large amount of absorption at 265 mμ. Finally, it was found that IX was a substrate for the enzymatic reaction.

DISCUSSION

Previous studies on the inhibition of adenosine deaminase by substituted cycloaliphatic purines revealed that for effective inhibition an amino or a methylamino group was required at the 6-position of the purine nucleus, with the amino group being more effective than a methylamino group. In addition, it was found that a substituent was required at the 9-position of the purine nucleus, and a 2′-hydroxy group on that substituent contributed to the binding more than a 3′-hydroxy or a 4′-hydroxymethyl group (6–8).

In the present investigation it also has been found that those nucleosides with a 6-amino group were inhibitors of adenosine deaminase. However, rather than finding the expected result—that the inhibition decreased in the order 6-amino > 6-methylamino > 6-dimethylamino—the effectiveness of inhibition decreased in the following order: 6-methylamino > 6-amino > 6-dimethylamino. At first glance, this reversal of order of effectiveness of inhibition might seem anomalous, but an explanation of this order of inhibition became apparent when the compounds were tested as substrates of adenosine deaminase. Thus, the 6-amino analog (IX) was a substrate of the enzyme, whereas the 6-methylamino (X) and the 6-dimethylamino (XI) analogs were exclusively inhibitors. Because IX is a substrate of adenosine deaminase, its apparent effectiveness as an inhibitor will be less than its real value because during the determination of the $[I/S]_{0.5}$, the concentration of IX is continually being decreased by the enzymatic reaction. The observation that IX is a substrate of adenosine deaminase poses an interesting question—"What are the structural requirements that decide whether a compound will be a substrate or an inhibitor of an enzyme?" This problem is certainly worthy of further investigation.

The authors have been interested in determining the mode of binding by the 6-amino group of the purine nucleus to the enzyme, and our results allow us to draw certain conclusions concerning this point. *A priori*, it could be suggested that one of the possible modes of binding is that the N-H group of adenosine forms a hydrogen bond with an unshared pair of electrons on the enzyme; or it could be suggested that the unshared pair of electrons on the amino group of adenosine are involved in bonding to the enzyme, possibly by means of a hydrogen bond. If it is assumed that the inhibitors IX, X, and XI bind to the active site of the enzyme, *i.e.*, the same site to which adenosine binds, then these data allow the exclusion of the first postulate above and suggest that the bonding by the group at the 6-position of the purine nucleus is through its unshared electrons. The reasoning is based on the following argument.

It does not appear that a basic group is necessary at the 6-position of the purine nucleus for binding since the 6-methoxy analog (XIII), which is essentially neutral, is a relatively good inhibitor. Since XIII does not possess a hydrogen at the 6-position capable of forming a strong hydrogen bond, we believe that it follows that the hydrogens on the amino group of the substrate, adenosine, do not participate in binding by means of hydrogen bonds. Consequently, we believe that the mode of binding to the enzyme by the group at the 6-position is either by means of its unshared electrons or by the inductive effect of that group on the purine nucleus. Such a conclusion appears to be reasonable, since XIV, a nucleoside with a hydrogen at the 6-position which is sterically smaller than an amino or methoxy group, is essentially inactive as an inhibitor.

Finally, it is apparent that the stereochemistry of the substituent on the 9-position of the purine nucleus can vary considerably from that of ribose without appreciably affecting binding to the enzyme. For example, in the present case, the hydroxy group at $C_{5'}$ is absent, and the hydroxy group at $C_{3'}$ is in the opposite configuration than found in the normal substrate, adenosine. These results support the previously stated view that the active site of adenosine deaminase has a large bulk tolerance in the area where the 9-substituent of adenosine analogs bind (6). Furthermore, an inspection of molecular models shows that the hydrogen of the $C_{3'}$-hydroxy group of the 5-deoxyxylofuranosylpurines can occupy an almost identical position as the hydrogen of the $C_{5'}$-hydroxy group in one of the conformations of adenosine. It, therefore, is possible that in compounds like IX, X, and XIII the hydrogen of the $C_{3'}$-hydroxy group is bound to the enzyme at that position where the hydrogen of the $C_{5'}$-hydroxy group of adenosine would be bound normally.

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Hypotensive Activity of Two Benzothiadiazine Derivatives

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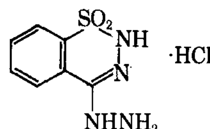
4-Hydrazino-1,2,3-benzothiadiazine-1,1 dioxide hydrochloride (EX 4211A) and 6-chloro-3,4-dihydro-3-(β-oxo-*n*-propyl)-7-sulfamyl-2H-1,2,4-benzothiadiazine 1,1-dioxide phthalazone azine (EX 5004) were studied in the rat and dog to elucidate the mechanism(s) by which these compounds produced hypotensive effects. EX 4211A and EX 5004 were potent hypotensive agents in the anesthetized rat, mildly hypotensive in the anesthetized normotensive dog, and produced a moderate degree of hypotension in unanesthetized renal hypertensive dogs. EX 5004 and EX 4211A appeared to produce their hypotensive effect by the lowering of peripheral resistance through a direct action on vascular smooth muscle.

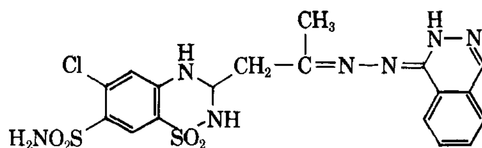
Chlorothiazide and other benzothiadiazine derivatives have been utilized to lower the blood pressure of hypertensive patients and to enhance the hypotensive activity of other antihypertensive compounds (1, 2). This present report describes the hypotensive activity and possible mechanisms of action of two benzothiadiazine derivatives[1]: EX 4211A, 4-hydrazino-1,2,3-benzothiadiazine-1,1 dioxide hydrochloride, and EX 5004, 6-chloro-3,4-dihydro-3-(β-oxo-*n*-propyl)-7-sulfamyl-2H-1,2,4-benzothiadiazine 1,1-dioxide phthalazone azine.

EXPERIMENTAL

Hypotensive Activity in Rats.—The compounds were evaluated for their hypotensive activity in anesthetized normotensive rats using a modification



EX 4211A



EX 5004

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[1] Kindly supplied by Lakeside Laboratories, Milwaukee Wis.