Potential Anticancer Agents.[1] LXXVI. Synthesis of Purine Nucleosides of β-D-Arabinofuranose

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The synthesis of 9-(β-D-arabinofuranosyl)adenine (VI) from 9-(β-D-xylofuranosyl)adenine is described. The arabinosides of hypoxanthine, 6-mercaptopurine, 6-chloropurine, and a number of other 6-substituted purines, as well as the arabinoside of purine, were also prepared, starting from 9-(β-D-arabinofuranosyl)adenine (VI).

There has been a growing interest in the last few years in nucleosides derived from β-D-arabinofuranose, this interest stemming mainly from certain interesting biological properties of the arabinosides of uracil and cytosine. Pizer and Cohen[2] observed that spongouridine[3] was not cleaved by the enzymes that rupture the base-sugar bond of uridine, although it was phosphorylated to the nucleotide by the enzymes that phosphorylated deoxyuridine. In addition, there was slight but detectable methylation of spongouridylic acid by the enzymes that methylate deoxyuridylic acid. Chu and Fischer[4a] observed that spongocytidine profoundly inhibited the metabolic conversion of cytidine, presumably as its diphosphate, to the corresponding 2′-deoxycytidine diphosphate. In addition, spongocytidine is a potent inhibitor of the growth of tumor cells in culture and also causes striking regression of well established tumors in mice.[4b]

These results with pyrimidine arabinosides made it of interest to synthesize some purine β-D-arabinosides in the hope that they, too, would exhibit some interesting biological activities. The syntheses of a number of purine β-D-arabinosides are described in this paper.

The *cis* arrangement of functional groups on C-1′:C-2′ of the spongonucleosides precludes a synthesis *via* a direct nucleoside condensation with a derivative of D-arabinose. Such a condensation gives the α-arabinoside.[5] An indirect method starting with a β-D-xyloside was utilized.[6]

Treatment of 9-(3′,5′-*O*-isopropylidene-β-D-xylofuranosyl)adenine (I)[7] with methanesulfonyl chloride gave the crystalline 9-(3′,5′-*O*-isopropylidene-2′-*O*-methanesulfonyl-β-D-xylofuranosyl)-adenine (II). Removal of the ketal blocking group of II proved to be unexpectedly difficult. Thus, treatment of II with 70% aqueous acetic acid at reflux temperatures for up to seven hours gave incomplete reaction, although such conditions were adequate for the deacetonation of other 3,5-*O*-isopropylidene xylofuranosides.[7] Conversion of II to III was accomplished by the use of 90% aqueous acetic acid at 100° for five hours, with no evidence for nucleoside cleavage. Epoxide formation from III was effected with methanolic sodium methoxide to give 9-(2′,3′-anhydro-β-lyxofuranosyl)adenine (VII), which could be purified via its picrate.

A number of different methods were investigated to convert the epoxide (VII) to the arabinoside (VI). Among the reagents investigated for this reaction, sodium acetate in acetic anhydride and acetic acid acetylated the nucleoside but did not open the epoxide ring, since base catalyzed deacetylation of the product gave back the starting anhydronucleoside (VII). Treatment of VII with aqueous sodium hydroxide brought about cleavage of the nucleoside link and an almost quantitative yield of adenine was obtained. The reaction of the nucleoside (VII) with sodium benzoate or sodium acetate in 95% aqueous *N*,*N*-dimethylformamide, however, effectively cleaved the epoxide ring to give in good yield a crude product from which the desired arabinoside (VI) could be isolated easily. It is interesting to note that the reaction of sodium benzoate in diethylene glycol dimethyl ether containing 5% water, using the same time and temperature conditions that were successful with *N*,*N*-dimethylformamide, gave only trace amounts (as shown by paper chromatography) of the desired arabinoside (VI).

There is ample precedent for the nucleophilic opening of a furanose epoxide such as VII to occur predominantly at C-3′.[8] Thus, the epoxide (VII) could be expected to give the arabinoside (VI)

(1) This work was carried out under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Public Health Service, Contract No. SA-43-ph-1892. The opinions expressed in this paper are those of the authors and are not necessarily those of the Cancer Chemotherapy National Service Center. For the preceding paper in this series, see M. E. Wain, E. M. Acton, B. R. Baker, and L. Goodman, *J. Org. Chem.*, **27**, 2905 (1962).

(2) L. I. Pizer and S. S. Cohen, Abstr. 136th Meeting, American Chemical Society 1959, p. 9-C; *J. Biol. Chem.*, **235**, 2387 (1960).

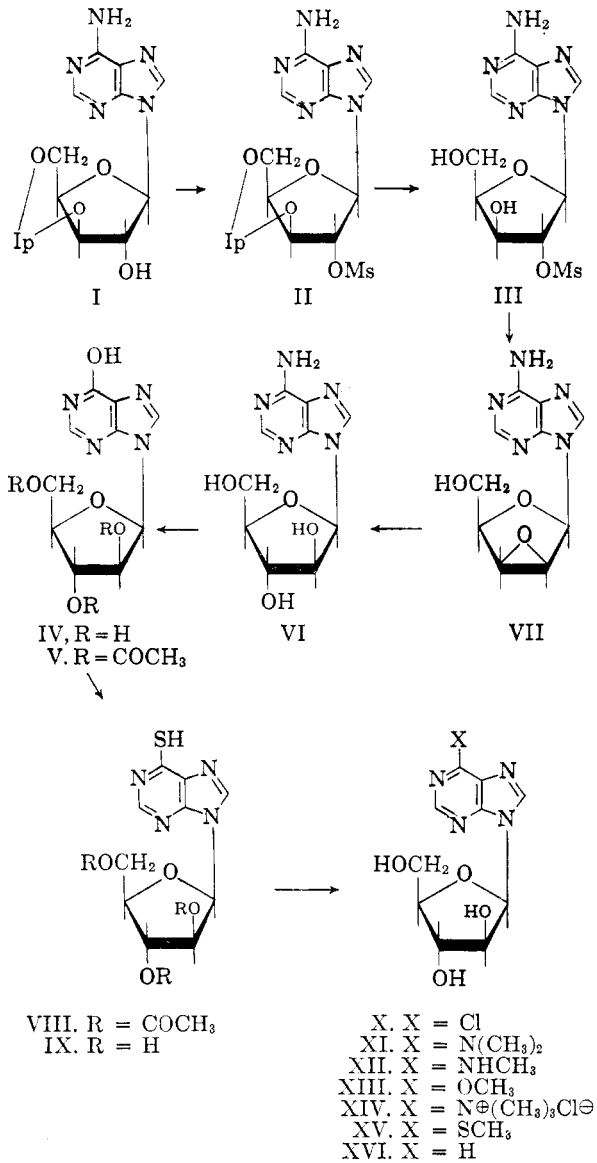
(3) The term "spongonucleosides" is a trivial name that is applied to nucleosides that contain the sugar β-D-arabinofuranose.

(4) (a) M. Y. Chu and G. A. Fischer, *Biochem. Pharm.*, **2**, 423 (1962); (b) J. S. Evans, E. A. Musser, G. D. Mengel, K. R. Forsblad, and J. H. Hunter, *Proc. Soc. Exp. Biol. Med.*, **106**, 350 (1961).

(5) N. W. Bristow and B. Lythgoe, *J. Chem. Soc.*, 2306 (1949).

(6) A preliminary communication from these laboratories on the synthesis of spongoadenosine has been published, see W. W. Lee, A. Benitez, L. Goodman, and B. R. Baker, *J. Am. Chem. Soc.*, **82**, 2648 (1960).

(7) A. Benitez, O. P. Crews, Jr., L. Goodman, and B. R. Baker, *J. Org. Chem.*, **25**, 1946 (1960).



rather than the isomeric xyloside[9] that would result from opening at C-2′. Paper chromatographic behavior, as well as a depression in the mixed melting point with authentic xyloside, proved conclusively that the main product of the epoxide opening was not the xyloside. A two-dimensional paper chromatogram of the crude reaction product, using water-saturated butanol in one direction and benzene–water–methanol (2:1:6) in the second direction, indicated that there were trace amounts of epoxide opening at C-2 to give the xyloside. This trace was easily removed by recrystallization of the crude product to give pure arabinoside (VI), which was now homogeneous in the two-dimensional paper chromatogram. A periodate titration confirmed that the C-2′:C-3′ hydroxyls existed in the expected *trans* relationship.[10] Further confirmation of the arabinose configuration for the sugar portion of VI was obtained by the hydrolysis of VI with hydrochloric acid. Paper chromatography of the hydrolysis product showed one reducing sugar which corresponded with D-arabinose and which was significantly different from D-xylose, D-ribose, and D-lyxose. For preparative purposes, it was possible to obtain spongoadenosine (VI) in a 78% over-all yield from the xyloside ketal (I) when the intermediates were used directly without prior purification.

It was of interest to prepare the arabinoside (IX) of 6-mercaptopurine, both for biological evaluation and for its synthetic versatility in the preparation of a variety of purine arabinosides. With this end in mind, spongoadenosine (VI) was treated with nitrous acid to give hypoxanthine arabinoside (IV). Acetylation of IV gave the triacetate (V), which was treated with phosphorus pentasulfide in dry pyridine to yield the triacetate (VIII) of 6-mercaptopurine arabinoside. Deacetylation of VIII with methanolic sodium methoxide gave 9-(β-D-arabinofuranosyl)-9*H*-purine-6-thiol (IX). The over-all yield of IX from spongoadenosine (VI) was 48% when crude intermediates were used without prior purification.

A recent communication by Robins[11] describes the synthesis of ribonucleosides of 6-chloropurine by the reaction of chlorine gas with the riboside of 6-mercaptopurine. Reaction of the arabinoside (IX) with chlorine gas gave a 50% yield of the 6-chloropurine arabinoside (X). The relatively low yield of product is primarily due to the great reactivity of the chlorine atom in X, resulting in handling losses during purification. It was observed that losses of 50% or more of the product (X) were suffered with each recrystallization from water, the most useful crystallization solvent.

That the chlorination reaction of the thiol (IX) proceeded in high yield was demonstrated when the crude chloropurine arabinoside (X) was treated directly with aqueous dimethylamine to give the 6-dimethylaminopurine arabinoside(XI) in a 95% yield from the 6-thiol (IX). Similarly, the 6-methylaminopurine arabinoside (XII) and the 6-methoxypurine arabinoside (XIII) were each obtained in 88% yield from the thiol (IX) by reaction of the intermediate chloronucleoside (X) with 40% aqueous methylamine and methanolic sodium methoxide, respectively. Reaction of the chloronucleoside (X) with ammonia gave spongoadenosine (VI), thus demonstrating that no rearrangements or migration of functional groups had occurred during the previous transformations.

The quaternary salt (XVIII), prepared from 6-chloropurine and trimethylamine, has been syn-

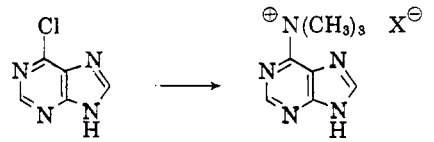
(8) (a) C. D. Anderson, L. Goodman, and B. R. Baker, *J. Am. Chem. Soc.*, **81**, 898 (1959); (b) R. E. Schaub and M. J. Weiss, *ibid.*, **80**, 4683 (1958).

(9) B. R. Baker and K. Hewson, *J. Org. Chem.*, **22**, 966 (1957).

(10) J. J. Fox, N. Yung, and A. Bendich, *J. Am. Chem. Soc.*, **79**, 2775 (1957).

(11) R. K. Robins, *ibid.*, **82**, 2654 (1960).

thesized[12] and found to be active against Ehrlich ascites and Epidermoid carcinoma DC5. It seemed interesting to prepare a nucleoside of such a compound from the available chloropurine arabinoside (X). An 89% yield of 9-(β-D-arabinofuranosyl)-6-trimethylammonium-9*H*-purine chloride (XIV) was obtained by the reaction of purified chloronucleoside (X) with anhydrous trimethylamine. The quaternary nucleoside (XIV) was



XVII XVIII. X = Cl
XIX. X = OCH_3

extraordinarily unstable and the mild heating necessary for recrystallization resulted in the loss of the elements of chloromethane to give the 6 dimethylaminonucleoside (XI). For this reason, the quaternary nucleoside (XIV) could not be recrystallized and it was necessary to use purified chloronucleoside (X) for its preparation.

An interesting variation of the unusual reactivity of quaternary nitrogenous purines of the type illustrated by XIV was observed during model experiments for the preparation of the 6-trimethylammoniumpurine arabinoside (XIV). In this case, the reaction of 6-chloropurine (XVII) with anhydrous trimethylamine gave a material, assumed to be the 6-trimethylammoniumpurine chloride (XVIII), which was homogeneous on paper chromatography. Recrystallization of crude XVIII from methanol gave a crystalline product which was still homogeneous on paper chromatography but had a broad melting point and analyzed for a 1:1 mixture of the chloride (XVIII) and the methoxide (XIX). Confirmatory evidence for the presence of XIX was obtained by the presence of a sharp singlet in the n.m.r. spectrum at 3.35 p.p.m., which is consistent with methoxyl and of the intensity to be expected for a 1:1 mixture of XVIII and XIX. In addition, the expected $N(CH_3)_3$ band was present at 3.83 p.p.m. A recent report by Lewis *et al.*[13] described the reaction of trimethylamine with 6-chloro-9-(tetrahydro-2-furyl)-9*H*-purine to give the trimethylpurinylammonium chloride. This compound showed significant antitumor activity against adenocarcinoma 755 in mice.

Methylation of the thiol (IX) with methyl iodide and potassium carbonate in dimethylsulfoxide gave an 82% yield of the 6-methylthiopurine arabinoside (XV). Purine arabinoside (XVI) was prepared in 77% yield by the desulfurization of the thiol (IX) with a sponge nickel catalyst.

Biological evaluation of these nucleosides is in progress.

Experimental[14]

9-(3′,5′,-*O*-Isopropylidene-2′-*O*-methanesulfonyl-β-D-xylofuranosyl)adenine (II).—A solution of 250 mg. (0.81 mmole) of 9-(3′,5′-*O*-isopropylidene-β-D-xylofuranosyl)adenine (I)[7] in 3 ml. of anhydrous pyridine was cooled to 0° and 0.11 ml. (1.4 mmoles) of methanesulfonyl chloride was added with stirring. The reaction mixture was stored at room temperature for 72 hr., then quenched by the addition of 26 ml. of water. The aqueous mixture was extracted with three 10-ml. portions of chloroform. The chloroform extracts were combined and washed with two 10-ml. portions of saturated aqueous sodium bicarbonate and 10 ml. of water. The organic layer was dried over magnesium sulfate, then evaporated to dryness *in vacuo* to give 240 mg. (77%) of crude product (II) that was suitable for the next step; $\lambda_{max(\mu)}^{Nujol}$ 3.05, 3.21 (NH_2), 8.46 (OSO_2). Recrystallization from water gave a 63% yield of the analytical sample, m.p. 212.0–212.5°, $[\alpha]^{25.9}D$ −77° (1.0% in methanol).

Anal. Calcd. for $C_{14}H_{19}N_5O_6S$: C, 43.6; H, 4.97; N, 18.2. Found: C, 43.4; H, 4.95; N, 18.1.

9-(2′-*O*-Methanesulfonyl-β-D-xylofuranosyl)adenine (III).—A solution of 100 mg. (0.26 mmole) of crude II in 1 ml. of 90% aqueous acetic acid was heated with stirring at 100° for 5 hr. The yellow solution was evaporated to dryness *in vacuo*. The last traces of acetic acid were removed by the addition and removal *in vacuo* of two 4-ml. portions of ethanol–toluene (1:1). The residue, which weighed 88 mg. (98%), was homogeneous as shown by paper chromatography,[14] with R_{Ad} 0.67 in solvent A and 1.42 in solvent B, and was suitable for the next step.

A solution of 82 mg. of crude III in 5 ml. of acetonitrile was treated with Norit, then filtered. The filtrate was diluted with 5 ml. of benzene, then concentrated *in vacuo* to 3 ml. and stored at room temperature to crystallize. The crystalline material weighed 44 mg. (49%), m.p. 170.5–171.0°; $\lambda_{max(\mu)}^{Nujol}$ 3.00, 3.16 (NH_2,OH), 8.49 (OSO_2).

Anal. Calcd. for $C_{11}H_{15}N_5O_6S$: C, 38.3; H, 4.38; N, 20.3; S, 9.27. Found: C, 38.4; H, 4.43; N, 20.2; S, 9.01.

9-(2′,3′-Anhydro-β-D-lyxofuranosyl)adenine (VII).—To a hot solution of 890 mg. (2.58 mmoles) of recrystallized methanesulfonate (III) in 20 ml. of absolute methanol was added a solution of 210 mg. (3.89 mmoles) of methanolic sodium methoxide. The reaction mixture was heated at reflux for 12 min., then concentrated to 5 ml. *in vacuo*. The mixture was cooled to room temperature and neutralized to pH 7 with glacial acetic acid. The methanolic solution was evaporated to dryness *in vacuo* to give the crude anhydronucleoside (VII), which was free of starting material as shown by paper chromatography (R_{Ad} 0.97 in solvent C) and which was of satisfactory purity to use in the next step.

To a solution of the above crude anhydronucleoside (VII) in 10 ml. of water was added 90 ml. of saturated aqueous picric acid. The aqueous solution was cooled at 0° for 3 hr.,

(12) J. P. Horowitz and V. K. Vaitkevicius, *Experimentia*, **17**, 552 (1961).

(13) L. R. Lewis, F. H. Schneider, and R. K. Robins, *J. Org. Chem.*, **26**, 3837 (1961).

(14) Melting points were taken on a Fisher-Johns apparatus and are uncorrected. Paper chromatograms were run by the descending technique on Whatman No. 1 paper in the following solvent systems: A, water-saturated *n*-butyl alcohol; B, 5% aqueous disodium hydrogen phosphate; C, *n*-butyl alcohol–methyl ethyl ketone–water (5:3:2); D, *n*-butyl alcohol–ethanol–water (5:1:1). Adenine was used as a standard and spot locations were expressed as R_{Ad} units with adenine at 1.00. The nucleoside spots were located by visual examination under ultraviolet light and the reducing sugar spots by an aniline citrate spray. The n.m.r. spectra were run in deuterium oxide, with 6.25% tetramethylsilane in carbon tetrachloride as an external standard.

then the insoluble picrate was collected by filtration. The picrate was washed with 16 ml. of ice-cold water, then was regenerated in 60 ml. of water in the usual manner,[15] using Dowex 2 (acetate), to give 450 mg. (70%) of nucleoside (VII) as a white solid, m.p. 200–207° dec. Recrystallization from 28 ml. of absolute ethanol gave 290 mg. of pure anhydronucleoside (VII), m.p. 205–206° dec., $[\alpha]^{29.5}D$ −13° (1.0% in water). This product moved as a single spot on paper chromatography in solvents A (R_{Ad} 0.54) and B (R_{Ad} 1.32).

Anal. Calcd. for $C_{10}H_{11}N_5O_3$: C, 48.2; H, 4.45; N, 28.1. Found: C, 48.2; H, 4.50; N, 27.9.

9-(β-D-Arabinofuranosyl)adenine (Spongoadenosine) (VI). A mixture of 100 mg. (0.40 mmole) of purified anhydronucleoside (VII) and 100 mg. (1.22 mmoles) of anhydrous sodium acetate in 5 ml. of 95% aqueous *N,N*-dimethylformamide was heated with stirring at 156° for 6 hr., then evaporated to dryness *in vacuo*. The crude arabinoside (VI) was free from starting material as shown by paper chromatography.

The residue was recrystallized from about 3 ml. of water to give 310 mg. (29%) of the arabinoside (VI), m.p. 257.0–257.5°; $[\alpha]^{27}D$ −5° (0.25% in water); $\lambda_{max}^{pH\ 1}$ 257.5 mμ (ε 12,700), $\lambda_{max}^{pH\ 7}$ 259 mμ (ε 13,400), $\lambda_{max}^{pH\ 13}$ 259 mμ (ε 14,000). This product moved as a single spot on paper chromatography in solvent A (R_{Ad} 0.47).

Anal. Calcd. for $C_{10}H_{13}N_5O_4 \cdot 0.4H_2O$: C, 43.8; H, 5.07; N, 25.5. Found: C, 43.9; H, 4.81; N, 25.5.

On a larger scale, 5.00 g. of the xyloside (I) was converted to 2.72 g. of recrystallized arabinoside (VI) for an over-all yield of 78%. In this case, no effort was made to purify the intermediates and the crude products were used directly in the subsequent reactions.

Hydrolysis of Spongoadenosine (VI).—A solution of 3 mg. of spongoadenosine (VI) in 2 ml. of 0.01 *N* aqueous hydrochloric acid was heated at reflux for 3 hr. The reaction mixture was evaporated to dryness *in vacuo* to give a residue which was chromatograhed on Whatman No. 1 paper using solvent D. The resulting chromatogram showed one spot at R_{Ad} 1.00 by ultraviolet examination and one spot at R_{Ad} 0.41 by an aniline citrate reducing sugar spray. The latter spot corresponded with D-arabinose. D-Xylose, D-ribose, and D-lyxose had R_{Ad} values of 0.46, 0.51, and 0.49, respectively.

9-(β-D-Arabinofuranosyl)hypoxanthine (IV).—A suspension of 2.70 g. (10.1 mmoles) of spongoadenosine (VI) and 4.54 g. (65.8 mmoles) of sodium nitrite in 135 ml. of water that contained 15 ml. of glacial acetic acid and 10.1 ml. (10.1 mmoles) of 1 *N* hydrochloric acid was stored in a stoppered flask at room temperature for 67 hr. By this time solution was complete and the reaction mixture was evaporated to dryness *in vacuo*. The last traces of acetic acid and hydrochloric acid were removed by the addition and removal *in vacuo* of an additional 50 ml. of water. The residue was recrystallized from 25 ml. of water to give 1.99 g. (73%) of the desired nucleoside (IV), m.p. 232.5–234.0°.

Two more recrystallizations from water gave the analytical sample, m.p. 243–245°; $[\alpha]^{26}D$ +5° (1% in 0.1 *N* aqueous sodium hydroxide); $\lambda_{max}^{pH\ 1,7}$ 249 mμ (ε 12,000), $\lambda_{max}^{pH\ 13}$ 254 mμ (ε 13,700). This product moved as a single spot on paper chromatography in solvent B with R_{Ad} 2.12.

Anal. Calcd. for $C_{10}H_{12}N_4O_5$: C, 44.8; H, 4.51; N, 20.9. Found: C, 44.4; H, 4.04; N, 20.7.

9-(2′,3′,5′-Tri-*O*-acetyl-β-D-arabinofuranosyl)hypoxanthine (V).—To a suspension of 50 mg. (0.19 mmole) of 9-(β-D-arabinofuranosyl)hypoxanthine (IV) in 3.0 ml. of dry pyridine was added 0.07 ml. (0.75 mmole) of acetic anhydride. The reaction mixture was stored at room temperature for 26 hr., by which time solution was complete. The excess acetic anhydride was decomposed with 0.25 ml. of methanol, then the reaction mixture was evaporated to dryness *in vacuo* to give 73.4 mg. (100%) of crude product, m.p. 228–231°.

Recrystallization from 2 ml. of water gave the analytical sample, m.p. 231.0–232.0°; $[\alpha]^{24}D$ −29° (1% in pyridine); $\lambda_{max}^{pH\ 1,7}$ 248 mμ (ε 12,200), $\lambda_{max}^{pH\ 13}$ 253 mμ (ε 13,500).

Anal. Calcd. for $C_{16}H_{18}N_4O_8$: C, 48.7; H, 4.60; N, 14.2. Found: C, 48.8; H, 5.00; N, 14.2.

9-(2′,3′,5′-Tri-*O*-acetyl-β-D-arabinofuranosyl)-9*H*-purine-6-thiol (VIII).—To a solution of 50 mg. (0.127 mmole) of triacetylarabinofuranosylhypoxanthine (V) in 2.5 ml. of dry pyridine was added 134 mg. (0.60 mmole) of phosphorus pentasulfide. The reaction mixture was heated with stirring at 135° for 4 hr. while protected from moisture. The resulting dark solution was poured into 50 ml. of hot water and the aqueous mixture was evaporated to dryness *in vacuo*. The residue was triturated with 2 ml. of cold water, then collected on a filter to give 49 mg. (94%) of a dark solid, m.p. 188–194°. This solid was free of starting material as shown by the absence of infrared absorption at 5.90 μ, attributable to the hypoxanthine, and also by paper chromatography in solvents B and C.

Recrystallization from water gave a nearly colorless solid, m.p. 217–218°; $[\alpha]^{22}D$ −39° (1% in pyridine); $\lambda_{max}^{pH\ 1}$ 321 mμ (ε 26,000), $\lambda_{max}^{pH\ 7}$ 319 mμ (ε 24,300), $\lambda_{max}^{pH\ 13}$ 310 mμ (ε 24,000).

Anal. Calcd. for $C_{16}H_{18}N_4O_7S$: C, 46.8; H, 4.42; N, 13.7; S, 7.81. Found: C, 46.2; H, 4.16; N, 13.9; S, 8.02.

9-(β-D-Arabinofuranosyl)-9*H*-purine-6-thiol (IX).—A solution of 397 mg. (0.97 mmole) of triacetate (VIII) in 12 ml. of methanol that contained 68.3 mg. (1.26 mmoles) of sodium methoxide was heated under reflux for 3.25 hr. while protected from moisture. The reaction mixture was cooled to room temperature, then carefully neutralized to pH 7 with glacial acetic acid. The neutralized solution was cooled at 0°, then filtered to yield 278 mg. (101%) of crude product (IX).

Purification was effected by treating a suspension of the crude product in 4 ml. of water with sufficient aqueous ammonia to cause solution. The ammoniacal solution was decolorized with Norit, then neutralized with acetic acid as described above, to give 219 mg. (79%) of product (IX) as a white solid that was homogeneous on paper chromatography in solvent C with R_{Ad} 0.80, and had m.p. 165–190° dec. (preheated block); $[\alpha]^{26}D$ −26° (1% in 0.1 *N* aqueous sodium hydroxide); $\lambda_{max}^{pH\ 1}$ 322 mμ (ε 23,600), $\lambda_{max}^{pH\ 7}$ 319 mμ (ε 24,500), $\lambda_{max}^{pH\ 13}$ 310 mμ (ε 23,800).

Anal. Calcd. for $C_{10}H_{12}N_4O_4S \cdot 0.4H_2O$: C, 41.2; H, 4.43; N, 19.2; S, 11.0. Found: C, 41.1; H, 4.74; N, 19.3; S, 11.0.

On a 5-g. scale, an over-all yield of 48% of the nucleoside thiol (IX) was obtained from spongoadenosine (VI) when the intermediates were used directly without purification.

9-(β-D-Arabinofuranosyl)-6-chloro-9*H*-purine (X).—A suspension of 100 mg. (0.35 mmole) of 9-(β-D-arabinofuranosyl)-9*H*-purine-6-thiol (IX) in 5 ml. of absolute methanol was cooled to −10° while protected from moisture. Chlorine gas was slowly bubbled through the cold mixture for 2 min., by which time solution was complete. The resulting yellow solution was stirred at −10° for about 5 min., then dry nitrogen was bubbled through the cold solution for 15 min., by which time excess chlorine had been removed as evidenced by the discharge of the yellow color.[16] Saturated methanolic ammonia (about 15 drops) was added to the reaction dropwise to neutralize the excess acid, then the solution was evaporated to dryness *in vacuo*. The residue was treated with 2 ml. of water and cooled at 0°, then filtered to yield 50 mg. (49%) of the desired nucleoside (X), which was homogeneous as shown by

(15) E. J. Reist, L. Goodman, R. R. Spencer, and B. R. Baker, *J. Am. Chem. Soc.*, **80**, 3962 (1958).

(16) If the excess chlorine gas is not removed as described, the subsequent neutralization with ammonia may cause the reaction to ignite spontaneously, presumably due to the formation of the highly unstable nitrogen trichloride.

paper chromatography and was suitable for subsequent reactions.

Recrystallization from 2 ml. of water gave 21 mg. of product, m.p. 171–173° dec.; $[\alpha]^{25}D$ +17° (1% in water); $\lambda_{max}^{pH\ 1,7}$ 264 mμ (ε 9830), $\lambda_{max}^{pH\ 13}$ 266 mμ (ε 10,000). The product was homogeneous in solvents A (R_{Ad} 2.01) and C (R_{Ad} 1.48).

Anal. Calcd. for $C_{10}H_{11}ClN_4O_4$: C, 41.9; H, 3.87; Cl, 12.4; N, 19.5. Found: C, 42.0; H, 4.15; Cl, 12.4; N, 19.6.

9-(β-D-Arabinofuranosyl)-6-dimethylamino-9*H*-purine (XI).—The chlorination of 100 mg. of the 6-thiol (IX) was carried out as described for the preparation of the 6-chloro nucleoside (X). After the excess chlorine had been removed with dry nitrogen, 2 ml. (11 mmoles) of 25% aqueous dimethylamine was added to the reaction mixture, which was then heated in a stainless steel bomb at 115° for 4.5 hr. The bomb was cooled to 0° and the contents were evaporated to dryness *in vacuo*. The residue was recrystallized from 4 ml. of water to give 99 mg. (95%) of product, m.p. 208–210°.

A second recrystallization from water gave the analytical sample, m.p. 210–211°; $[\alpha]^{19}D$ −7° (1% in methanol); $\lambda_{max}^{pH\ 1}$ 268 mμ (ε 16,000), $\lambda_{max}^{pH\ 7}$ 275 mμ (ε 15,700), $\lambda_{max}^{pH\ 13}$ 276 mμ (ε 15,200). The product was homogeneous on paper chromatography in solvent A with R_{Ad} 1.88.

Anal. Calcd. for $C_{12}H_{17}N_5O_4$: C, 48.8; H, 5.80; N, 23.7. Found: C, 48.8; H, 6.07; N, 23.9.

9-(β-D-Arabinofuranosyl)-6-methylamino-9*H*-purine (XII) was prepared in 88% yield from the 6-thiol (IX) by the same general procedure that was used for the preparation of the 6-dimethylamine (XI), with 40% aqueous methylamine substituted for 25% aqueous dimethylamine. The analytical sample, after recrystallization from water, had m.p. 201.5–202.5°; $[\alpha]^{25}D$ +10° (1% in methanol); $\lambda_{max}^{pH\ 1}$ 262.5 mμ (ε 15,500), $\lambda_{max}^{pH\ 7}$ 266 mμ (ε 13,800), $\lambda_{max}^{pH\ 13}$ 266 mμ (ε 14,400). The product was homogeneous on paper chromatography in solvents A (R_{Ad} 1.06) and C (R_{Ad} 1.12).

Anal. Calcd. for $C_{11}H_{15}N_5O_4$: C, 47.0; H, 5.38; N, 24.9. Found: C, 47.2; H, 5.72; N, 25.2.

9-(β-D-Arabinofuranosyl)-6-methoxy-9*H*-purine (XIII).—The reaction of 450 mg. of the 6-thiol (IX) was carried out as described for the preparation of the 6-chloro nucleoside (X). After the excess chlorine had been removed with dry nitrogen, 15 ml. of 1 *N* methanolic sodium methoxide was added to the clear solution and the solution was heated at reflux for 0.5 hr. The reaction mixture was cooled to 0° and adjusted to *p*H 6 with concd. hydrochloric acid, then evaporated to dryness *in vacuo*. The residue was extracted with five 25-ml. portions of hot ethyl acetate. The hot extracts were combined, filtered, and concentrated to 20 ml. To the ethyl acetate solution was added 4 drops of water,[17] then the solution was cooled at 0° and filtered to give 0.42 g. (88%) of nucleoside (XIII), m.p. 100–104°, resolidified 110–115°, remelted 155–165°.

A second crystallization from wet ethyl acetate gave the analytical sample, m.p. 104–108°, resolidified 109–118°, remelted 175–179°; $[\alpha]^{27}D$ +19° (1% in water); $\lambda_{max}^{pH\ 1}$ 251 mμ (ε 10,400), $\lambda_{max}^{pH\ 7}$ 251.5 mμ (10,900), $\lambda_{max}^{pH\ 13}$ 252 mμ (ε 11,100). The product was homogeneous on paper chromatography in solvents A (R_{Ad} 1.49) and C (R_{Ad} 1.36).

Anal. Calcd. for $C_{11}H_{14}N_4O_5 \cdot H_2O$: C, 44.0; H, 5.37; N, 18.7. Found: C, 44.2; H, 5.48; N, 18.5.

9-(β-D-Arabinofuranosyl)-6-trimethylammonium-9*H*-purine Chloride (XIV).—A solution of 100 mg. (0.35 mmole) of analytically pure 9-(β-D-arabinofuranosyl)-6-chloro-9*H*-purine (X) in 30 ml. of dry, freshly distilled tetrahydrofuran was cooled to 0°, then added slowly to 4 ml. of freshly distilled trimethylamine. The reaction was stored at room temperature for 18 hr., then the supernatant liquid was decanted from the product that had separated from the solution. The precipitated solid was triturated with two additional 7-ml. portions of tetrahydrofuran, then dried in a vacuum desiccator at room temperature to give 108 mg. (89%) of the nucleoside (XIV), which was homogeneous on paper chromatography and had R_{Ad} 0.12 in solvent A, R_{Ad} 2.64 in solvent B, and R_{Ad} 0.27 in solvent C; $[\alpha]^{24}D$ +13° (1% in water); $\lambda_{max}^{pH\ 1,7}$ 264.5 mμ (ε 11,000), $\lambda_{max}^{pH\ 13}$ 253 mμ (ε 17,000).

Anal. Calcd. for $C_{13}H_{20}ClN_5O_4$: C, 45.2; H, 5.83; Cl, 10.3; N, 20.3. Found: C, 44.7; H, 5.79; Cl, 10.6; N, 19.8.

This nucleoside was extremely hygroscopic and heat sensitive. When it was heated, it lost the elements of chloromethane to give the 6-dimethylamino nucleoside (XI), which was identified by paper chromatography.

The nuclear magnetic resonance spectrum had a peak with $\overset{\oplus}{N}CH_3$ δ value (in p.p.m.) of 3.93 in addition to the usual peaks assignable to the sugar and purine protons.

6-Trimethylammoniumpurine Chloride(methoxide) (XVIII, XIX).—A mixture of 300 mg. (1.94 mmoles) of 6-chloropurine (XVII) and 4 ml. of anhydrous trimethylamine was stored in a stainless steel bomb at room temperature for 2.5 hr. The excess trimethylamine was allowed to evaporate at room temperature and the residue was transferred to a filter as a suspension in petroleum ether (b.p. 62–70°). The filter cake was washed with 3 ml. of petroleum ether, then dried to give 343 mg. (83%) of the quaternary base (XVIII), m.p. 175–180° (gas evolution); λ_{max}^{water} 267.5 mμ (ε 7990). The crude product was homogeneous on paper chromatography with R_{Ad} 0.49 in solvent A, R_{Ad} 2.06 in solvent B, and R_{Ad} 0.59 in solvent C.

Recrystallization from methanol gave 115 mg. of crystalline material, m.p. 165–232°; $\lambda_{max}^{pH\ 1}$ 266 mμ (ε 7900), $\lambda_{max}^{pH\ 7}$ 268 mμ (ε 6750), $\lambda_{max}^{pH\ 13}$ 273.5 mμ (ε 6710). This material travelled as a single spot on paper chromatography in solvents A and B, with R_{Ad} 0.48 and 2.11, respectively. The chromatogram in solvent C showed an elongated spot, R_{Ad} 0.45–0.63.

Anal. Calcd. for $C_8H_{12}ClN_5$ (49%) + $C_9H_{15}N_5O$ (51%): C, 48.4; H, 6.46; Cl, 8.24; N, 33.1; OCH_3, 7.6. Found: C, 48.0; H, 6.46; Cl, 8.26; N, 33.0; OCH_3, 7.8.

The nuclear magnetic resonance spectrum had peaks with $\overset{\oplus}{N}(CH_3)_3$ δ values (in p.p.m.) of 3.83 and $\overset{\ominus}{O}CH_3$ 3.35.

9-(β-D-Arabinofuranosyl)-6-methylthio-9*H*-purine (XV).—To a stirred mixture of 100 mg. (0.35 mmole) of 6-thiol (IX) and 49 mg. (0.36 mmole) of anhydrous potassium carbonate in 1 ml. of dimethyl sulfoxide was added 69 mg. (0.03 ml., 0.49 mmole) of methyl iodide. The mixture was stirred at room temperature for 1.5 hr. and at 45° for 0.75 hr. The reaction mixture was diluted with 1.5 ml. of water and neutralized to pH 7 with 1 *N* hydrochloric acid. The solution was evaporated to dryness *in vacuo* and the gummy residue was crystallized from 2 ml. of water to give 86 mg. (82%) of the product (XV), m.p. 99–101°, which was homogeneous on paper chromatography in solvents A and C with R_{Ad} values of 2.39 and 2.56, respectively. Recrystallization from water gave the analytical sample with m.p. 98.5–101.0°; $[\alpha]^{25}D$ +11° (1% in methanol); $\lambda_{max}^{pH\ 1}$ 288 mμ (ε 14,200), 293.5 mμ (ε 15,400); $\lambda_{max}^{pH\ 7}$ 287.5 mμ (ε 16,300), 292.5 mμ (ε 16,300); $\lambda_{max}^{pH\ 13}$ 287 mμ (ε 17,600, 292.5 mμ (ε 17,100).

Anal. Calcd. for $C_{11}H_{14}N_4O_4S \cdot 1/3H_2O$: C, 43.4; H, 4.86; N, 18.4; S, 10.5. Found: C, 43.3; H, 4.86; N, 18.1; S, 10.4.

9-(β-D-Arabinofuranosyl)purine (XVI).—A mixture of 200 mg. of the 6-thiol (IX) and 2.0 g. of Davison sponge nickel[18] in 23 ml. of water was stirred at reflux for 4 hr. The reaction mixture was filtered through Celite and the filter cake was washed with 5 ml. of hot water. The combined filtrate and washings were evaporated to dryness *in vacuo*

(17) The nucleoside crystallizes only as a monohydrate.

(18) Sponge nickel catalyst, Davison Chemical Co., Cincinnati 29, Ohio.

to give 136 mg. (77%) of crude product, m.p. 243–245° dec., which was homogeneous on paper chromatography in solvent A with R_{Ad} 1.00. Recrystallization from water gave the analytical sample, m.p. 242–243°; $[\alpha]^{22}D$ +27° (1% in water); $\lambda_{max}^{pH\ 1}$ 263 mμ (ε 5740), $\lambda_{max}^{pH\ 7}$ 263 mμ (ε 7310), $\lambda_{max}^{pH\ 13}$ 264 mμ (ε 7780).

Anal. Calcd. for $C_{10}H_{12}N_4O_4$: C, 47.6; H, 4.80; N, 22.2. Found: C, 47.9; H, 4.91; N, 22.1.

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Potential Anticancer Agents.[1] LXXVII. Synthesis of Nucleosides of Purine-6-thiol (6-Mercaptopurine) Containing "Fraudulent" Sugars

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Syntheses are described for the preparation of 6-mercaptopurine nucleosides (VI, XI, and XXI) containing 2-*S*-ethyl-2-thio-β-D-arabinofuranose, 3-*S*-ethyl-3-thio-β-D-xylofuranose, and 6-deoxy-β-D-allofuranose, respectively. Selective desulfurizations of VI and XI gave 9-(2-*S*-ethyl-2-thio-β-D-arabinofuranosyl)-9*H*-purine (VII) and 9-(3-*S*-ethyl-3-thio-β-D-xylofuranosyl)-9*H*-purine (XII), respectively. Complete desulfurization of XI afforded 9-[3-deoxy-β-D-ribo(xylo)furanosyl]-9*H*-purine (XIII).

Extensive investigations have been carried out on the biological activity of 6-mercaptopurine (6-MP) and its ribonucleoside.[4] There is strong evidence, at least in certain systems, that both 6-MP and its ribonucleoside are active as the ribonucleotide.[4b] Nucleosides of 6-MP that contain a sugar other than D-ribofuranose cannot form this same nucleotide without a preliminary cleavage of the nucleoside to 6-MP. Such nucleosides could possess different spectra of biological activity from 6-MP and could conceivably be active against 6-MP-resistant tumors.

The present interest[1] in nucleosides of β-D-arabinofuranose and the relative availability in these laboratories[5] of the 2-thioethyl-β-D-arabinoside (II) made it of interest to prepare the 6-MP derivative for biological evaluation. The corresponding 3-thioethyl-β-D-xyloside (XI) was also prepared, since modifications of the functional groups and their configurations at C-3′ of the nucleosides have given biologically active compounds.[6] Also included in these syntheses was the 6-MP analog of 9-(6-deoxy-β-D-allofuranosyl)adenine (XVII) because of its obvious similarity to the active 6-MP ribonucleoside.

The preceding communication[1] from these laboratories described the synthesis of 9-β-D-arabinofuranosyl-9*H*-purine-6-thiol by a sequence of reactions starting with 9-β-D-arabinofuranosyladenine. Similar reaction sequences were utilized to prepare the above-mentioned nucleosides of 6-MP.

The reaction of nitrous acid with 9-(3-*S*-ethyl-3-thio-β-D-xylofuranosyl)adenine (I) gave an 82% yield of crystalline 9-(3-*S*-ethyl-3-thio-β-D-xylofuranosyl)hypoxanthine (VIII), which was acetylated to give a quantitative yield of the diacetate (IX). Thiation of IX with phosphorus pentasulfide gave the 6-thiol diacetate (X), which was deacetylated directly with methanolic sodium methoxide to give 9-(3-*S*-ethyl-3-thio-β-D-xylofuranosyl)-9*H*-purine-6-thiol (XI) in 55% over-all yield from VIII.

It was of interest to determine whether the reactivities of the two sulfur atoms of XI were sufficiently different to permit a selective desulfurization of the 6-thiol without concomitant removal of the 3′-thioethyl group. There is precedent to suggest that such a selective desulfurization is possible. Fox *et al.*[7] have desulfurized the riboside of 6-MP to the naturally occurring 9-(β-D-ribofuranosyl)-9*H*-purine by using two grams of Raney nickel catalyst per gram of nucleoside in water at 100° for three and one half hours. Such conditions

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(4) See (a) R. W. Brockman, G. G. Kelley, P. Stutts, and V. Copeland, *Nature*, **191**, 469 (1961), and (b) M. T. Hakala and C. A. Nichol, *J. Biol. Chem.*, **234**, 3224 (1959) for leading references.

(5) C. D. Anderson, L. Goodman, and B. R. Baker, *J. Am. Chem. Soc.*, **81**, 3967 (1959).

(6) For a brief discussion of the effect of modification of the sugar moiety in nucleosides from the naturally occurring D-ribose, see E. J. Reist, L. Goodman, R. R. Spencer, and B. R. Baker, *ibid.*, **80**, 3962 (1958).

(7) J. J. Fox, I. Wempen, A. Hampton, and I. L. Doerr, *ibid.*, **80**, 1669 (1958).