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

ELMER J. REIST,[2] ALLEN BENITEZ, WILLIAM W. LEE, B. R. BAKER,[3] AND LEON GOODMAN

Life Sciences Division, Stanford Research Institute, Menlo Park, California

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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

(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 E. J. Reist, A. Benitez, L. Goodman, B. R. Baker, and W. W. Lee, *J. Org. Chem.*, **27**, 3274 (1962).

(2) To whom enquiries should be sent.

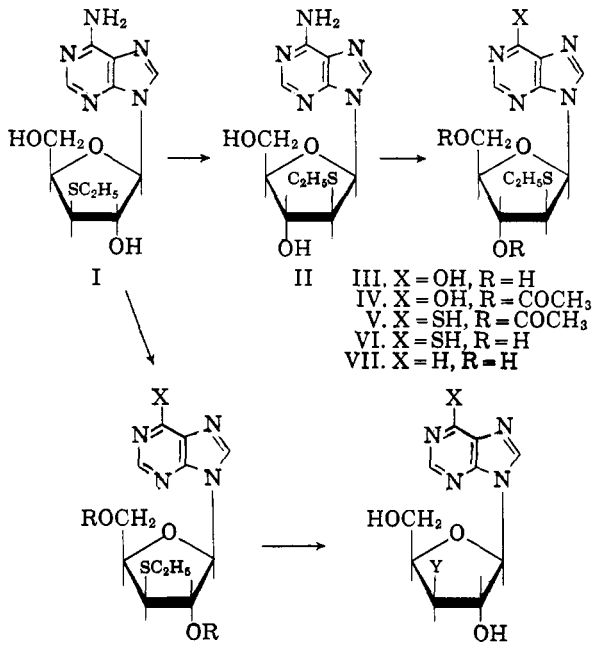
(3) Present address, School of Pharmacy, University of Buffalo, Buffalo 14, New York.

(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).



I II III. X = OH, R = H
IV. X = OH, R = $COCH_3$
V. X = SH, R = $COCH_3$
VI. X = SH, R = H
VII. X = H, R = H

VIII. X = OH; R = H
IX. X = OH; R = $COCH_3$
X. X = SH; R = $COCH_3$
XI. X = SH; R = H
XII. X = H; R = H
XIII. X = H; Y = H
XIV. X = Cl; Y = SCH_2CH_3
XV. X = Cl; Y = $SCl_2CH_2CH_3$
XVI. X = $N(CH_3)_2$; Y = $SOCH_2CH_3$

are completely inadequate to accomplish the desulfurization of the ethylthio nucleoside (II) and starting material is recovered unchanged. Use of 10 g. of Davison sponge nickel[8] per gram of the 6-thiol (XI) in refluxing ethanol for one hour gave after recrystallization a 50% yield of 9-(3-*S*-ethyl-3-thio-β-D-xylofuranosyl)-9*H*-purine (X-II). There was no detectable amount of the completely desulfurized product (XIII). In order to effect complete desulfurization, it was necessary to use a ratio of sponge nickel[8] to nucleoside of 20:1 in methoxyethanol at 100° with a hydrogen atmosphere for twenty four hours. Under these conditions, a 72% yield of crude but chromatographically homogeneous 9-(3-deoxy-β-D-ribofuranosyl)-9*H*-purine (XIII) was obtained.[9]

The successful preparation of nucleosides of 6-chloropurine by the reaction of chlorine with the appropriate nucleoside of 6-MP has been reported.[1,11] The versatility of the 6-chloro substituent on purine for the preparation of a variety of 6-substituted purines made it of interest to investigate the chlorination of XI to give the analogous 6-chloro nucleoside. Chlorination of the second sulfur atom which is present as a thioethyl group on the sugar moiety of XI was conceivable.[12,13] Nevertheless, the successful selective desulfurization of the 6-thiol of XI with Raney nickel encouraged an attempt to effect a selective chlorination of the 6-thiol of XI without affecting the 3′-thioethyl group. The product isolated from the reaction of chlorine with XI, however, could not be satisfactorily characterized and contained excessive amounts of chlorine. Since the ultraviolet spectrum of the chlorination product was satisfactory for a 6-chloropurine nucleoside, it is assumed that the excess chlorine was due to attack by chlorine on the 3′-thioethyl group to give a product such as the chlorinated thioethyl nucleoside (XV). That such a reaction had occurred was further substantiated by the reaction of the crude chloro nucleoside (XV) with aqueous dimethylamine. The resulting 6-dimethylamino nucleoside gave analytical figures consistent with the sulfoxide (XVI).[14]

The preparation of 9-(2-*S*-ethyl-2-thio-β-D-arabinofuranosyl)-9*H*-purine-6-thiol (VI) from 2′-thioethyladenosine (II)[5] was accomplished by a sequence of reactions similar to the sequence used for the preparation of the 3′-thioethyl-6-thiol (XI). Deamination of II with nitrous acid gave 9-(2-*S*-ethyl-2-thio-β-D-arabinofuranosyl)-hypoxanthine (III) in 73% yield. The solubility of the 2′-thioethylinosine (III) was significantly different from that of the analogous 3′-thioethyl derivative (VIII). The 2′-thioethyl derivative (III) crystallized out of the nitrous acid reaction medium, whereas the 3′-thioethyl derivative (VIII) remained in solution under these conditions. It was possible to utilize this difference in solubility in the reaction medium to eliminate the tedious isolation of pure 2′-thioethyladenosine (II) from the mixture of 3′- and 2′-thioethyladenosines[5] (I and II, respectively). When this mixture was treated with nitrous acid in aqueous acetic acid, pure 9-(2-*S*-ethyl-2-thio-β-D-arabinofuranosyl)hypoxanthine (III) was obtained in 55% yield starting from 3′-thioethyladenosine (I).

Acetylation of III gave the diacetate IV, which was thiated with phosphorus pentasulfide, then deblocked with methanolic sodium methoxide to afford 9-(2-*S*-ethyl-2-thio-β-D-arabinofuranosyl)-9-*H*-purine-6-thiol (VI) in a 42% yield from III. The 2′-thioethyl series was much more susceptible to cleavage of the nucleoside during the thiation than was the analogous 3′-thioethyl series. There was much more darkening during the thiation itself

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

(9) These conditions were also adequate to bring about the complete desulfurization of both 3′-ethylthioadenosine (I) and 2′-ethylthioadenosine (II) to give 3′-deoxyadenosine and 2′-deoxyadenosine, respectively, with no detectable traces of the starting materials. It had been noted previously[10] that a catalyst-nucleoside ratio of 15:1 in refluxing 2-methoxyethanol under an hydrogen atmosphere for 6 hours gave incomplete desulfurization of II.

(10) W. W. Lee, A. Benitez, C. D. Anderson, L. Goodman, and B. R. Baker, *J. Am. Chem. Soc.*, **83**, 1906 (1961).

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

(12) H. Böhme and H.-J. Gran, *Ann.*, **577**, 68 (1952).

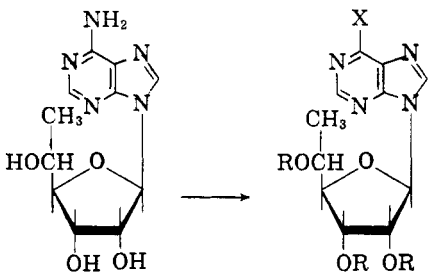
(13) (a) T. Zincke and W. Frohneberg, *Ber.*, **42**, 2721 (1909); (b) T. Zincke and W. Frohneberg, *ibid.*, **43**, 837 (1910).

(14) Zincke and Frohneberg[13] document several cases for the hydrolysis of dihalosulfides to the corresponding sulfoxide.

and 6-MP was detectable in the crude thiation product from the 2′-thioethyl series.

Selective desulfurization was carried out as described for the 3′-thioethyl series and a 72% yield of 9-(2-*S*-ethyl-2-thio-β-D-arabinofuranosyl)-9*H*-purine (VII) was obtained from the 6-thiol (VI). Attempts to effect the complete desulfurization of the 2′-thioethyl-6-thiol (VI) to deoxyribofuranosylpurine gave a noncrystalline material whose ultraviolet spectrum indicated it to contain about 50% of a purine nucleoside.

Deamination of 9-(6-deoxy-β-D-allofuranosyl)-adenine (XVII)[6] with nitrous acid gave 9-(6-deoxy-β-D-allofuranosyl)hypoxanthine (XVIII) as an amorphous solid that could not be crystallized. Acetylation of crude XVIII, however, afforded 57% (from XVII) of the crystalline triacetate (XIX). Reaction of the triacetate (XIX) with



XVII

XVIII. X = OH, R = H
XIX. X = OH, R = $COCH_3$
XX. X = SH, R = $COCH_3$
XXI. X = SH, R = H

phosphorus pentasulfide followed by deacetylation of the resulting 6-thiol triacetate (XX) with methanolic sodium methoxide gave 9-(6-deoxy-β-D-allofuranosyl) - 9*H* - purine - 6 - thiol (XXI). Attempts to prepare the 6-chloro or 6-dimethylamino derivative from XXI and desulfurization of XXI gave impure products that could not be crystallized.

These nucleosides are currently undergoing biological evaluation.

Experimental[15]

9-(3-*S*-Ethyl-3-thio-β-D-xylofuranosyl)hypoxanthine (VIII).—The reaction of 4.16 g. of 9-(3-*S*-ethyl-3-thio-β-D-xylofuranosyl)adenine (I)[5] with sodium nitrite in hydrochloric acid and acetic acid in the manner described for the preparation of 9-(β-D-arabinofuranosyl)hypoxanthine[1] gave 3.42 g. (82%) of product, m.p. 197–203°, which was homogeneous on paper chromatography in solvents B and C with R_{Ad} 1.82 and 1.26, respectively. Recrystallization from water gave the analytical sample, m.p. 203–205° $[\alpha]^{29.5}D$ −70° (1% in 0.1 *N* sodium hydroxide).

Anal. Calcd. for $C_{12}H_{16}N_4O_4S$: C, 46.2; H, 5.16; N, 17.9. Found: C, 46.0; H, 5.32; N, 17.8.

9-(2,5-Di-*O*-acetyl-3-*S*-ethyl-3-thio-β-D-xylofuranosyl)-hypoxanthine (IX).—Acetylation of 300 mg. of the hypoxanthine derivative (VIII) with acetic anhydride in pyridine in the manner described for the preparation of 9-(2,3,5-tri-*O*-acetyl-β-D-arabinofuranosyl)hypoxanthine[1] gave a quantitative yield of the crude diacetate (IX) that was free of hydroxyl absorption at 2.9 μ in the infrared and was satisfactory for the thiation reaction.

Recrystallization from water gave the analytical sample, m.p. 187.0–188.5°; $[\alpha]^{22}D$ −67° (1% in chloroform); $\lambda_{max}^{pH\ 1,7}$ 249 mμ (ε 12,100), $\lambda_{max}^{pH\ 13}$ 254 (ε 13,300).

Anal. Calcd. for $C_{16}H_{20}N_4O_6S \cdot 1/4H_2O$: C, 47.9; H, 5.15; N, 14.0; S, 8.00. Found: C, 48.0; H, 5.52; N, 14.1; S, 7.81.

9-(3-*S*-Ethyl-3-thio-β-D-xylofuranosyl)-9*H*-purine-6-thiol (XI).—The reaction of the diacetate (IX) with phosphorus pentasulfide in pyridine in the manner described for the preparation of 9-(2,3,5-tri-*O*-acetyl-β-D-arabinofuranosyl)-9*H*-purine-6-thiol[1] gave a 78% yield of crude 6-thiol diacetate (X), m.p. 224–228°, that was free of starting material as shown by the absence of the infrared band at 5.85 μ assignable to a carbonyl at the 6-position. The crude 6-thiol diacetate (X) could be recrystallized from acetonitrile; however, efforts to obtain a satisfactory analytical sample were unsuccessful.

Anal. Calcd. for $C_{16}H_{20}N_4O_5S_2$: C, 46.6; H, 4.89; N, 13.6; S, 15.6. Found: C, 47.6; H, 4.81; N, 14.5; S, 15.6.

Deacetylation of the crude 6-thiol diacetate (X) with methanolic sodium methoxide in the manner described for the preparation of 9-(β-D-arabinofuranosyl)-9*H*-purine-6-thiol[1] gave a 78% yield of the 6-thiol (XI), m.p. 188–190°, that was homogeneous on paper chromatography in solvents A and D, with R_{Ad} 1.61 and 1.30, respectively. Recrystallization from water gave pure XI, m.p. 191.5–192.5°; $[\alpha]^{21}D$ −90° (1% in 0.1 *N* sodium hydroxide); $\lambda_{max}^{pH\ 1}$ 322.5 mμ (ε 24,400), $\lambda_{max}^{pH\ 7}$ 319 mμ (ε 23,900), $\lambda_{max}^{pH\ 13}$ 311 mμ (ε 23,900).

Anal. Calcd. for $C_{12}H_{16}N_4O_3S_2$: C, 43.9; H, 4.91; N, 17.1; S, 19.5. Found: C, 43.8; H, 4.80; N, 17.0; S, 19.5.

9-(3-*S*-Ethyl-3-thio-β-D-xylofuranosyl)-9*H*-purine (XII).—A mixture of 200 mg. of the 6-thiol (XI) and 2 g. of Davison sponge nickel[8] in 20 ml. of water was heated with stirring at 80° for 1 hr. The hot reaction was filtered through Celite and the filter cake was washed with 6 ml. of hot water. The combined filtrate and washings were evaporated to dryness *in vacuo* to give 127 mg. of crude product (XII) that was homogeneous on paper chromatography in solvents A and D, with R_{Ad} 1.55 and 1.23, respectively. Crystallization from acetonitrile gave 90 mg. (50%) of product, m.p. 150–151°. Recrystallization from acetonitrile gave the analytical sample, m.p. 152.0–152.7°; $[\alpha]^{22}D$ −48° (1% in water); $\lambda_{max}^{pH\ 1}$ 263 mμ (ε 5640), $\lambda_{max}^{pH\ 7,13}$ 264 mμ (ε 7150).

Anal. Calcd. for $C_{12}H_{16}N_4O_3S$: C, 48.6; H, 5.44; N, 18.9; S, 10.8. Found: C, 48.7; H, 5.68; N, 19.1; S, 10.6.

9-(3-Deoxy-β-D-ribo(xylo)furanosyl)-9*H*-purine (XIII).—A mixture of 200 mg. of the 6-thiol (XI) and 4.0 g. of Davison sponge nickel,[8] which had been washed with 2-methoxyethanol, in 30 ml. of 2-methoxyethanol was stirred at 100° under an hydrogen atmosphere for 24 hr. The hot mixture was filtered through a Celite pad and the filter cake was washed with two 6-ml. portions of hot 2-methoxyethanol. The combined filtrate and washings were evaporated to dryness *in vacuo* to give 75 mg. (72%) of crude product (XIII), m.p. 160–170°. Recrystallization from 2.5 ml. of absolute ethanol gave the analytical sample, m.p. 192.0–

(15) Melting points were taken on a Fisher-Johns apparatus and are uncorrected. Optical rotations were measured with a Standard polarimeter Model D attachment to the Beckman DU spectrophotometer calibrated with sucrose solutions. Paper chromatograms were run with water-saturated butyl alcohol (solvent A), 5% aqueous disodium phosphate (solvent B), butanol–acetic acid–water (4:1:5) (solvent C), and benzene–methanol–water (2:6:1) (solvent D) by the descending technique on Whatman No. 1 paper. The spots were located by visual examination with an ultraviolet lamp. Adenine was used as a standard and spot locations were expressed as R_{Ad} units with adenine at R_{Ad} 1.00.

192.5°; $[\alpha]^{25}D$ −38° (1% in water); $\lambda_{max}^{pH\ 1}$ 263 mμ (ε 5350), $\lambda_{max}^{pH\ 7,13}$ 263 mμ (ε 6900).

Anal. Calcd. for $C_{10}H_{12}N_4O_3$: C, 50.8; H, 5.12; N, 23.7. Found: C, 50.9; H, 5.43; N, 23.8.

Reaction of 9-(3-*S*-Ethyl-3-thio-β-D-xylofuranosyl)-9*H*-purine-6-thiol (XI) with Chlorine.—A suspension of 100 mg. (0.30 mmole) of XI in 4 ml. of absolute methanol was cooled to −10° while protected from moisture. Chlorine gas was bubbled through the suspension for 6 min., by which time solution was complete. The reaction solution was kept at 0° for an additional 5 min., then the yellow color was discharged by flushing the solution with dry nitrogen for 10 min. A solution of 2 ml. of 25% aqueous dimethylamine was added to the mixture and the reaction was heated in a steel bomb at 115° for 4.5 hr., then evaporated to dryness *in vacuo*. The residue was dissolved in 10 ml. of water and extracted with four 5-ml. portions of chloroform. The combined chloroform extracts were washed with 5 ml. of water and then dried over magnesium sulfate and evaporated to dryness *in vacuo*. The residue weighed 78 mg. Crystallization from benzene gave 45 mg. of solid, m.p. 190–193°, that was homogeneous on paper chromatography, with R_{Ad} 2.14 (solvent A) and 1.91 (solvent B). Recrystallization from isopropyl alcohol gave white crystals, m.p. 191–192°; λ_{max}^{Nujol} 9.68 μ (C—O—C, >S→O); $\lambda_{max}^{pH\ 8}$ 278 mμ (spot cut from paper chromatogram in solvent B).

Anal. Calcd. for $C_{14}H_{21}N_5O_4S$: C, 47.3; H, 5.92; N, 19.7; S, 9.01. Found: C, 47.5; H, 6.19; N, 19.4; S, 9.17.

9-(2-*S*-Ethyl-2-thio-β-D-arabinofuranosyl)hypoxanthine (III).—A solution of 5.43 g. of 9-[3(2)-chloro-3(2)-deoxy-2(3)-*S*-ethyl-2(3)-thio-β-D-arabino(xylo)furanosyl]-adenine[5] and 16.2 g. of sodium acetate trihydrate in 105 ml. of 2-methoxyethanol was heated at reflux under a nitrogen atmosphere for 3 hr., then evaporated to dryness *in vacuo*. The residue was dissolved in 150 ml. of hot water and continuously extracted with 250 ml. of chloroform for 66 hr. A second continuous extraction was carried out with an additional 250 ml. of chloroform. The chloroform layers were combined and evaporated to dryness to give 5.13 g. (100%) of crude 9-[2(3)-*S*-ethyl-2(3)-thio-β-D-arabino-(xylo)furanosyl]adenine (I and II).[5]

The crude 6-amine mixture (I and II) (5.13 g., *ca.* 16.5 mmoles) was dissolved in 50 ml. of water containing 16.5 ml. (16.5 mmoles) of 1.0 *N* aqueous hydrochloric acid. To this solution was added 6.9 g. of sodium nitrite and 24 ml. of glacial acetic acid. The reaction mixture was stored at room temperature for 64 hr., by which time some product had crystallized. The reaction was cooled at 0° for 3 hr., then the crystalline product was collected. Additional crops were collected by concentration of the mother liquors for a total yield of 2.97 g. of III (55% based on I) of product that was identical in every respect with authentic III, which was prepared in 73% yield by treatment of pure 9-(2-*S*-ethyl-2-thio-β-D-arabinofuranosyl)adenine (II)[5] with nitrous acid in aqueous acetic acid.

The analytical sample, prepared from pure II, was recrystallized from water and had m.p. 228°, resolidified 230–300°; $[\alpha]^{19}D$ −106° (1% in 0.1 *N* sodium hydroxide); $\lambda_{max}^{pH\ 1}$ 251 mμ (ε 11,800), $\lambda_{max}^{pH\ 7}$ 251 mμ (ε 12,400), $\lambda_{max}^{pH\ 13}$ 254 mμ (ε 13,600). The product was homogeneous on paper chromatography in solvents A, B, and C, with R_{Ad} 1.22, 2.13, and 1.31, respectively.

Anal. Calcd. for $C_{12}H_{16}N_4O_4S$: C, 46.2; H, 5.16; N, 17.9; S, 10.2. Found: C, 46.0; H, 5.00; N, 18.0; S, 10.5.

9-(3,5-Di-*O*-acetyl-2-*S*-ethyl-2-thio-β-D-arabinofuranosyl)-hypoxanthine (IV).—Acetylation of 100 mg. of the hypoxanthine nucleoside (III) with acetic anhydride in pyridine in the manner described for the preparation of 9-(2,3,5-tri-*O*-acetyl-β-D-arabinofuranosyl)hypoxanthine[1] gave 129 mg. (100%) of crude diacetate (IV), m.p. 195–197°. Two recrystallizations from water gave the analytical sample, m.p. 191.5–193.5°; $[\alpha]^{20}D$ −51° (1% in chloroform); λ_{max}^{pH} 250 mμ (ε 12,300), $\lambda_{max}^{pH\ 7}$ 249 mμ (ε 12,700), $\lambda_{max}^{pH\ 13}$ 254 mμ (ε 13,600).

Anal. Calcd. for $C_{16}H_{20}N_4O_6S$: C, 48.5; H, 5.09; N, 14.1; S, 8.09. Found: C, 48.1; H, 5.24; N, 14.2; S, 8.51.

9-(2-*S*-Ethyl-2-thio-β-D-arabinofuranosyl)-9*H*-purine-6-thiol (VI).—To a solution of 100 mg. (0.252 mmole) of the blocked nucleoside (IV) in 4 ml. of dry pyridine was added 226 mg. (1.02 mmoles) of phosphorus pentasulfide. The mixture was heated with stirring at 125–130° for 3 hr. while protected from moisture. The dark reaction mixture was cooled to 15°, then slowly poured into a vigorously stirred solution of 250 mg. of sodium bicarbonate in 30 ml. of water. The aqueous solution was extracted with four 10-ml. portions of chloroform. The chloroform extracts were combined and washed with five 20-ml. portions of water to remove all the 6-MP, then were dried over magnesium sulfate and evaporated to dryness *in vacuo* to give 92 mg. of crude blocked 6-thiol (V), which was free of starting material and 6-MP, as shown by infrared spectroscopy.

Deacetylation of 82 mg. of the crude diacetate (V) with methanolic sodium methoxide gave, after recrystallization from water, 35 mg. (42% overall yield from IV) of crystals; m.p. 165–177° dec. A second recrystallization gave 25 mg. of product, m.p. 199–200°; $[\alpha]^{26}D$ −164° (1% in 0.1 *N* sodium hydroxide; $\lambda_{max}^{pH\ 1}$ 323 mμ (ε 24,400), $\lambda_{max}^{pH\ 7}$ 321 mμ (ε 24,300), $\lambda_{max}^{pH\ 13}$ 3.12 mμ (ε 23,000). The product moved as a single spot on paper chromatography in solvents A and B with R_{Ad} 1.68 and 1.71, respectively.

Anal. Calcd. for $C_{12}H_{16}N_4O_3S_2$: C, 43.9; H, 4.91; N, 17.1; S, 19.5. Found: C, 44.0; H, 4.80; N, 17.3; S, 19.2.

9-(2-*S*-Ethyl-2-thio-β-D-arabinofuranosyl)-9*H*-purine (VII).—The desulfurization of 200 mg. of the 6-thiol (VI) with 2 g. of Davison sponge nickel[8] in 20 ml. of water was performed as described for the preparation of the 3′-*S*-ethyl xyloside (XII) to give 129 mg. (72%) of crude product, m.p. 205–206°. Recrystallization from water gave the analytical sample, m.p. 204.5–204.8°; $[\alpha]^{23}D$ −40° (1% in methanol); $\lambda_{max}^{pH\ 1}$ 262.5 mμ (ε 5000), $\lambda_{max}^{pH\ 7}$ 266.5 mμ (ε 6370), $\lambda_{max}^{pH\ 13}$ 265 mμ (ε 6370). The product was homogeneous on paper chromatography with R_{Ad} 2.58 (solvent A) and 2.12 (solvent B).

Anal. Calcd. for $C_{12}H_{16}N_4O_3S \cdot 1/5H_2O$: C, 48.1; H, 5.51; N, 18.7; S, 10.7. Found: C, 48.1; H, 5.56; N, 18.8; S, 10.6.

9-(6-Deoxy-2,3,5-tri-*O*-acetyl-β-D-allofuranosyl)hypoxanthine (XIX).—A mixture of 1.00 g. of 9-(6-deoxy-β-D-allofuranosyl)adenine (XVII)[6] was treated with 1.68 g. of sodium nitrite in 3.6 ml. of 1 *N* aqueous hydrochloric acid and 6 ml. of acetic acid in the manner described for the synthesis of 9-(β-D-arabinofuranosyl)hypoxanthine.[1] The reaction mixture was evaporated to dryness *in vacuo* and the residue was dried by the addition and removal *in vacuo* of 20 ml. of toluene. The residue was treated with 1.38 ml. of acetic anhydride and 40 ml. of dry pyridine, then worked up in the manner described for the preparation of 9-(2,3,5-tri-*O*-acetyl-β-D-arabinofuranosyl)hypoxanthine[1] to give 840 mg. (57%) of product, m.p. 218–242°, after one crystallization from water. The analytical sample from a previous reaction had m.p. 246.5–247.5°; $[\alpha]^{26}D$ −30° (0.6% in chloroform); $\lambda_{max}^{pH\ 1,7}$ 248 mμ (ε 11,100), $\lambda_{max}^{pH\ 13}$ 253.5 mμ (ε 12,200).

Anal. Calcd. for $C_{17}H_{20}N_4O_8$: C, 50.0; H, 4.94; N, 13.7. Found: C, 49.7; H, 5.24; N, 13.9.

9-(6-Deoxy-β-D-allofuranosyl)-9*H*-purine-6-thiol (XXI).—The thiation of 6.14 g. of the triacetate (XIX) with phosphorus pentasulfide was carried out in the manner described for the preparation of 9-(2,3,5-tri-*O*-acetyl-β-D-arabinofuranosyl)-9*H*-purine-6-thiol.[1] After the reaction mixture was decomposed with water and evaporated to dryness *in vacuo*, the residue was triturated with cold water, then recrystallized once from ethanol to give 4.73 g. (74%) of crude 6-thiol triacetate (XX), m.p. 207–212°, which was

free of starting material according to paper chromatography and infrared spectroscopy. Deacetylation of 150 mg. of the triacetate (XX) with methanolic sodium methoxide in the usual fashion gave 75 mg. (71%) of the desired product (XXI), m.p. 211.0–212.5° dec. Recrystallization from water gave the analytical sample, m.p. 227–228° dec.; $[\alpha]^{33}D$ −80° (1% in 0.1 *N* sodium hydroxide); $\lambda_{max}^{pH\ 1}$ 322.5 mμ (ε 23,000), $\lambda_{max}^{pH\ 7}$ 318 mμ (ε 18,500), $\lambda_{max}^{pH\ 13}$ 311.5 mμ (ε 23,000). The product was homogeneous on paper chromatography with R_{Ad} 1.74 (solvent B).

Anal. Calcd. for $C_{11}H_{14}N_4O_4S$: C, 44.3; H, 4.73; N, 18.8; S, 10.7. Found: C, 44.1; H, 4.64; N, 18.9; S, 10.9.

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Potential Anticancer Agents. LXXVIII.[1] Nonclassical Antimetabolites. IV.[2] Synthesis of Compounds Related to 4-(Iodoacetamido)salicylic Acid, an Exo-Alkylating Irreversible Inhibitor

B. R. BAKER,[3] WILLIAM W. LEE,[4] ABELARDO P. MARTINEZ, LEONARD O. ROSS, AND LEON GOODMAN

Life Sciences Division, Stanford Research Institute, Menlo Park, California

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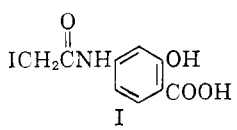
A number of compounds that are related to 4-(iodoacetamido)salicylic acid, an exo-alkylating irreversible enzyme inhibitor, have been synthesized in order to make them available for enzyme studies. These compounds are derivatives of oxanilic and salicylic acids that contain alkylating groups such as haloacyl, haloacetamido, and nitrogen mustard functions.

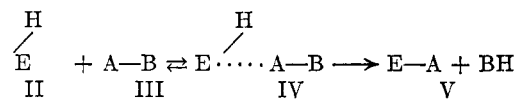
Nonclassical antimetabolites—relatively large molecules compared to the substrate—capable of inhibiting the lactic acid dehydrogenase (LDH) catalyzed reduction of pyruvate by reduced diphosphopyridine nucleotide (DPNH) have been found.[5] Examples are such compounds as salicylate, oxanilate, and phenylpyruvate, with I_{50} values[6] of 19, 14, and 21, respectively.

These compounds also inhibited the glutamic acid dehydrogenase (GDH) catalyzed conversion of L-glutamate to α-oxoglutarate with I_{50} values of 20, 19, and 36, respectively. Therefore, 4-(iodoacetamido)salicylic acid (I) was investigated as a possible irreversible inhibitor of these two enzymes.



I

In a previous paper,[2] it was shown that I was an irreversible inhibitor of both LDH and GDH. Strong evidence was presented[2] that this inhibitor complexed reversibly with the active site of these enzymes, then became irreversibly bound by alkylation of the enzyme adjacent to the active site; this phenomenon was predicted earlier[7] and was termed "exo-alkylation." The phenomenon of exo-alkylation is shown diagramatically by the sequence II–V. An important facet of this problem



was to eliminate the possibility that II and III reacted bimolecularly with direct formation of V without intervention of the reversible complex, IV; this type of irreversible inhibition was termed "tail-alkylation."[2] In the case of I and GDH, only exo-alkylation took place and there was no measurable amount of tail-alkylation.[2] Similarly, I and LDH interacted by the exo-alkylation route, but the possibility of a small amount of tail-alkylation could not be eliminated.[8]

A series of compounds related to I that might be more selective for one enzyme over the other were needed. These compounds can be grouped as follows: (1) variation of the bridge length in the reversible complex (IV) between the halogen and

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(3) School of Pharmacy, University of Buffalo, Buffalo 14, New York.

(4) To whom inquiries should be sent.

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(6) An I_{50} value is defined[5] as the millimolar concentration of inhibitor necessary to give 50% reversible inhibition of an enzyme in the presence of 1 millimolar concentration of substrate.

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