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Synthesis of 9-β-D-Xylofuranosyl-6-mercaptopurine and 9-β-D-Xylofuranosylguanine 5′-Phosphate

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An attempt was made to synthesize 9-β-D-xylofuranosylguanine (VI) by condensation of N^2,$N^{9(or7)}$-diacetylguanine (I) and 1,2,3,5-tetra-O-acetyl-D-xylofuranose (II) by a fusion method. However the main product obtained was 7-xylofuranosylguanine (III). Compound VI was successfully synthesized by deamination of 2-chloroadenine xyloside derivative (IV) with nitrous acid followed by amination with ammonia. VI was enzymati cally phosphorylated to give 9-β-D-xylofuranosylguanine 5′-phosphate. This Compound was found to have a flavoring activity.

Catalytic hydrogenation of 2-chlorohypoxanthine derivative (V) with palladium-charcoal afforded hypoxanthine xyloside (IX), which, after acetylation, was chlorinated with phosphorus oxychloride to give the chloro compound (XII). This was converted by reaction with thiourea and subsequent ammoniacal treatment to 9-β-D-xylofuranosyl-6-mercaptopurine (XIII).

Recent work[2] in our laboratories has shown that the flavoring activities of 2-substituted inosine 5′-phosphates varied with the kind of 2-substituent in synergistic effect with monosodium L-glutamate (MSG) and that the isopropylidene derivative of 5′-nucleotide lost its flavoring activity.[2a] The activity was also found to be lost when the ribose moiety of 5′-inosinic acid or 5′-guanylic acid was replaced by glucopyranosyl[3] or hydroxybutyl group.[4] In view of the fact that 2′-deoxy-5′-inosinic and 2′-deoxy-5′-guanylic acids have a flavoring activity,[5] the 3′-hydroxyl group in the ribofuranose moiety may be essential to the emergence of the activity. Therefore, it became desirable to synthesize 9-β-D-xylofuranosylguanine 5′-phosphate (XIV), in which the configuration of the hydroxyl gorup at position 3′ was changed, and to examine its flavoring activity.

In the present paper authors wish to report the synthesis of 9-β-D-xylofuranosylguanine (VI) and its biochemical phosphorylation. In addition, the recent report of LePage, *et al.*[6] on the metabolism of 9-β-D-xylofuranosyl-6-mercaptopurine-^{35}S and its prolongation of survival time of mice bearing S-180 acites tumor cells led us to prepare 9-β-D-xylofuranosyl-6-mercaptopurine (XIII) in order to examine its antitumor activity in more detail.

When N^2,$N^{9(or7)}$-diacetylguanine (I)[7] and 1,2,3,5-tetra-O-acetyl-D-xylofuranose (II)[8] were condensed directly by the fusion method[9] and subsequently deacetylated, the crystalline

1) Location: *Suzuki-cho, Kawasaki.*
2) *a*) A. Yamazaki, I. Kumashiro, and T. Takenishi, *Chem. Pharm. Bull.* (Tokyo), **16**, 338 (1968); *b*) I. Kumashiro, A. Yamazaki, T. Meguro, T. Takenishi, and T. Tsunoda, *Biotechnol. Bioeng.*, **X**, 303 (1968); *c*) S. Yamaguchi, T. Yoshikawa, S. Ikeda, and T. Ninomiya, *Agr. Biol. Chem.*, **32**, 729 (1968).
3) A. Nohara, K. Imai, and M. Honjo, *Chem. Pharm. Bull.* (Tokyo), **14**, 491 (1966).
4) A. Yamazaki, *Chem. Pharm. Bull.* (Tokyo), **17**, 1268 (1969).
5) Y. Nakao and K. Ogata, Presented at the Kanto-Branch Meeting of the Agricultural Chemical Society of Japan, Tokyo, November 1960.
6) K. Sato, G.A. LePage, and A.P. Kimball, *Cancer Res.*, **26**, 741 (1966).
7) Reported by Y. Ishido, A. Hosono, Y. Nagasawa, K. Iwabuchi, S. Isome, A. Maruyama, and T. Sato at the 18th Annual Meeting of the Chemical Society of Japan, Tokyo, April 1965, Abstract, p. 224.
8) E.J. Reist and L. Goodman, *Biochemistry*, **3**, 15 (1964).
9) T. Sato, T. Shimadate, and Y. Ishido, *Nippon Kagaku Zasshi*, **81**, 1440 (1960).

product was obtained in 18% yield. This compound was proved to be an anomeric mixture of 7-D-xylofuranosylguanine (III) on the basis of paper chromatography, nuclear magnetic resonance (NMR) spectrum, and the similarity of its ultraviolet absorption spectra to those of 7-methylguanine.[10] Paper chromatographic and spectral examination of the mother liquor indicated the formation of 9-D-xylofuranosylguanine in very low yields, but it was not isolated.

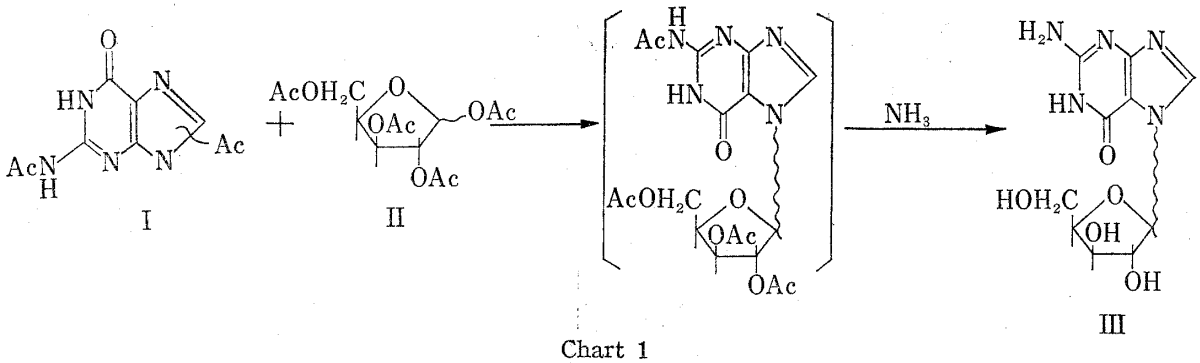

Chart 1

Another approach *via* a versatile intermediate, 2,6-dichloropurine xyloside derivative, appeared much more promising for obtaining VI. Since it was previously reported by Reist and Goodman[8] that 9-β-D-arabinofuranosylguanine was prepared from the above intermediate, this synthetic route was applied in part to the preparation of VI. 2-Chloro-6-amino-9-(3′,5′-O-isopropylidene-β-D-xylofuranosyl)purine (IV) was treated with nitrous acid in aqueous acetic acid to give 2-chloro-6-hydroxy-9-β-D-xylofuranosylpurine (V) in 55.3% yield after purification with Dowex 50 W×4. Amination of V with ammonia in a sealed tube at 150° produced VI in 32% yield. Similarly, V was aminated with methylamine and dimethylamine to afford N^2-methyl-9-β-D-xylofuranosylguanine (VII) and N^2,N^2-dimethyl-9-β-D-xylofuranosylguanine (VIII), respectively.

Since the phosphorylation at 5′-position of VI by a chemical method required a considerable amount of the nucleotide precursor, 2′,3′-di-O-acetyl derivative, the biochemical phosphorylation of VI was carried out by the enzyme, nucleoside phosphotransferase, which was prepared from *Pseudomonas trifolii*.[11] In this case, *p*-nitrophenyl phosphate was used as a phosphate donor. The phosphorylation by this enzyme has already been reported to occur only at 5′-position of a ribonucleoside in good yield and in the case of VI, the yield of phosphorylation was 86.3%. After being purified by ion-exchange chromatography, XIV was isolated as the ammonium salt. The structure of XIV was verified by giving a positive test for sodium periodide and by complete hydrolysis of XIV with snake venom 5′-nucleotidase to VI and inorganic phosphate. The ultraviolet absorption spectra showed characteristic curve similar to 5′-guanylic acid. The compound XIV thus obtained was found to have a flavoring activity and its synergistic effect with MSG was almost comparable[12] to that of 5′-inosinic acid. This fact, therefore, showed that, when the configuration of 3′-hydroxyl group was changed from down to up in 5′-guanylic acid, the activity was retained, although the synergistic flavoring strength with MSG was affected.

Catalytic hydrogenolysis of V over palladium-charcoal afforded 9-β-D-xylofuranosylhypoxanthine (IX) in 5% yield, which was acetylated with acetic anhydride in pyridine to give 9-(2′,3′,5′-tri-O-acetyl-β-D-xylofuranosyl)hypoxanthine (X). Alternatively, X was readily obtained by the simplified procedure of a silyl method[13] from the trimethylsilyl derivative (XI) of hypoxanthine and II.

10) W. Pfleiderer, *Ann.*, **647**, 167 (1961).
11) K. Mitsugi, K. Komagata, M. Takahashi, H. Iizuka, and H. Katagiri, *Agr. Biol. Chem.*, **28**, 586 (1964).
12) Private communication by Mr. Ninomiya, *et al.* in our laboratories.
13) T. Nishimura and I. Iwai, *Chem. Pharm. Bull.* (Tokyo), **12**, 352 (1964); T. Nishimura, B. Shimizu, and I. Iwai, *ibid.*, **12**, 1471 (1964).

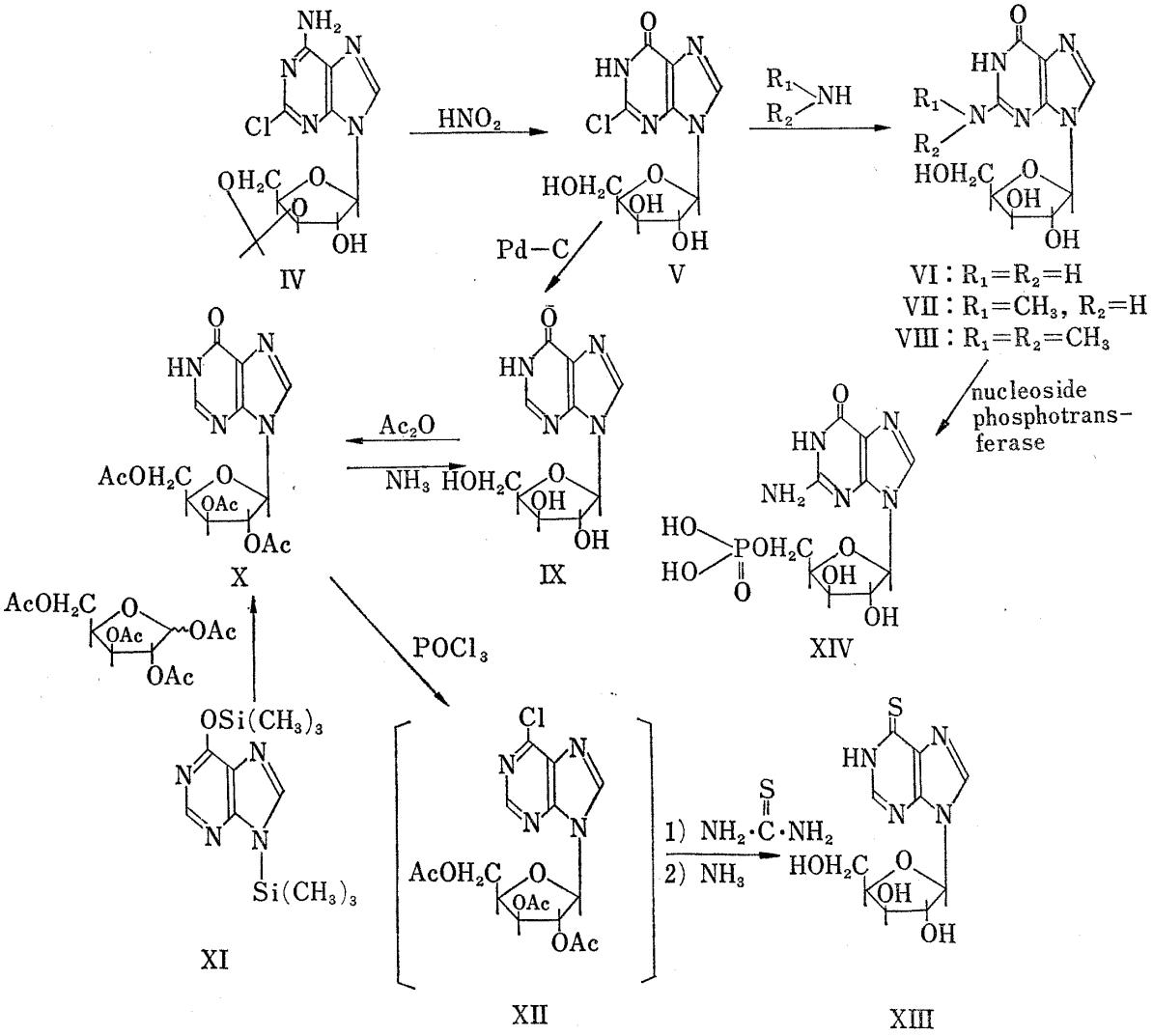

Chart 2

The chlorination of X with phosphorus oxychloride produced the glassy chloro derivative (XII), which in turn was converted by reaction with thiourea and subsequent ammoniacal treatment to 9-β-D-xylofuranosyl-6-mercaptopurine (XIII).

The biochemical activities of the synthesized compounds are now under investigation.

Experimental[14]

7-D-Xylofuranosylguanine (III)——A mixture of diacetylguanine[7] (I, 5 g, 2.12 mmoles) and 1,2,3,5-tetra-O-acetyl-D-xylofuranose (II, 8.8 g, 2.76 mmoles)[8] was heated at inside temperature of 170—180° for 30—40 min in an oil bath under aspirator vacuum. After cooling, MeOH (100 ml) was added and the unchanged diacetylguanine was filtered off. The filtrate, after decolorized with charcoal, was concentrated to dryness. The resulting gummy product was dissolved in 350 ml of MeOH saturated with NH_3 at 0° and the solution was allowed to stand at refrigerator overight. The solvent was removed *in vacuo* and the residue was crystallized from a small amount of MeOH. Recrystallization from 70 ml of H_2O gave 1.1 g (18.5%) of white crystals. mp>250°. Paper chromatography in solvent C showed two spots at *Rf* values, 0.51 and 0.60. The ultraviolet absorption spectra indicated $\lambda_{max}^{pH\,1}$ mμ: 252, 270 (s); $\lambda_{max}^{pH\,6}$ mμ: 243, 286; $\lambda_{max}^{pH\,11}$

14) All melting points are uncorrected. Ultraviolet spectra were taken with a Hitachi EPS-2 automatic recording spectrophotometer. The NMR spectra were measured with a Varian A-60 using tetramethylsilane as an internal standard. Paper chromatography was carried out on Toyo Filter Paper No. 51 by the ascending method. Solvent systems were A, *n*-BuOH–AcOH–H_2O (4:1:1, v/v); B, iso-PrOH–sat. $(NH_4)_2SO_4$–H_2O (2:79:19, v/v); and C, isobutylic acid–0.5N NH_4OH (10:6, v/v).

mμ: 240 (s), 283; $\lambda_{max}^{pH\ 13}$ mμ: 242 (s), 283 distinguishable from guanosine. An examination of NMR spectrum showed two singlets at 8.83 and 8.88 ppm, indicating the presence of two different ring protons. $[\alpha]_D^{28}$ −32.8° (*c*=0.5, 0.1N NaOH.) *Anal.* Calcd. for $C_{10}H_{13}O_5N_5 \cdot \frac{1}{2}H_2O$: C, 41.10; H, 4.83; N, 24.00. Found: C, 40.91; H, 4.79; N, 24.40.

2-Chloro-6-hydroxy-9-β-D-xylofuranosylpurine (V)——2-Chloro-6-amino-9-(3′,5′-O-isopropylidene-β-D-xylofuranosyl) purine[8] (IV, 3 g) and sodium nitrite (4.8 g) were dissolved in 75 ml of H_2O, 90 ml of glacial acetic acid was added, and the mixture was allowed to stand at room temperature for 3 days. After the solvent was removed *in vacuo*, EtOH was added and the mixture was concentrated. The treatment was repeated several times. Finally the residue was dissolved in 50 ml of H_2O and the solution was run onto a Dowex 50W×4 (H^+ form, 100—200 mesh) resin column (5.2×87 cm). After washing with H_2O, the column was eluted with about 15 liters of H_2O. The ultraviolet absorbing fractions were collected and evaporated *in vacuo*. The residue was crystallized from H_2O to give colorless crystals; Yield 1.5 g (55%); mp 205—206°; $[\alpha]_D^{27.5}$ −24.4° (*c*=1, H_2O); *Rf*: 0.38 (solvent A); UV $\lambda_{max}^{pH\ 1}$ mμ (ε): 251 (13000); $\lambda_{max}^{pH\ 6}$ mμ (ε): 253 (12600); $\lambda_{max}^{pH\ 13}$ mμ (ε): 295.5 (11500). *Anal.* Calcd. for $C_{10}H_{11}O_5N_4Cl$: C, 39.68; H, 3.66; N, 18.51. Found: C, 39.76; H, 3.86; N, 18.41.

9-β-D-Xylofuranosylguanine (VI)——One gram of V was added to 50 ml of MeOH saturated with NH_3 at 0°, and the mixture was heated in an autoclave at 150° for 3 hr. Concentration of the solution under reduced pressure gave the crude product, which was crystallized from 20 ml of H_2O and 4 drops of EtOH to give colorless crystals, yield 300 mg (32%); mp 241—243° (decomp.); $[\alpha]_D^{27}$ −31.2° (*c*=0.5, H_2O); *Rf*: 0.12 (solvent A); UV $\lambda_{max}^{pH\ 1}$ mμ (ε): 256 (12600), 275 (s); $\lambda_{max}^{pH\ 6}$ mμ (ε): 253.5 (13200), 270 (s); $\lambda_{max}^{pH\ 13}$ mμ (ε): 266 (10300). *Anal.* Calcd. for $C_{10}H_{13}O_5N_5 \cdot \frac{1}{2}H_2O$: C, 41.10; H, 4.83; N, 24.00. Found: C, 41.20; H, 4.96; N, 23.60.

N^2-Methyl-9-β-D-xylofuranosylguanine (VII)——A solution of V (1 g) in 40 ml of 30% aqueous methylamine was heated in an autoclave at 150° for 5 hr. After removal of the solvent *in vacuo*, the yellowish residue was dissolved in 50 ml of H_2O and allowed to stand at room temperature. The resulting crystals were filtered and crystallized from a small amount of H_2O, giving 300 mg (30.5%) of the product; mp 262—263° (decomp.); $[\alpha]_D^{28.5}$ −20.0° (*c*=0.5, H_2O): *Rf*: 0.29 (solvent A); UV $\lambda_{max}^{pH\ 1}$ mμ (ε): 255 (13000), 280 (s); $\lambda_{max}^{pH\ 6}$ mμ (ε): 253.5 (13600), 275 (s); $\lambda_{max}^{pH\ 13}$ mμ (ε): 254.5 (9200). *Anal.* Calcd. for $C_{11}H_{15}O_5N_5 \cdot H_2O$: C, 41.90; H, 5.43; N, 22.22. Found: C, 41.28; H, 5.08; N, 22.95.

N^2,N^2-Dimethyl-9-β-D-xylofuranosylguanine (VIII)——A solution of V (700 mg) in 30 ml of 30% aqueous dimethylamine was heated in an autoclave at 150° for 8 hr. The solvent was removed under reduced pressure to afford a gummy product, which was dissolved in 5 ml of H_2O and allowed to stand at room temperature. The precipitate was filtered and recrystallized from H_2O to yield 299 mg (41.6%) of a pure sample; mp 223—225°; $[\alpha]_D^{27}$ −23.2° (*c*=0.5, H_2O); *Rf*: 0.30 (solvent A); UV $\lambda_{max}^{pH\ 1}$ μ (ε): 263 (16400), 290 (s); $\lambda_{max}^{pH\ 6}$ mμ (ε): 259.5 (16200), 283 (s); $\lambda_{max}^{pH\ 13}$ mμ (ε): 222 (22300), 261 (13100). *Anal.* Calcd. for $C_{12}H_{17}O_5N_5 \cdot \frac{1}{3}H_2O$: C, 45.42; H, 5.61; N, 22.07. Found: C, 45.61; H, 5.58; N, 22.57.

9-β-D-Xylofuranosylhypoxanthine (IX)——One gram of V was hydrogenated with 500 mg of 10% Pd-C in 30 ml of H_2O at room temperature until no more hydrogen was absorbed. After the catalyst was filtered off, the filtrate was neutralized with 1N NaOH and passed through a column (2.0×25 cm) of Dowex 50W×4 (H^+ form, 100—200 mesh). The column was eluted with H_2O. The fractions containing ultraviolet absorbing material were collected and evaporated to dryness *in vacuo*. The residue was dissolved in 5 ml of H_2O and, to this solution about 30 ml of acetone was added. The resulting precipitate solidified, after being allowed to stand in 3—4 days at room temperature. The solid was collected, washed with EtOH and dried *in vacuo* over P_2O_5 at room temperature. Yield 45 mg (5.0%); mp 223—224° (decomp.); $[\alpha]_D^{23.5}$ −43.0° (*c*=0.5, H_2O); *Rf*: 0.16 (solvent A); UV $\lambda_{max}^{pH\ 1}$ mμ (ε): 248.5 (10600); $\lambda_{max}^{pH\ 6}$ mμ (ε): 249 (10400); $\lambda_{max}^{pH\ 13}$ mμ (ε): 253.5 (9300). *Anal.* Clacd. for $C_{10}H_{12}O_5N_4 \cdot \frac{1}{2}H_2O \cdot \frac{1}{2}C_3H_6O$: C, 45.10; H, 5.26; N, 18.29. Found: C, 45.03; H, 4.89; N, 17.98.

9-(2′,3′,5′-Tri-O-acetyl-β-D-xylofuranosyl)hypoxanthine (X)——A) Compound IX (1 g) was dissolved in a mixture of pyridine (35 ml) and acetic anhydride (30 ml) and the mixture was allowed to stand at room temperature overnight. EtOH (95 ml) was added and the solvent was removed *in vacuo*. This procedure was repeated several times to decompose acetic anhydride completely. The residue was crystallized from EtOH, giving a crude product. Recrystallization from MeOH afforded an analytically pure sample; yield 650 mg (44.2%); mp 225—226°.

B) A solution of hypoxanthine (5.44 g) in hexamethyldisilazane (15 g) was refluxed for 10 hr and then evaporated to dryness *in vacuo*, leaving a syrup. A mixture of the above syrup, 1,2,3,5-tetra-O-acetyl-D-xylofuranose (14 g), and a catalytic amount of sulfamic acid (50 mg) was heated at 145° for 35 min in an oil bath under aspirator vacuum. To this mixture, MeOH (50 ml) was added and the mixture was evaporated to dryness. The syrup thus obtained was dissolved in 150 ml of $CHCl_3$ and the solution was wahsed with H_2O until the water layer became neutral. The organic layer was dried with anhydrous Na_2SO_4 and evaporated to give a syrup, which was crystallized from 20 ml of EtOH. Recrystallization from MeOH afforded a pure sample, which showed no melting point depression with that of prepared by method A. Yield: 7.84 g (49.6%); mp 225—226°; $[\alpha]_D^{28.5}$ −50.0° (*c*=0.5, DMF–EtOH (1:1, v/v)); *Rf*: 0.63 (solvent A); UV

$\lambda_{max}^{pH\,1}$ mμ: 251; $\lambda_{max}^{pH\,13}$ mμ: 254. *Anal.* Calcd. for $C_{16}H_{18}O_8N_4$: C, 48.73; H, 4.60; N, 14.21. Found: C, 48.65; H, 4.85; N, 14.04.

Deacetylation of compound X prepared by method B with NH_3 in MeOH at room temperature gave 9-β-D-xylofuranosylhypoxanthine which was identical with an authentic material (IX); mp 223—224°; $[\alpha]_D^{29}$ −43.0° (c=1, H_2O).

9-β-D-Xylofuranosyl-6-mercaptopurine (XIII)——A solution of X (1.39 g) and N,N-dimethylaniline (3.5 ml) in $POCl_3$ (16 ml) was heated to reflux in an oil bath for 15 min. The reaction mixture was poured into 100 ml of ice water and the resulting chloro compound (XII) was extracted with three 70 ml portions of $CHCl_3$. After being washed with cold 1N HCl and then with H_2O and dried, the chloroform extracts were concentrated to syrup *in vacuo* and the residue was dissolved in anhydrous EtOH. This solution was again concentrated to dryness and the process was repeated twice. To a solution of the above syrup (1.25 g) in 40 ml of EtOH was added 0.3 g of thiourea. The mixture was refluxed for 2 hr and then concentrated *in vacuo*. H_2O (50 ml) was added and the product was extracted with three 100 ml portions of $CHCl_3$. After being washed and dried, the chloroform extracts were evaporated under reduced pressure. MeOH (100 ml) saturated with NH_3 at 0° was added and the mixture was allowed to stand at room temperature overnight. Evaporation of the solvent gave a crystalline product, which was recrystallized from H_2O to afford 0.384 g (38.4%) of a pure sample;[15] mp 211—212° (decomp.); $[\alpha]_D^{29}$ −80.0° (c=0.5, 0.1N NaOH); UV $\lambda_{max}^{pH\,1}$ mμ (ε): 227 (9700), 324 (18900); $\lambda_{max}^{pH\,13}$ mμ (ε): 233.5 (13500), 312 (20700). *Anal.* Calcd. for $C_{10}H_{12}O_4N_4S$: C, 42.25; H, 4.26; N, 19.71. Found: C, 42.22; H, 4.30; N, 19.88.

9-β-D-Xylofuranosylguanine 5′-Phosphate (XIV)——The reaction mixture containing 365 mg of the compound VI (1.288 mmole), 1.673 g of sodium *p*-nitrophenylphosphate as a phosphate donor, 18.6 mg of $ZnSO_4 \cdot 7H_2O$, acetate buffer (pH 4.5) (250 mmole) and 128.8 mg of intact cells of *Pseudomonas trifolii* (IAM 1555) as an enzyme source was incubated in a final volume of 64.4 ml at 40°, for 24 hr. The amount of 9-β-D-xylofuranosylguanine 5′-phosphate formed was 403 mg as free acid (yield: 86.3%).

The reaction mixture was centrifuged to remove the bacterial cells and the supernatant fluid was passed through a column (3.8×82 cm) of Dowex 1×4 ($HCOO^-$ form, 100—200 mesh). The column was washed with H_2O, during which time the unreacted xylosylguanine was eluted. The elution was followed with 0.2M HCOOH and 0.15M $HCOONH_4$ by collecting each 100 ml fraction. The effluents from fractions No. 480 to No. 610 were combined and evaporated to dryness under reduced pressure. The residue was sublimed *in vacuo* (at 2 mmHg) at 70—80° in water bath. After the crude compound was dissolved in 80 ml of H_2O a small insoluble material was filtered off. The filtrate was adjusted to pH 9.0 with 1N NH_4OH and concentrated *in vacuo* to about 20 ml. Addition of a half volume of EtOH gave a product, which was isolated by centrifugation as an amorphous powder. This was dried *in vacuo* over P_2O_5 at room temperature for 3 hr; yield 225 mg (36.5%); mp>240°; the migrating distance in paper electrophoresis (10% AcOH buffer, 800 v/cm, 2 hr): 2.9 cm; paper chromatography in solvent B indicated one spot at *Rf* 0.34. UV $\lambda_{max}^{pH\,1}$ mμ: 256; $\lambda_{max}^{pH\,13}$ mμ: 258—267. *Anal.* Calcd. for $C_{10}H_{17}O_8N_6P \cdot HCOONH_4 \cdot 2H_2O$: C, 27.56; H, 5.47; N, 20.46; P, 6.46. Found: C, 28.02; H, 5.56; N, 19.98; P, 6.07.

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15) 9-β-D-Xylofuranosyl-6-mercaptopurine-^{35}S was previously synthesized[6] by thiation of X with P_2S_5-^{35}S, but there was no description of its chemical and physical properties.