Synthesis in Nucleoside Antibiotics. II. Facile Synthesis of Nebularine and Its Analogs by a Modified Fusion Procedure[1]

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Nebularine and its N-7-position isomer have been synthesized simultaneously by the fusion method. A series of its analogs modified in the sugar moiety to D-xylofuranose, D-ribopyranose, and D-glucopyranose has been synthesized. The β configuration of these compounds has been assigned from their nmr spectra and ORD curves. A report on the tumor-inhibitory activity test of some of these compounds is included.

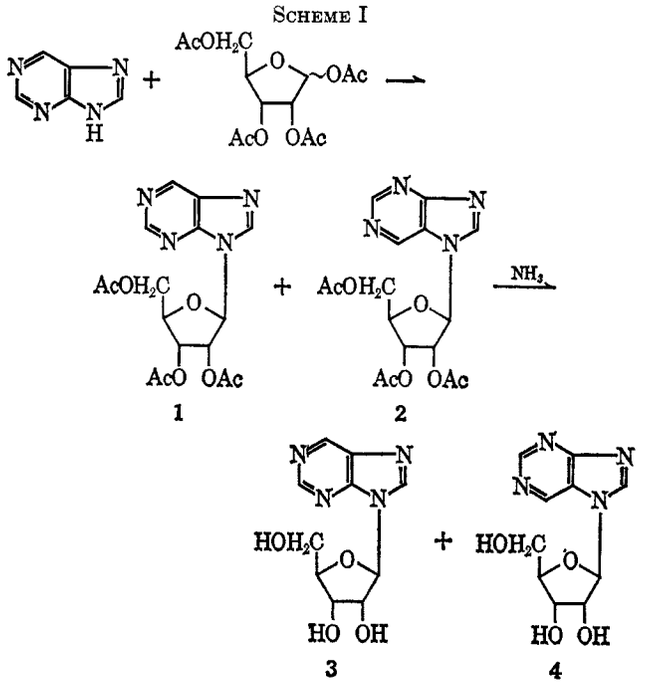
Of the naturally occurring nucleoside antibiotics, nebularine is of special interest as the simplest member of the purine nucleosides and because of its biological activity against mouse Sarcoma 180 and mycobacteria.[2,3] In addition, the increasing importance of the role played by such nucleoside antibiotics in protein and nucleic acid biosynthesis prompted us to develop a convenient method of synthesis of nebularine and its analogs.[4]

Two syntheses of nebularine have been reported. The first synthesis, by Brown and Weliky, used the Davoll–Lowy method, and the yield is calculated to be 34% starting from the chloromercury complex of purine and 2,3,5-tri-*O*-acetyl-D-ribofuranosyl chloride.[5] In another synthesis reported by Fox and his coworkers the over-all yield is calculated to be 32% starting from inosine.[6]

With the aim of developing a more satisfactory synthesis with fewer stages, the applicability of the modified fusion procedure has been examined, using bis(*p*-nitrophenyl)hydrogen phosphate as the catalyst.[7] During the course of this investigation, it has been found, for the first time, that nebularine and its N-7-position isomer are synthesized simultaneously by this procedure. Effort has also been directed to synthesize the nebularine analogs in which the sugar moiety is altered to D-xylofuranose, D-ribopyranose, and D-glucopyranose, including alteration in configuration or ring size or both. Previously, the utility of purine in the fusion procedure has been demonstrated in the synthesis of some of the purine glycosides such as 9-(2′-deoxy-α- and-β-D-ribofuranosyl and 2′-deoxy-α- and -β-D-ribopyranosyl)purine.[8] The utility seems to be largely due to its fusibility in the fusion reaction.

Fusion of purine[9] and tetra-*O*-acetyl-D-ribofuranose[10] in the presence of catalytic amounts of bis(*p*-nitrophenyl) hydrogen phosphate gave an oil, which was shown, by thin layer chromatography, to be a mixture of unreacted starting materials and two condensation products. The mixture was resolved by silicic acid column chromatography into unreacted sugar, 9-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)purine (**1**), and another nucleoside which was subsequently characterized as 7-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)purine (**2**). The yields of **1** and **2** were 70 and 20%, respectively, on the basis of reacted sugar. Without further purification these compounds **1** and **2** were deacetylated with methanolic ammonia to give 9-β-D-ribofuranosylpurine (**3**, nebularine) and 7-β-D-ribofuranosylpurine (**4**, isonebularine), respectively (Scheme I). Compound **3** was compared directly with a sample of natural nebularine which was isolated from *Streptomyces yokosukaensis*.[3a] The two compounds had the same melting point (182–183°), which showed no depression on admixture, and were identical in every respect examined.

SCHEME I



Compound **4** melted at 184–185°; the melting point showed depression on admixture with natural nebularine. The result of gas chromatographic analysis[11,12] indicated that the compound consisted of

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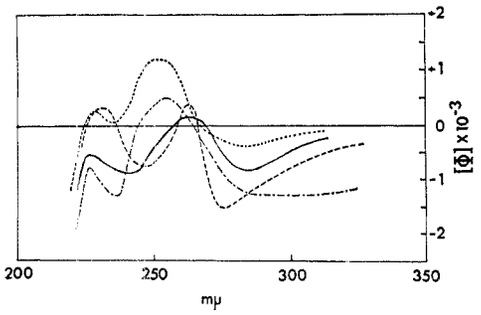

Figure 1.—ORD curves of nebularine (**3**) (——), 9-β-D-xylofuranosylpurine (**5**) (- - - -), 9-β-D-ribopyranosylpurine (**7**) (-·-·-·-), and 9-β-D-glucopyranosylpurine (**9**) (. . . . .).

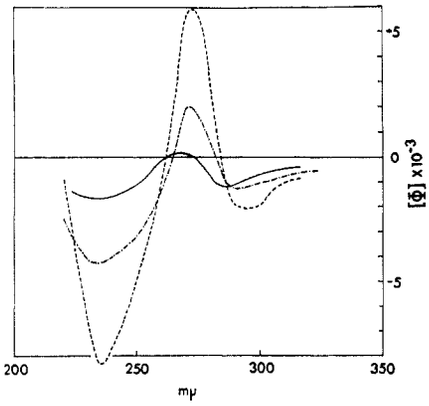

Figure 2.—ORD curves of 7-β-D-ribofuranosylpurine (**4**) (-·-·-·-), 7-β-D-xylofuranosylpurine (**6**) (——), and 7-β-D-ribopyranosylpurine (**8**) (- - - -).

ribose and purine, and elemental analysis validated the formula $C_{10}H_{12}O_4N_4$. The ultraviolet spectra ($\lambda_{max}^{H_2O}$ 264.0, $\lambda_{max}^{N\text{-}HCl}$ 258.0, $\lambda_{max}^{pH\ 9.18}$ 265.0 mμ) were similar to those of 7-methylpurine among the spectra of 1-, 3-, 7- and 9-methylpurine.[13-15] Thus, this compound was determined as 7-D-ribofuranosylpurine. Moreover the physicochemical constants were in accord with those of 7-β-D-ribofuranosylpurine synthesized by the mercury method.[16] The nmr spectrum of the compound was in agreement with the structure assigned.

Similarly, the fusion of purine with tetra-*O*-acetyl-D-xylofuranose[17] gave 9-β-D-xylofuranosylpurine (**5**) and 7-β-D-xylofuranosylpurine (**6**), whereas that with tetra-*O*-acetyl-D-ribopyranose[10] gave 9-β-D-ribopyranosylpurine (**7**) and 7-β-D-ribopyranosylpurine (**8**), but only 9-β-D-glucopyranosylpurine (**9**) was obtained in reaction with penta-*O*-acetyl-β-D-glucopyranose.[18] No N-7 isomer was detected on examination by tlc of the reaction mixture.

By the chloromercury method, 9-D-ribopyranosylpurine has been synthesized previously in the over-all yield of 13% from purine.[19] Although the earlier workers made no assignment of the configuration, the earlier and present preparations seem to give identical nucleosides. It should be noted, however, that the earlier preparation appears to be slightly contaminated as judged by melting point and ultraviolet spectral criteria. Because the chloromercury method normally yields several by-products,[20] isolation of the desired compound from the reaction product requires careful purification procedure such as chromatography.

The position of attachment of the sugar moiety to the purine ring was established as N-9 for **5**, **7**, and **9**, whereas N-7 was established for **6** and **8** by comparison of the ultraviolet spectra with those of the corresponding N-9- or N-7-methyl counterparts described above.

The β configuration of the ribo- and glucopyranosylpurines (**7**, **8**, and **9**) was assigned by measurements of the nmr spectra. The $H_{1'}$ signals in **7**, **8**, and **9** appeared as doublets of $J_{1'2'}$ = 9 cps, which indicated the diaxial orientation of $H_{1'}$ and $H_{2'}$ with a projected angle of about 180° between the $C_{1'}$ and $C_{2'}$ carbon–hydrogen bond.[21-26] Such large values of $J_{1'2'}$ in **7**, **8**, and **9** exclude the possibilities of α- and β-D-ribo- and glucopyranoside in *IC* conformation and of the α-D anomers in *CI* conformation; the one possibility remaining is of β-D anomers in *CI* conformation.[23,24,27] The nmr spectra of N-9- and N-7-xylofuranosylpurine (**5** and **6**) indicated the $J_{1'2'}$ values of 2.0 and 2.2 cps, respectively. Such values are low enough to assign the β configuration to both compounds. Similar assignments have been reported by several investigators.[24,28,29]

Since compounds **3** and **4** showed $J_{1'2'}$ values of 5.5 cps, we were unable to assign the configurations from the value. The identity of **4** and 7-β-D-ribofuranosylpurine synthesized by the mercury method[16] and that of **3** and nebularine,[5,6] however, were established by comparisons of their physicochemical properties, so that the β configuration was assigned.

Those assignments stated above were also supported by the results of ORD measurements. ORD curves of these compounds in the N-9 and N-7 series are given in Figure 1 and Figure 2, respectively. In each series of these unsubstituted purine derivatives, the second Cotton effect is negative, while a real trough before the first peak is absent. These results parallel those of Ulbricht, *et al.*,[30] and strongly support the assignment of a β configuration to these nucleosides.

These assignments are also in accord with Baker's *trans* rule, although the detailed reaction mechanism of the fusion method is not exactly the same as that of the mercury method.

Physicochemical properties of the purine nucleosides are listed in Table I.

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TABLE I

PHYSICOCHEMICAL PROPERTIES OF PURINE NUCLEOSIDES

Compd	Mp, °C	$[\alpha]_D$, deg (c, water)	λ_{max}, mμ H_2O	H-HCl	pH 9.18
3	182–183	−46.8 (2 at 35°)	262.5	262.8	263.0
4	184.5–185	−37.8 (2 at 38°)	264.0	258.0	265.0
5	163.5–164.5	−35.2 (2 at 39°)	262.5	263.3	263.5
6	150–151	−31.6 (1 at 23°)	264.5	258.0	264.5
7	256–257	−23.6 (2 at 23°)	262.5	262.5	262.5
8	241–242		264.5	258.0	262.5
9	205–205.5	−1.5 (0.8 at 11.5°)	261.5	262.5	262.5

A tumor-inhibitory activity test was carried out with modified antibiotics against Ehrlich ascites carcinoma in the mice. Tumor-inhibitory action of 9-β-D-glucopyranosylpurine was noticed a dose level of 19 mg/kg/day, whereas none of the other nucleosides showed any activity. Details of the results will be fully discussed elsewhere.

Experimental Section

All melting points were determined on Yanagimoto micro-melting point apparatus and corrected.

Spectra.—The ultraviolet absorption spectra were obtained from a Model 139 Hitachi/Perkin-Elmer spectrophotometer. The infrared spectral data were obtained with a Model AR-275 Shimadzu spectrometer. Proton magnetic resonance spectra were recorded on a Varian A-60 spectrometer in D_2O with DDS as the internal standard. The optical rotatory dispersions were measured at room temperature on aqueous solutions with a Model 185 Yanagimoto spectropolarimeter.

Thin Layer Chromatography (tlc).—Aluminum oxide G (supplied by E. Merck A. G., Darmstadt) was used for tlc. After development with ethyl acetate, the plates were examined under an ultraviolet lamp and then sprayed with a solution of ammonium metavanadate (2 g) in 50% sulfuric acid (50 ml) and heated.

9-(2′,3′,5′-Tri-*O*-acetyl-β-D-ribofuranosyl)purine (1) and 7-(2′,3′,5′-Tri-*O*-acetyl-β-D-ribofuranosyl)purine (2).—A finely pulverized mixture of 2 g of purine[9] and 4.1 g of 1,2,3,5-tetra-*O*-acetyl-D-ribofuranose[10] was melted at 170–180° in an oil bath. To the mixture was added 200 mg of anhydrous bis(*p*-nitrophenyl) hydrogen phosphate[7] (dried at 100° for 30 min under a pressure of 1 mm) and mixed well. The mixture was kept at 170–180° for 15 min under reduced pressure (water pump). The reaction mixture was extracted with chloroform. The chloroform layer was washed with water, dried over sodium sulfate, and then evaporated to dryness yielding 5.2 g of oil. The crude product was dissolved in ethyl acetate, and the solution was placed on the top of a column, 49 cm in length and 3-cm i.d., packed with 180 g of silicic acid (100 mesh), supplied by Mallinckrodt Chemical Works. Then elution was carried out with ethyl acetate at a flow rate of 0.3 ml/min. The effluent was collected in 15-ml fractions using a fraction collector. Measuring the optical densities of the fractions, at 260 mμ, a chromatogram showing three peaks was obtained. From fractions 14–23, corresponding to the first peak, 0.6 g of unreacted sugar was recovered. From fractions 26–40 corresponding to the second peak, 2.09 g of **1** was obtained as oil (70% yield based on reacted sugar), λ_{max}^{EtOH} 262.5 mμ. From the third fractions, 56–81, 0.60 g of **2** was obtained as oil (20% yield based on reacted sugar), λ_{max}^{EtOH} 265.0 mμ. These compounds were fully characterized after deacetylation.

9-β-D-Ribofuranosylpurine (3, Nebularine).—A solution of 1.7 g of **1** in 70 ml of methanolic ammonia was kept at 0° for 1 day. The solution was evaporated to dryness, and recrystallization of the residue from methanol gave 886 mg of **3** as colorless needles: yield 79%; mp 182–183° (lit. 181–182°,[2,5] 180–181°[3]); $[\alpha]_D^{35}$ −46.8° (*c* 2, water) {lit. $[\alpha]_D^{25}$ −47.5° (*c* 2, water)[2]}; $\lambda_{max}^{H_2O}$ 262.5 mμ (ε 7020); $\lambda_{max}^{1\,N\,HCl}$ 262.8 mμ (5900); $\lambda_{max}^{pH\,9.18}$ 263.0 mμ (ε 7110) (lit.[3] $\lambda_{max}^{H_2O}$ 263 mμ; $\lambda_{max}^{0.1\,N\,HCl}$ 262 mμ; $\lambda_{max}^{0.1\,N\,NaOH}$ 263 mμ).

Anal. Calcd for $C_{10}H_{12}O_4N_4$: C, 47.62; H, 4.80; N, 22.22. Found: C, 47.67; H, 4.87; N, 22.37.

7-β-D-Ribofuranosylpurine (4, Isonebularine).—Compound **2** (298 mg) was treated with 30 ml of methanolic ammonia as described above. The methanolic solution was evaporated to dryness, and recrystallization of the residue from methanol gave 167 mg of **4** as colorless needles: yield 84%; mp 184.5–185°; $[\alpha]_D^{38}$ −37.8° (*c* 2, water); $\lambda_{max}^{H_2O}$ 264.0 mμ (ε 7480); $\lambda_{max}^{1\,N\,HCl}$ 258.0 mμ (ε 6900); $\lambda_{max}^{pH\,9.18}$ 265.0 mμ (ε 7300) (lit.[16] $\lambda_{max}^{pH\,5}$ 265.0 mμ; $\lambda_{max}^{pH\,0}$ 258 mμ).

Anal. Calcd for $C_{10}H_{12}O_4N_4$: C, 47.62; H, 4.80; N, 22.22. Found: C, 47.59; H, 4.83; N, 22.21.

9-(2′,3′,5′-Tri-*O*-acetyl-β-D-xylofuranosyl)purine and 7-(2′,3′,5′-Tri-*O*-acetyl-β-D-xylofuranosyl)purine.—A mixture of 2 g of purine and 4.1 g of 1,2,3,5-tetra-*O*-acetyl-D-xylofuranose[17] was fused in a manner similar with that described for the preparation of **1** and **2**. The oily crude product (5.1 g) was placed on the top of the column, 49 cm in length and 2.2-cm i.d., packed with 190 g of aluminum oxide (degree of activity 1) supplied by E. Merck A. G., Darmstadt. The elution was carried out with ethyl acetate at a flow rate of 0.6–0.7 ml/min and collected in 15-ml fractions. Measuring the optical densities of the fractions at 260 mμ, fractions 10–21 were combined and evaporated to dryness. From the residual oil, 2.0 g of unreacted sugar was recovered. From fractions 25–73. 1.58 g of the N-9 derivative was obtained as oil in a 63% yield based on reacted sugar, λ_{max}^{EtOH} 263.0 mμ. The fractions 143–337 gave 213 mg of the N-7 derivative as oil in a 8.5% yield based on reacted sugar, λ_{max}^{EtOH} 264.5 mμ.

9-β-D-Xylofuranosylpurine (5).—9-(2′,3′,5′-Tri-*O*-acetyl-β-D-xylofuranosyl)purine (1.5 g) was treated with 70 ml of methanolic ammonia as described above. The methanolic solution was evaporated to give an oil. Recrystallization from ethanol containing small amounts of ethyl acetate gave 556 mg (74% yield) of **5** as fine colorless needles: mp 163.5–164.5°; $[\alpha]_D^{39}$ −35.2° (*c* 2, water); $\lambda_{max}^{H_2O}$ 262.5 mμ (ε 7120); $\lambda_{max}^{1\,N\,HCl}$ 263.3 mμ (ε 5690), $\lambda_{max}^{pH\,9.18}$ 263.5 mμ (ε 7330).

Anal. Calcd for $C_{10}H_{12}O_4N_4$: C, 47.62; H, 4.80; N, 22.22. Found: C, 47.59; H, 4.93; N, 22.21.

7-β-D-Xylofuranosylpurine (6).—7 (2′,3′,5′-Tri-*O*-acetyl-β-D-xylofuranosyl)purine (213 mg) was treated with 20 ml of methanolic ammonia as described above. The methanolic solution was evaporated to dryness, and the residue was dissolved in ethanol containing small amounts of ethyl acetate. After storage for 6 months in a refrigerator, 60 mg (42.3% yield) of **6** was obtained as a colorless powder. The analytical sample was further recrystallized from ethanol: mp 150–151°; $[\alpha]_D^{23}$ −31.6° (*c* 2, water); $\lambda_{max}^{H_2O}$ 264.5 mμ (ε 7310); $\lambda_{max}^{1\,N\,HCl}$ 258.0 mμ (ε 6500); $\lambda_{max}^{pH\,9.18}$ 264.5 mμ (ε 6430).

Anal. Calcd for $C_{10}H_{12}O_4N_4$: C, 47.62; H, 4.80; N, 22.22. Found: C, 47.65; H, 4.93; N, 21.96.

9-(2′,3′,4′-Tri-*O*-acetyl-β-D-ribopyranosyl)purine and 7-(2′,3′,4′-Tri-*O*-acetyl-β-D-ribopyranosyl)purine.—A finely pulverized mixture of 1.0 g of purine and 2.0 g of 1,2,3,4-tetra-*O*-acetyl-D-ribopyranose[10] was fused in a similar manner to that described above. The oily crude product (2.29 g) was applied to a column, 28 cm in length and 2.5-cm i.d. packed with 100 g of alumina, eluted with ethyl acetate at a flow rate of 0.7 ml/min, and collected in 8-ml fractions. Each fraction was examined by the tlc, and 1.3 g of unreacted sugar was recovered from fractions 15–21. From fractions 40–205, 705 mg of the N-9 derivative was obtained as nearly colorless needles, 84.7% yield based on reacted sugar. The analytical sample was further recrystallized from methanol containing small amounts of *n*-hexane: mp 173.5–174.5°; λ_{max}^{EtOH} 262.0 mμ.

Anal. Calcd for $C_{16}H_{18}O_7N_4$: C, 50.79; H, 4.80; N, 14.81. Found: C, 51.08; H, 5.00; N, 14.59.

From fractions 276–302, 100 mg of the N-7 derivative was obtained as an oil, 12% yield based on reacted sugar.

9-β-D-Ribopyranosylpurine (7).—9-(2′,3′,4′-Tri-*O*-acetyl-β-D-ribopyranosyl)purine (200 mg) was treated with 30 ml of methanolic ammonia. The methanolic solution was evaporated to dryness, and recrystallization of the residue from methanol gave 176 mg (88.0% yield) of **7** as colorless needles: mp 256–257°; $[\alpha]_D^{23}$ −23.6° (*c* 2, water); $\lambda_{max}^{H_2O}$ 262.5 mμ (ε 2700); $\lambda_{max}^{1\,N\,C}$ 262.5 mμ (ε 6300); $\lambda_{max}^{pH\,9.18}$ 262.5 mμ (ε 8700).

Anal. Calcd for $C_{10}H_{12}O_4N_4$: C, 47.62; H, 4.80; N, 22.22. Found: C, 47.33; H, 4.74; N, 22.17.

7-β-D-Ribopyranosylpurine (8).—7-(2′,3′,4′-Tri-*O*-acetyl-β-D-ribopyranosyl)purine (100 mg) was treated with 30 ml of methanolic ammonia. The methanolic solution was evaporated to dryness, and recrystallization of the residue from methanol gave 45 mg (67.5% yield) of **8** as colorless needles: mp 241–242°; $\lambda_{max}^{H_2O}$ 264.5 mμ (ε 7300); $\lambda_{max}^{1\,N\,HCl}$ 258.0 mμ (ε 6500); $\lambda_{max}^{pH\,9.18}$ 264.5 mμ (ε 7550).

Anal. Calcd for $C_{16}H_{12}O_4N_4$: C, 47.62; H, 4.80; N, 22.22. Found: C, 47.59; H, 4.59; N, 22.05.

9-(2′,3′,4′,6′-Tetra-*O*-acetyl-β-D-glucopyranosyl)purine.—A finely pulverized mixture of 1.4 g of purine and 3.2 g of 1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranose[18] was fused in a similar manner to that described above. Recrystallization of the crude product from methanol and ethyl acetate gave 360 mg (8.4 % yield) of the titled compound as colorless needles: mp 199.5–200°; λ_{max}^{EtOH} 261.5 mμ.

Anal. Calcd for $C_{19}H_{22}O_9N_4$: C, 50.60; H, 4.92; N, 12.44. Found: C, 50.51; H, 4.91; N, 12.32.

9-β-D-Glucopyranosylpurine (9).—9-(2′,3′,4′,6′-Tetra-*O*-acetyl-β-D-glucopyranosylpurine (277 mg) was treated with 40 ml of methanolic ammonia as described above. The methanolic solution was evaporated to dryness, and recrystallization from ethanol and water gave 171 mg (98.5% yield) of 9 as colorless platelets: mp 205–205.5°; $[\alpha]^{11.5}D$ −1.5° (*c* 0.8, water); $\lambda_{max}^{H_2O}$ 261.5 mμ (ε 7810); $\lambda_{max}^{1\ N\ HCl}$ 262.5 mμ (ε 11,280); $\lambda_{max}^{pH\ 9.18}$ 262.5 mμ (ε 11,300).

Anal. Calcd for $C_{11}H_{14}O_5N_4$: C, 45.30; H, 5.19; N, 19.24. Found: C, 45.71; H, 5.21; N, 19.13.

Registry No.—1, 15981-63-2; 2, 15981-64-3; 3, 550-33-4; 4, 2149-71-5; 5, 15981-67-6; 6, 15981-68-7; 7, 15981-71-2; 8, 15981-69-8; 9, 15981-70-1; 9-(2′,3′,5′-tri-*O*-acetyl-β-D-xylofuranosyl)purine, 15981-47-2; 7-(2′,3′,5′-tri-*O*-acetyl-β-D-xylofuranosyl)purine, 15981-44-9; 9-(2′,3′,4′-tri-*O*-acetyl-β-D-ribopyranosyl)-purine, 15981-45-0; 9-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl)purine, 15981-46-1.

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The Proton Magnetic Resonance Spectra of Pentofuranose Derivatives

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The proton magnetic resonance spectra of a number of derivatives of D-arabinose, D-lyxose, D-ribose, and D-xylose in their furanose forms have been measured. The pmr parameters have been shown to be of diagnostic value in structural studies and form the basis for speculations regarding the conformations of these substances in solution. A novel example of "virtual long-range coupling," involving a hydroxyl hydrogen, is discussed.

The large difference between spin–spin coupling constants for vicinal hydrogens in a saturated six-membered ring, dependent on whether these hydrogens are axial–axial (J = *ca.* 6–11 Hz), axial–equatorial, or equatorial–equatorial (J = *ca.* 1–5.4 Hz),[2] has led to the solution of a large number of structural and stereochemical problems involving such ring systems.[3] Parallel success for five-membered ring compounds has not been so marked. This is in part due to the paucity of data on compounds containing these rings as well as the much greater complexity of the problem of conformation resulting from the large number of "extreme" conformations which are possible for five-membered rings[4] and the resultant uncertainty in the projected bond angles for vicinal hydrogens. Nevertheless, a number of problems have been solved and information is accumulating on the pmr parameters of variously substituted five-membered ring systems.[3b,5]

As part of a broad study of derivatives of sugars in the furanose form,[6] we have examined the pmr spectra of a number of acylated pentofuranoses with the aim of providing data for structural and stereochemical problems and with the view of relating the observed coupling constants to possible conformations.

For most of the compounds studied, the difference in chemical shift of H-1 and H-2 and of H-3 and H-4 is large compared with the couplings $J_{1,2}$ and $J_{3,4}$, respectively. For a number of the compounds, the difference in chemical shift between H-4 and H-5,H-5′ is sufficiently large to result in the C-5 methylene protons having no observable first-order effects upon the signals due to H-3. In such cases, the hydrogens attached to the ring could be considered independently of H-5,-H-5′ and they may be classified as an AKLX system.[7] For others, H-4, H-5, and H-5′ form a strongly coupled system (*i.e.*, the difference in chemical shift of these three protons is less than, or of the same order as, the spin–spin coupling between them) which results in a broadening of the signals due to H-3; in such cases, H-3 may be said to experience virtual long-range coupling with H-5,H-5′.[8] In order to obtain spectra amenable to first-order analysis, acyloxy groups on C-2 and C-3 were chosen such that the difference in chemical shift of H-2 and H-3 was several times the value of the coupling constant, $J_{2,3}$. This procedure introduced the problem of comparing coupling constants for CH–CH fragments bearing different sub-

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