Purine Nucleosides. XVIII. The Direct Utilization of Unsaturated Sugars in Nucleoside Syntheses. The Conformation and Structure of Certain 9-(2'-Deoxyribopyranosyl)purines Prepared from D-Arabinal[1]

Eldon E. Leutzinger, William A. Bowles,[2] Roland K. Robins, and Leroy B. Townsend[3]

Contribution from the Department of Chemistry, Arizona State University, Tempe, Arizona, and Department of Chemistry, University of Utah, Salt Lake City, Utah 84112. Received August 10, 1967

Abstract: The direct acid-catalyzed fusion of 6-chloropurine (I) or 2,6-dichloropurine (II) with 3,4-di-O-acetyl-D-arabinal provided a 40–50% crude yield of crystalline acetylated nucleoside derivatives. The major products were shown to be the corresponding 6-chloro- or 2,6-dichloro-9-(3',4'-di-O-acetyl-α- and β-2'-deoxy-D-ribopyranosyl)purine which were separated into pure anomers by preparative layer chromatography and fractional crystallization. The structures of the products were verified by an independent synthesis from the acid-catalyzed fusion of 1,3,4-tri-O-acetyl-2-deoxy-β-D-ribopyranose and the appropriate purine, which yielded a similar anomeric mixture of the corresponding 9-(3',4'-di-O-acetyl-2'-deoxyribopyranosyl)purine in about 30% yield, presumably *via* a common intermediate carbonium ion at C_1. The conformation and anomeric configuration has been assigned with the assistance of proton magnetic resonance studies utilizing the double-resonance or proton–proton spin decoupling technique. The ready availability of these 2'-deoxynucleosides from D-arabinal provides extremely useful synthetic intermediate nucleosides for the preparation of many interesting 9-(2'-deoxy-D-ribopyranosyl)purines of known anomeric assignment. A number of these derivatives obtained by functional group replacement on the purine moiety are reported.

The possibility of employing a glycal derivative directly in nucleoside synthesis was first suggested by Robins, *et al.*,[4] in a model study utilizing various purines and 2,3-dihydropyran and 2,3-dihydrofuran.[5,6] The fusion procedure of nucleoside synthesis[7-10] has proved very successful in recent years in our own laboratory and suggested the possibility of employing an appropriately acetylated glycal directly in an attempt to prepare 2'-deoxyribopyranosylpurines by a fusion procedure. The use of a 1,2-unsaturated sugar derivative directly has several distinct advantages. For instance, previous procedures have utilized glycals as starting material for subsequent conversion to the requisite 2-deoxy-D-pyranosyl halides which have then been employed in the condensation with the mercury salt of a purine[11,12] or pyrimidine base.[13,14]

In the present work the conversion to the 2-deoxypyranosyl halide is eliminated. The problems often encountered in removing contaminating mercury from the final nucleoside[15-17] are also avoided. The low yields of 2'-deoxyribopyranosylpurines previously reported have made the separation into pure anomers extremely difficult.

In the present procedure the requisite purine and 3,4-di-O-acetyl-D-arabinal have been fused to give the corresponding 9-(3',4'-di-O-acetyl-2'-deoxy-D-ribopyranosyl)purine as an anomeric mixture in excellent yield. A preliminary account of this work has appeared.[7] The present work is especially significant in view of the fact that several 2'-deoxypyranosyl nucleosides have demonstrated specific inhibition of certain nucleoside phosphorylases.[14,18,19] A mixture of 6-chloropurine (I) and 3,4-di-O-acetyl-D-arabinal was fused at 120° (inside temperature) for approximately 1 hr in the presence of a catalytic amount of sulfanilic acid. After separation of isomers, 18.5% 6-chloro-9-(3',4',di-O-acetyl-2'-deoxy-α-D-ribopyranosyl)purine (V) and 5.28% 6-chloro-9-(3',4'-di-O-acetyl-2'-deoxy-β-D-ribopyranosyl)purine (III) were obtained. Methylation studies[20,21] of 6-chloropurine and other chloropurines

(1) This work was supported in part by Contract No. PH 43-65-1041 with the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Public Health Service, and in part by Research Grants CA-04008-07 and CA-08109 from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service.

(2) In partial fulfillment for the requirements for the Ph.D. Thesis, W. A. Bowles, Arizona State University, 1964.

(3) Author to whom inquiries may be addressed at the Department of Chemistry, University of Utah, Salt Lake City, Utah 84112.

(4) R. K. Robins, E. F. Godefroi, E. C. Taylor, L. R. Lewis, and A. Jackson, *J. Am. Chem. Soc.*, **83**, 2574 (1961).

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

(6) W. A. Bowles, F. H. Schneider, L. R. Lewis, and R. K. Robins, *J. Med. Chem.*, **6**, 471 (1963).

(7) M. J. Robins, W. A. Bowles, and R. K. Robins, *J. Am. Chem. Soc.*, **86**, 1251 (1964), and references cited therein.

(8) W. A. Bowles and R. K. Robins, *ibid.*, **86**, 1252 (1964).

(9) R. J. Rousseau, L. B. Townsend, and R. K. Robins, *Chem. Commun.*, 265 (1966), and references cited therein.

(10) (a) Y. H. Pan, R. K. Robins, and L. B. Townsend, *J. Heterocyclic Chem.*, **4**, 246 (1967); (b) R. L. Tolman, R. K. Robins, and L. B. Townsend, *ibid.*, **4**, 230 (1967).

(11) H. Zinner and E. Wittenburg, *Chem. Ber.*, **95**, 1866 (1962).

(12) J. J. Novak and F. Sorm, *Collection Czech. Chem. Commun.*, **27**, 902 (1962).

(13) W. W. Zorbach and G. J. Durr, Jr., *J. Org. Chem.*, **27**, 1474 (1962).

(14) G. Etzold and P. Langen, *Chem. Ber.*, **98**, 1988 (1965).

(15) J. Skoda, I. Bartosek, and F. Sorm, *Collection Czech. Chem. Commun.*, **27**, 906 (1962).

(16) E. Walton, F. W. Holly, G. E. Boxer, R. F. Nutt, and S. R. Jenkins, *J. Med. Chem.*, **8**, 659 (1965).

(17) G. L. Tong, W. W. Lee, and L. Goodman, *J. Org. Chem.*, **32**, 1984 (1967).

(18) P. Langen and G. Etzold, *Biochem. Z.*, **339**, 190 (1963).

(19) M. Zimmerman, *Biochem. Biophys. Res. Commun.*, **16**, 605 (1964).

(20) A. G. Beaman and R. K. Robins, *J. Org. Chem.*, **28**, 2310 (1963).

(21) G. B. Elion, *ibid.*, **27**, 2478 (1962).

Table I. Ultraviolet Absorption Spectra[a] for Certain 2'-Deoxy-D-ribopyranosylpurine Nucleosides

X	Y	R	Nucleoside	pH 1 λ_{max}	pH 1 ϵ_{max}	pH 11 λ_{max}	pH 11 ϵ_{max}	Other solvent λ_{max}	Other solvent ϵ_{max}	
H	Cl	Ac	V(α)	263	8,390	264	10,700	263	11,400	Ethanol
H	Cl	Ac	III(β)	263	8,370	263	10,700	263	11,400	Ethanol
H	NH$_2$	H	VIII(α)	256.5	15,600	258	15,900	258	15,700	Water
H	NH$_2$	H	VII(β)	256.5	16,400	258	17,300	258	16,500	Water
H	SH	H	X(α)	320	22,900	309	22,100	314	21,800	Water
						233	14,600	228	11,100	Water
H	SH	H	IX(β)	320	23,800	309	22,500	317	23,600	Water
						233	14,200	226	9,930	Water
Cl	Cl	Ac	VI(α)	274	9,350	273	14,400	274	10,500	Ethanol
Cl	Cl	Ac	IV(β)	274	9,550	274	14,400	274	10,400	Ethanol
Cl	NH$_2$	H	XI(α)	263.5	13,700	263.5	14,500	263.5	14,500	Water

[a] Spectra were determined on a DK-2 ultraviolet absorption spectrometer.

have produced only 7- and 9-methyl derivatives, and on this basis N-1 and N-3 were excluded as possible sites of glycosidation. Therefore, the actual site of glycosidation was readily ascertained as occurring at N-9 for
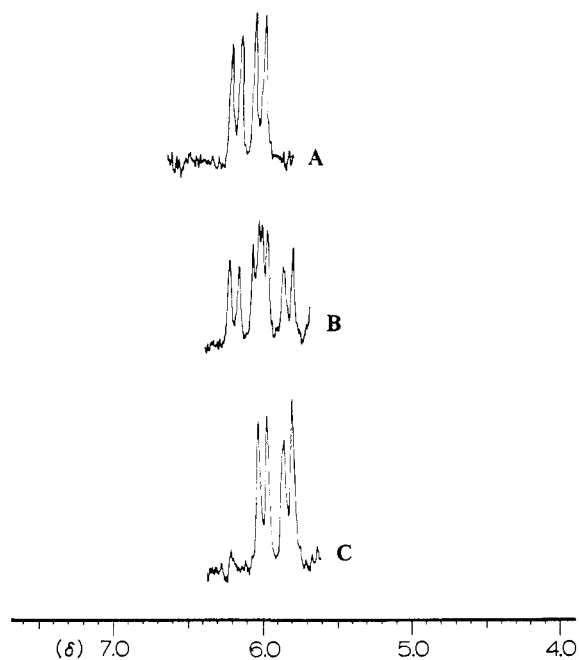

Figure 1. Splitting patterns and chemical shifts observed for the anomeric proton (H1') of 6-chloro-9-(3',4'-di-O-acetyl-2'-deoxy-β-D-ribopyranosyl)purine (III) and 6-chloro-9-(3',4'-di-O-acetyl-2'-deoxy-α-D-ribopyranosyl)purine (V): A. 100% α; B, 50% α and 50% β; C, 100% β.

these nucleosides by a comparison of their ultraviolet absorption spectra (Table I) with the ultraviolet absorption spectra of 6-chloro-9-methylpurine[22] and 6-chloro-7-methylpurine.[23]

(22) R. K. Robins and H. H. Lin, J. Am. Chem. Soc., 79, 490 (1957).
(23) R. N. Prasad and R. K. Robins, ibid., 79, 6401 (1957).

The crystalline anomeric mixture of 6-chloro-9-(3',4'-di-O-acetyl-2'-deoxy-D-ribopyranosyl)purine was successfully separated by fractional recrystallization to provide two chromatographically pure nucleosides melting at 205–207 and 159–160°. The assignment of anomeric configuration for several nucleosides has been recently accomplished[24-26] utilizing pmr spectroscopy since it has been observed that the absorption peak for the anomeric proton of a C1'-C2' cis-nucleoside appeared at a lower field (ca. δ 0.1–0.5 difference) than the corresponding anomeric proton of a C1'-C2' trans-nucleoside. Although the 2'-deoxy function in the present investigation precludes the formation of either a cis or trans anomeric configuration, an inspection of the pmr spectra for the above two nucleosides revealed a definite chemical shift between their anomeric proton absorption peaks. The nucleoside melting at 205–207° exhibited a quartet for the anomeric proton centered at δ 6.18 while the nucleoside melting at 159–160° exhibited a quartet for the anomeric proton centered at δ 6.03. On the basis of the above chemical shift data and their specific rotations,[27] the nucleoside melting at 205–207°, [α]^{26}D +21.8° (c 0.75, acetone), was initially assigned the α configuration (V) and the nucleoside melting at 159–160°, [α]^{26}D −33.6° (c 1.0, ethyl acetate), the β configuration (III). In fact, the pmr spectrum of III and V can be used to determine the anomeric purity or impurity of this series of nucleosides which is essential since, although III and V were chromatographically pure, there still existed the definite possibility of anomeric impurity. A visual inspection in the δ 6–6.5 region of the pmr spectra of III and V (Figure 1) reveals that although a slight overlap of the absorption peaks (quartets) for the anomeric protons

(24) K. R. Darnall and L. B. Townsend, J. Heterocyclic Chem., 3, 37 (1966).
(25) T. Nishimura and B. Shimizu, Chem. Pharm. Bull. (Tokyo), 13, 803 (1965).
(26) K. I. Imai, A. Nohara, and M. Honjo, ibid., 14, 1377 (1966).
(27) C. S. Hudson, J. Am. Chem. Soc., 31, 66 (1909); W. Pigman, Ed., "The Carbohydrates," Academic Press Inc., New York, N. Y., 1957, p 70.

does occur, the presence of an anomeric impurity can still be readily ascertained.
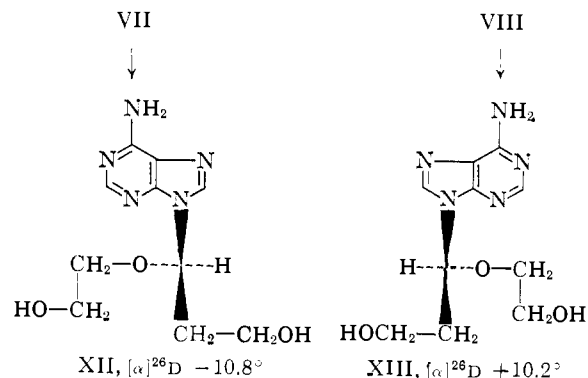
The acid-catalyzed fusion of 6-chloropurine (I) with 1,3,4-tri-O-acetyl-2-deoxy-β-D-ribopyranose[28] also gave a crystalline mixture of III and V. Fractional crystallization of this mixture afforded pure III and V which were shown to be identical with the two nucleosides obtained (vide supra) from the fusion of 6-chloropurine (I) and 3,4-di-O-acetyl-D-arabinal.

Treatment of III and V with alcoholic ammonia at 110–120° gave 6-amino-9-(2'-deoxy-β-D-ribopyranosyl)-purine (VII) and 6-amino-9-(2'-deoxy-α-D-ribopyranosyl)purine (VIII), respectively. The preparation of 6-amino-9-(2'-deoxy-β-D-ribopyranosyl)purine (VII), mp 262–264°, has been previously reported[11] to occur in low yield via a condensation of the mercury salt of 6-benzamidopurine and 3,4-di-O-p-tolyl-2-deoxy-D-ribo-pyranosyl chloride and also by direct glycosidation[29] of adenine. Although an anomeric mixture of VII and VIII was obtained by Zinner and Wittenburg[11] the α-anomer (VIII) was not isolated as a pure crystalline substance. Thus the anomeric assignment of the product with mp 262–264° was an arbitrary assignment based on the negative specific rotation of $[\alpha]^{20}$D −17.8°. In the present work a compound (VII) was obtained, mp 266–267°, $[\alpha]^{25}$D −17.0 (c 0.6, water), which possessed similar properties to those assigned 6-amino-9-(2'-deoxy-β-D-ribopyranosyl)purine (VII) as described by Zinner and Wittenburg,[11] including R_f values. The corresponding α-anomer (VIII) obtained from V melted at 232–235°, $[\alpha]^{25}$D +6° (c 1.0, water). Treatment of VII and VIII with dilute hydrochloric acid effected a facile cleavage of the glycosidic bond, and chromatography of the reaction mixture established the presence of adenine and 2-deoxy-D-erythro-pentose (2-deoxy-ribose).

As early as 1949, Davoll and Lythgoe[30] prepared the 2'-deoxyribopyranosyl derivative of theophylline via the Fischer–Helferich condensation of 3,4-di-O-acetyl-2-deoxy-D-ribopyranosyl chloride with the silver salt of theophylline. Although these workers[30] were able to separate the product into α- and β-anomers no assignment of configuration of the anomers could be made, since it was observed that the relative values of the optical rotations of the two theophylline nucleosides reversed upon deacetylation. These authors concluded that the isorotation rules have limited validity in this type of compound. A number of examples of other 2'-deoxynucleosides[31] have more recently been observed to violate Hudson's rules. In view of this uncertainty an effort was made to obtain additional supporting evidence for the anomeric structural assignment of the 2'-deoxynucleosides prepared in the present investigation.

The anomeric assignment of certain ribofuranosyl-imidazo[4,5-c]pyridines,[32] ribofuranosylpurines,[33] and

hexopyranosylpurines[34] has been previously accomplished by periodate oxidation followed by sodium borohydride reduction. Treatment of 6-amino-9-(2'-deoxy-β-D-ribopyranosyl)purine (VII) and 6-amino-9-(2'-deoxy-α-D-ribopyranosyl)purine (VIII) with sodium periodate effected a cleavage of the C3'–C4' bond and afforded the dialdehyde derivatives. Reduction of these dialdehyde derivatives with sodium borohydride furnished the corresponding alcohol derivatives which should be a dl pair with optical rotations of equal magnitude but opposite sign. Thus 6-amino-9-(2'-deoxy-

VII   VIII

NH₂   NH₂

CH_2—O---|---H   H---|---O—CH_2
HO—CH_2 CH_2OH
      CH_2—CH_2OH $HOCH_2$—CH_2

XII, $[\alpha]^{26}$D −10.8°   XIII, $[\alpha]^{26}$D +10.2°

β-D-ribopyranosyl)purine (VII), $[\alpha]^{25}$D −17.0°, produced XII, $[\alpha]^{26}$D −10.8°, and 6-amino-9-(2'-deoxy-α-D-ribopyranosyl)purine (VIII), $[\alpha]^{25}$D +6.0°, produced XIII, $[\alpha]^{26}$D +10.2°. This firmly established that VII and VIII are indeed an anomeric pair, and consequently so are III and V, but failed to furnish any additional support for their actual anomeric assignment.

Conformational analysis was initiated on the premise that additional support for the anomeric assignments might be possible utilizing certain features of the carbohydrate moiety other than the anomeric proton. The large coupling constants $(J_{1,2})$ of 9.0–10.0 cps observed for the anomeric proton in the pmr spectra of VII and VIII indicated that the anomeric proton must be axially oriented[10a,35–37] with the bulky adenine group assuming the equatorial position in both anomers. A visual inspection (Figure 2) of the four ideal chair conforma-
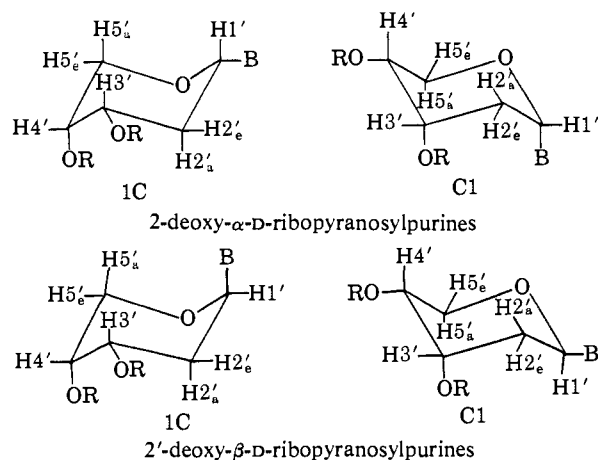

2-deoxy-α-D-ribopyranosylpurines

2'-deoxy-β-D-ribopyranosylpurines

Figure 2. Ideal chair conformations for 2'-deoxy-D-ribopyranosyl-purines.

(28) H. Zinner and E. Wittenberg, Chem. Ber., 94, 2072 (1961).

(29) N. Nagasawa, I. Kumashiro, and T. Takenishi, J. Org. Chem., 32, 251 (1967).

(30) J. Davoll and B. Lythgoe, J. Chem. Soc., 2526 (1949).

(31) R. H. Iwamoto, E. M. Acton, and L. Goodman, J. Med. Chem., 6, 684 (1963); M. Hoffer, R. Duschinsky, J. Fox, and N. Yung, J. Am. Chem. Soc., 81, 4112 (1959); R. U. Lemieux and M. Hoffer, Can. J. Chem., 31, 110 (1961).

(32) R. J. Rousseau, L. B. Townsend, and R. K. Robins, Biochemistry, 5, 756 (1966).

(33) R. S. Wright, G. M. Tener, and H. G. Khorana, J. Am. Chem. Soc., 80, 2004 (1958).

(34) L. M. Lerner and P. Kohn, J. Med. Chem., 7, 655 (1964).

(35) Z. Samek and J. Farkas, Collection Czech. Chem. Commun., 30, 2149 (1965).

(36) R. W. Lenz and J. P. Heeschen, J. Polymer Sci., 51, 247 (1961).

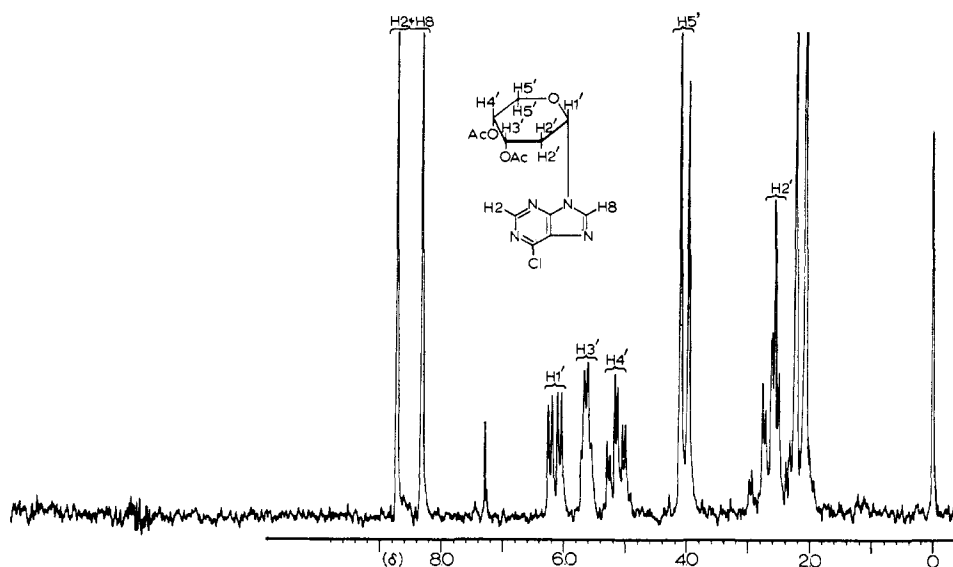(37) B. Coxon and H. G. Fletcher, Chem. Ind. (London), 662 (1964).

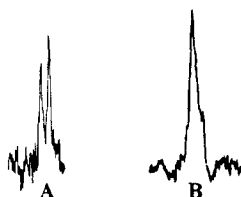Figure 3. Pmr spectrum of 6-chloro-9-(3',4'-di-O-acetyl-2'-deoxy-α-D-ribopyranosyl)purine (V).

Figure 4. Splitting pattern observed for the anomeric proton (H1') of 6-chloro-9-(3',4'-di-O-acetyl-2'-deoxy-α-D-ribopyranosyl)purine (V): (A) with H2$_a$' decoupled; (B) with H2$_a$' and H2$_e$' decoupled.

tions possible for 2'-deoxy-D-ribopyranosylpurines revealed that only two forms possess vicinal diaxial protons residing at C1' and C2', one being α (1C) and the other β (C1). Therefore the β-anomer (VII) should reside primarily in the C1 conformation. The alternate chair conformations can also be further excluded since their existence would be improbable due to several additional instability factors,[38-40] e.g., bulky purine group in axial position, transannular, or 1,3-diaxial interaction, etc.

The detailed structure of several carbohydrate derivatives has been recently[41,42] elucidated utilizing the proton–proton spin decoupling or double-resonance technique.[43] It was obvious that since the α- and β-anomers prepared in this investigation exist in different conformations, there should be certain salient features which are characteristic of the individual conformations and would permit a definite assignment of anomeric configuration by utilization of the proton–proton spin decoupling technique. Therefore a proton–proton spin decoupling study of 6-chloro-9-(3',4'-di-O-acetyl-2'-deoxy-α-D-ribopyranosyl)purine (V) was initiated in efforts to assign a more specific conformation to V and to obtain additional support for the previously as-

signed anomeric configuration. A visual inspection (Figure 3) of the pmr spectrum of V reveals that it should be amenable toward double resonance studies since the absorption peaks are well defined with a reasonable chemical shift occurring between them. The initial problem was the actual assignment of protons to the observed absorption peaks (Table II).

Table II. Chemical Shifts for the Ring Protons in the Carbohydrate Moiety of 6-Chloro-9-(3',4'-di-O-acetyl-2'-deoxy-α- and -β-D-ribopyranosyl)purine (V and III)

Proton	Chemical shift,[a] δ	
	α anomer, V	β anomer, III
H1'	6.18	6.03
H2$_a$', H2$_e$'	2.50	2.50
H3'	5.65 ⎱	5.32, multiplet
H4'	5.16 ⎰	
H5$_a$',H5$_e$'	4.03	4.14

[a] Relative to the internal standard TMS.

The quartet at δ 6.18 was assumed to be and was assigned to the anomeric proton (H1') on C1'. Irradiation of the high-field side of the multiplet centered at δ 2.50 caused a collapse of the quartet (anomeric proton) located at δ 6.18 to a doublet ($J_{H1',H2e'}$ = 3.7 cps) due to decoupling of the axial C2' proton (H2$_a$'). This was to be expected since it has been previously established[44-46] that while axial acetoxy group occurs at a lower field than a similar equatorial acetoxy group, an axial hydrogen generally tends to produce a signal in the pmr spectra at a higher applied magnetic field than a similar equatorial hydrogen. Irradiating the low-field side of the δ 2.50 multiplet collapsed the quartet at δ 6.18 to a singlet (Figure 4), a result which can be explained by decoupling both protons (H2$_a$' and H2$_b$') at position C2' and on this basis the multiplet at δ 2.50 was assigned to the two protons residing at C2'. A collapse of the quartet at δ 5.65 to a doublet with a coupling constant of 2.6 ± 0.2 cps, corresponding to

(38) R. U. Lemieux, Advan. Carbohydrate Chem., 9, 1 (1954).
(39) O. Hassel and B. Ottar, Acta Chem. Scand., 1, 929 (1947).
(40) E. A. Davidson, "Carbohydrate Chemistry," Holt, Rinehart and Winston Inc., New York, N. Y., 1967, p 46.
(41) H. Agahigian, G. D. Vickers, M. H. Von Saltza, J. Reid, A. I. Cohen, and H. Gautheir, J. Org. Chem., 30, 1085 (1965).
(42) B. Coxon, H. J. Jennings, and K. A. McLauchlan, Tetrahedron, 23, 2395 (1965).
(43) L. D. Hall, Advan. Carbohydrate Chem., 19, 51 (1964).

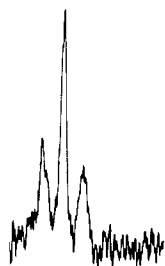(44) R. U. Lemieux, R. K. Kullnig, H. J. Berstein, and W. G. Schneider, J. Am. Chem. Soc., 80, 6098 (1958).
(45) R. J. Ferrier and M. F. Singleton, Tetrahedron, 18, 1143 (1962).
(46) L. D. Hall, Tetrahedron Letters, 1457 (1964).

Figure 5. Splitting pattern observed for H4' after decoupling H3'.

Figure 6. Newman projections of the carbohydrate moiety of 6-chloro-9-(3',4'-di-O-acetyl-2'-deoxy-α-D-ribopyranosyl)purine (V) as viewed down the C2'-C3' bond and the C3'-C4' bond.

a dihedral angle[47] of approximately 54°, was accomplished by irradiating the multiplet at δ 2.50. Therefore, the absorption peak (quartet) at δ 5.65 was assigned to the methine proton at C3'. The sextet centered at δ 5.16 was collapsed into a slightly distorted triplet (Figure 5) by irradiating (H3') 30 cps to a lower field (δ 5.65) indicating that the two C5' protons (H5$_a$' and H5$_e$') must be in a chemically similar environment. Further indication of the similarity existing between the H5$_a$' and H5$_e$' protons was observed when a collapse of the doublet at δ 4.03 to a singlet occurred on irradiation of the sextet (H4') centered at δ 5.16. It is of interest that this would indicate a complete lack of geminal coupling[48] for the two protons residing at C5'. This completed the initial assignment of absorption peaks in the pmr spectrum of V since the sextet (δ 5.16) was assigned to the C4' proton (H4') and the doublet at δ 4.03 to the two C5' protons (H5$_a$' and H5$_e$').

The quartet (anomeric proton) at δ 6.18 which possessed coupling constants (Table III) of 9.1 and 3.9 cps, which correspond to dihedral angles of 174 ± 6 and 46 ± 1°, respectively, can be explained by approximate axial–axial and axial–equatorial interaction between the protons residing at C1' and C2'. The occurrence

Figure 7. Newman projection of the carbohydrate moiety of 6-chloro-9-(3',4'-di-O-acetyl-2'-deoxy-α-D-ribopyranosyl)purine (V) as viewed down the C4'-C5' bond.

sults, and then further coupling of the C4' proton with the C3' proton would give rise to a multiplicity of six.

The δ 5.65 quartet assigned to H3' in the pmr spectrum of V could arise by distortion of the deoxyribopyranose ring in such a way as to couple H3' with H4' and with only one of the C2' protons (H2$_a$') which could occur if the coupling constant between H3' and H2$_e$' was very small or approaching zero. These proton relationships can best be seen by examination of the Newman projections (Figure 6) looking down the C2'–C3' bond and the C3'–C4' bond. A coupling constant of $J_{H2_a',H3'}$ = 6.9 ± 0.3 cps as part of the δ 5.65 quartet corresponds to a calculated dihedral angle of approximately 156° between the protons H2$_a$' and H3'. This deviation in the H2$_a$'–H3' dihedral angle from the normal 180° causes the H2$_e$'–H3' dihedral angle to increase to approximately 84° which corresponds to a coupling constant of $J_{H2_e',H3'}$ = −0.2 ± 0.1 cps. Although these calculations must be used with extreme caution, it is sufficient to say that the H2$_e$', H2$_a$', and H3' protons in the carbohydrate moiety possess the approximate relationship shown (Figure 6) in the Newman projections.

The average coupling constant, $J_{H4',H5_a'}$ = 8.0 ± 0.1 cps, for the doublet at δ 4.06 in the pmr spectrum of V, corresponds to a calculated average dihedral angle between H4' and H5$_a$' of approximately 8°. This dihedral angle represents a considerable deviation from the normal 60° expected for an equatorial–axial relationship of the H4' and H5$_a$' protons in the ideal chair conformation. The coupling constant $J_{H3',H4'}$ = 2.9 ± 0.1 cps, measured from the sextet in the pmr spectrum of V, corresponds to a calculated dihedral angle between H3' and H4' of approximately 52° which is in good agreement with that (54°) calculated from the coupling constant taken from the doublet observed for the C3' proton after decoupling of both C2' protons. These decoupling data show that while the dihedral angle between the H4' and H5' protons are distorted considerably from the normal 60°, the dihedral angle

Table III. Observed Coupling Constants and Calculated Dihedral Angles for 6-Chloro-9-(3',4'-di-O-acetyl-2'-deoxy-α-D-ribopyranosyl)purine (V)

Proton split a	Splitting proton b	Coupling constant, J_{ab}, cps	Dihedral angle,[a] φ, deg, between protons H_a, H_b
H1'	H2$_a$'	9.1 ± 0.2	174 ± 6
	H2$_e$'	3.9 ± 0.2	46 ± 1
	H2$_e$'	3.7 ± 0.2	47 ± 1 (decoupled pmr)
H3'	H4'	3.2 ± 0.1	50 ± 1
	H4'	2.6 ± 0.2	54 ± 2 (decoupled pmr)
	H2$_a$'	6.9 ± 0.3	156 ± 3
	H5$_a$', H5$_e$	8.0 ± 0.1	8 ± 2
H4'	H3'	2.9 ± 0.1	52 ± 1
H5$_a$'	H4'	8.0 ± 0.1	8 ± 2
H5$_e$'	H4'	−0.1 ± 0.1	112 ± 2

[a] Calculated from the Karplus equation,[47] $J_{HH'}$ = $A \cos^2 \phi$ − 0.28, where A = 8.5 for 0° ≤ φ ≤ 90° and A = 9.5 for 90° ≤ φ ≤ 180°.

of the sextet (δ 5.16) can be explained if coupling of the C4' proton with the two similar C5' protons produced a triplet, which is in agreement with the decoupling re-

(47) R. J. Abraham, L. D. Hall, L. Hough, and K. A. McLaughlan, J. Chem. Soc., 3699 (1962).
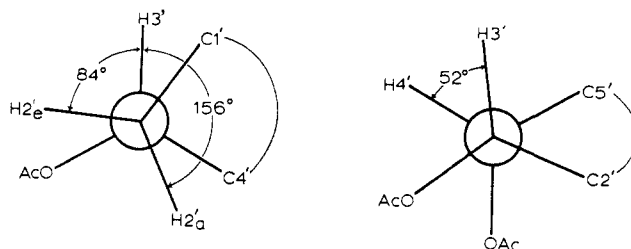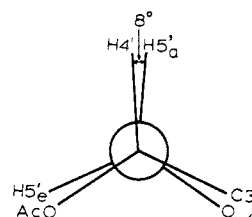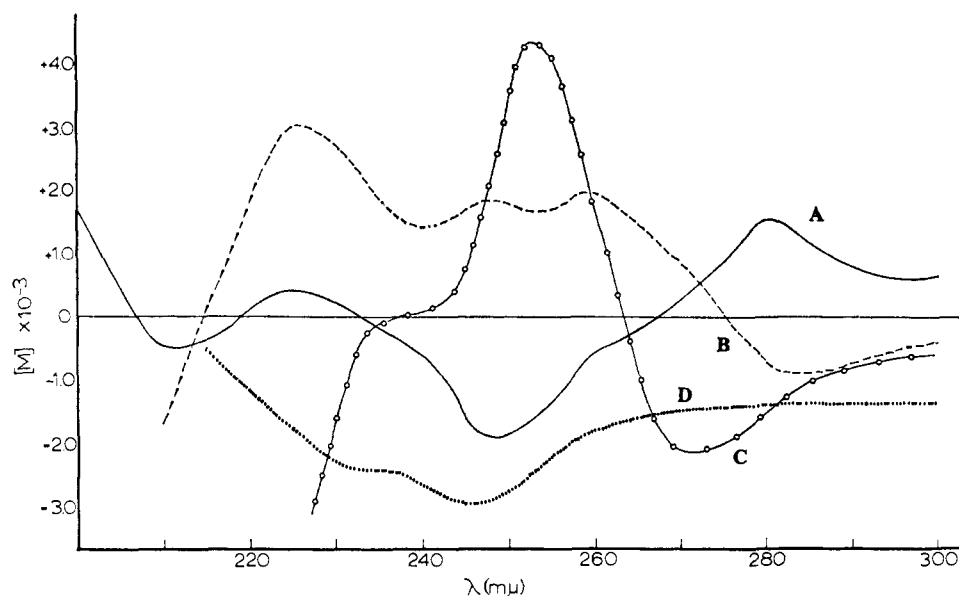(48) D. Horton and W. N. Turner, J. Org. Chem., 30, 3387 (1965).

Figure 8. ORD curves: A, 6-chloro-9-(3',4'-di-O-acetyl-2'-deoxy-α-D-ribopyranosyl)purine (V) (————); B, 6-chloro-9-(3',4'-di-O-acetyl-2'-deoxy-β-D-ribopyranosyl)purine (III) (— — — —); C, 6-amino-9-(2'-deoxy-α-D-ribopyranosyl)purine (VIII) (-o-o-o-); D, 6-amino-9-(2'-deoxy-β-D-ribopyranoyl)purine (VII) (• • • •).

between the H3' and H4' protons deviate very little from 60°, as shown in the Newman projections (Figures 6 and 7) looking down the C3'–C4' bond and the C4'–C5' bond. This could be the result of the unfavorable situation (eclipsing of the C3' and C4' acetoxy groups) caused by moving C4' into a position of coplanarity with C3' and C5'.

In order to explain these observed proton relationships in the carbohydrate moiety of V, the C5' methylene group must vacate a normal resting position in the ideal chair form of the 1C conformation and assume a new position coplanar or approaching coplanarity with the ring oxygen atom and the carbon atom C4'. Further distortion of the ring into a boat form is highly unlikely because of the bow–stern interaction repulsion energy generated and by the formation of a number of other eclipsed interactions.

A study of molecular models demonstrated that it would be difficult to explain the observed splitting pattern for the C3' proton in the pmr spectrum of V by either a perfect or a distorted C1 conformation. A perfect C1 conformation should give rise to a sextet for the C3' proton since it would be coupled to the two C2' protons, resulting in a triplet, and further coupling with the C4' proton would give rise to a multiplicity of six. If it were assumed that the C1 conformation existed in a manner similar to that observed for the 1C conformation, one would expect that a more complicated splitting pattern than a quartet for the C3' proton might result. Even if only a quartet for the C3' proton were to arise from a distorted C1 conformation it would be expected to have a smaller coupling constant, $J_{H3',H4'}$, since the equatorial C3' proton would be coupled to the axial C4' proton and to the equatorial C2' proton. It is of interest that III displayed the normal geminal coupling between the H5$_a$' and H5$_e$' protons (octet) and would tend to indicate less flexibility of the ring than that observed for V.

The marked differences, other than the splitting pattern observed for the anomeric proton, in the pmr spectra of III and V together with the foregoing discussion

indicates that III and V, which have been shown to be a true anomeric pair, must definitely assume different conformations. Obviously, any attempt to make an absolute assignment of conformation to III and V is dependent on the differences in the splitting patterns for the C3' and C4' protons. This has proven to be extremely difficult since the C3' and C4' protons in the pmr spectrum of III occur at the same chemical shift and attempts to resolve the H3' and H4' resonances by the use of different solvents have proved unfruitful. Due to the unresolved C3' and C4' protons in the pmr spectrum of III, it is not possible to describe with any certainty how the C1 conformation is distorted from the ideal chair form, but it would probably be similar to that postulated for the 1C conformation of V.
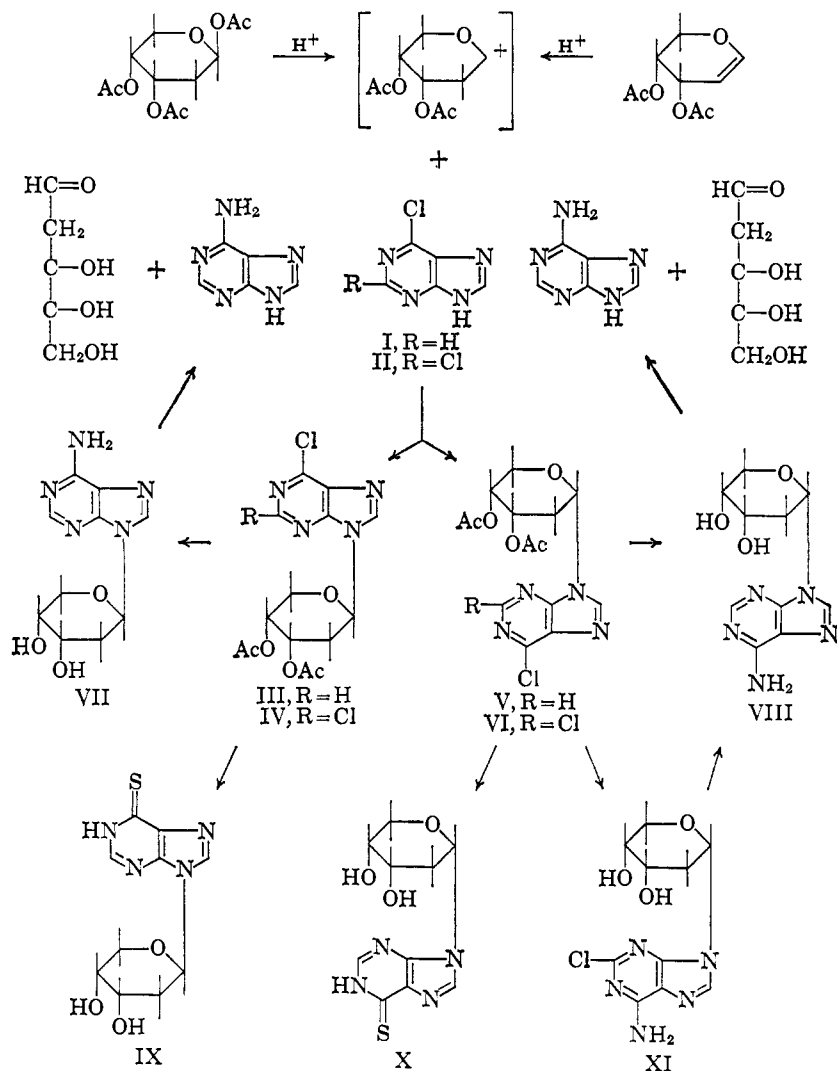
The results of the pmr and spin decoupling studies described in this investigation leave little doubt that there is appreciable distortion in the deoxyribopyranose ring of the nucleosides under consideration here. The flexibility of these conformations might be anticipated because of the absence of groups other than hydrogens at the C2' and C5' positions. Molecular models show that with these positions occupied by larger groups, flexing the carbohydrate ring could cause serious group interactions which might serve to restrict the ring to a more rigid conformation. The preparation of nucleosides which fulfill these requirements is under present investigation in this laboratory.

A method utilized for the determination of anomeric configuration for certain nucleosides, which has recently found considerable support, is optical rotatory dispersion (ORD). The assignment of anomeric configuration has been established or substantiated for numerous α- and β-anomers of pyrimidine and purine furanosides on the basis of the sign of the Cotton effect.[49-53] The

(49) T. L. V. Ulbricht, T. R. Emerson, and R. J. Swan, Tetrahedron Letters, 1561 (1966).
(50) T. R. Emerson, R. J. Swan, and T. L. V. Ulbricht, Biochem. Biophys. Acta, 22, 505 (1966).
(51) T. L. V. Ulbricht, J. P. Jennings, P. M. Scopes, and W. Klyne, Tetrahedron Letters, 695 (1964).

OAc

H⁺ ⇌ ⟶ ⇌ H⁺

AcO OAc AcO OAc AcO OAc

+

HC=O
CH₂
C—OH   +   NH₂   Cl   NH₂   +   HC=O
C—OH   CH₂
CH₂OH   R   C—OH
                                                C—OH
                    I, R=H   CH₂OH
                    II, R=Cl

NH₂   Cl   NH₂

R—   AcO   HO

                          OAc   OH

HO   AcO   R—

OH   OAc

VII   III, R=H   V, R=H   VIII
          IV, R=Cl   VI, R=Cl

S

HN   N

            HO   HO

HO   OH   OH

OH   Cl—

IX   X   XI

NH₂

correlation of anomeric configuration for certain pyrimidine pyranosides with the Cotton effects exhibited by their ORD curves has been reported for only a few pyrimidine pyranosides. They appear to generally follow the same rules which apply to the pyrimidine furanosides and, therefore, by analogy a purine pyranoside would also probably follow the same rules as a purine furanoside. It is of considerable interest that it has been shown that the absence of a hydroxyl group at position C2' of certain pyrimidine and purine furanosides changes the magnitude of the amplitude of the Cotton effect but produces no change in the sign. The ORD curves[54] (Figure 8) for 6-chloro-9-(3',4'-di-O-acetyl-2'-deoxy-β-D-ribopyranosyl)purine (III) and 6-chloro-9-(3',4'-di-O-acetyl-2'-deoxy-α-D-ribopyranosyl)purine (V) exhibit a negative and positive Cotton effect, respectively, which is in complete accord with the Cotton effects reported for purine furanosides. However, the ORD curves (Figure 8) obtained[55] for 6-amino-9-(2'-deoxy-β-D-ribopyranosyl)purine (VII) and 6-amino-9-(2'-deoxy-α-D-ribopyranosyl)purine (VIII) both exhibit a negative Cotton effect for the first extremum

(λ 247 and 271 mμ, respectively). One possible explanation for this anomalous behavior is the increased flexibility of the carbohydrate ring due to the removal of the acetyl groups. It is of interest that a visual inspection (Figure 8) in the 245–255-mμ region reveals a large negative Cotton effect for the β-anomer and a large positive Cotton effect for the α-anomer. The ORD curves obtained for 2,6-dichloro-9-(3',4'-di-O-acetyl-2'-deoxy-β-D-ribopyranosyl)purine (IV) and 2,6-dichloro-9-(3',4'-di-O-acetyl-2'-deoxy-α-D-ribopyranosyl)purine (VI) exhibited Cotton effects (negative and positive, respectively) in accord with those observed for III and V.

These results would indicate that an increase in flexibility of the conformation of the carbohydrate ring may well influence the optical rotary dispersion. To our knowledge these are the first reported ORD curves for purine 2'-deoxypyranosides.

On treatment of 6-chloro-9-(3',4'-di-O-acetyl-2'-deoxy-β-D-ribopyranosyl)purine (III) and 6-chloro-9-(3',4'-di-O-acetyl-2'-deoxy-α-D-ribopyranosyl)purine (V) with a refluxing methanolic sodium hydrosulfide solution there was observed a facile replacement of the 6-chloro group with concomitant deacetylation which furnished 9-(2'-deoxy-β-D-ribopyranosyl)purine-6-thione (IX) and 9-(2'-deoxy-α-D-ribopyranosyl)purine-6-thione (X), respectively. The pmr spectra of IX and X exhibited a coupling constant ($J_{H1',H2a}'$ = 9.0–10.0

(52) T. R. Emerson, R. J. Swan, and T. L. V. Ulbricht, Biochemistry, 6, 843 (1967).
(53) I. Fric, J. Smejkal, and J. Farkas, Tetrahedron Letters, 75 (1966).
(54) Daniel W. Miles, Ph.D. Thesis, Department of Chemistry, University of Utah, June 1967, p 124.
(55) The authors wish to thank Mr. Cheong Kam-Khow and Professor Henry Eyring for these ORD spectra.

cps) which would indicate that IX and X exist primarily in the same conformation as VII and VIII. This facile nucleophilic displacement of the 6-chloro group provides an important route for the preparation of other 9-(2'-deoxy-D-ribopyranosyl)purines of known anomeric configuration.

A mixture of 2,6-dichloropurine (II) and 3,4-di-O-acetyl-D-arabinal was fused at 125° in the presence of a catalytic amount of sulfanilic acid to furnish a good yield of nucleoside material. This mixture was separated by preparative thin layer chromatography to afford two major components with an ultraviolet absorption spectra consistent with a 9-substituted 2,6-dichloropurine.[56,57] One nucleoside possessed a melting point of 224–225° and $[\alpha]^{26}$D $+27.0°$, while the other nucleoside had a melting point of 185–186° and $[\alpha]^{26}$D $-12.5°$. On the basis of specific rotations the nucleoside with a melting point of 224–225° and a positive specific rotation was assigned the structure 2,6-dichloro-9-(3',4'-di-O-acetyl-2'-deoxy-α-D-ribopyranosyl)purine (VI) and the other nucleoside with a negative specific rotation the structure 2,6-dichloro-9-(3',4'-di-O-acetyl-2'-deoxy-α-D-ribopyranosyl)purine (IV). The pmr spectra (carbohydrate portion) of IV and VI were essentially identical with the pmr spectra (carbohydrate portion) of III and V and were presumed to possess similar conformations. It is of interest that the preparation of α-nucleosides has recently achieved considerable significance in view of the isolation and characterization[58] of the first α-nucleotide from yeast RNA. Furthermore, α-2'-deoxythioguanosine has recently demonstrated[59] the ability to be incorporated *in vivo* into the DNA of *Mecca* lymphosarcoma ascites cells. Fusion of 2,6-dichloropurine (II) with 1,3,4-tri-O-acetyl-D-ribopyranose in the presence of a catalytic amount of p-toluenesulfonic acid also produced an anomeric mixture of IV and VI. This anomeric mixture was separated by fractional recrystallization instead of preparative layer chromatography but still furnished pure IV and VI identical in all respects with IV and VI prepared previously (*vide supra*). Additional support for the assignment of anomeric configuration to IV and VI seemed desirable since this assignment was based solely on specific rotations. Treatment of VI with methanolic ammonia at room temperature furnished 6-amino-2-chloro-9-(2'-deoxy-α-D-ribopyranosyl)purine (XI). Catalytic dehalogenation of XI produced a nucleoside which was shown to be identical with 6-amino-9-(2'-deoxy-α-D-ribopyranosyl)purine (VIII) prepared from 6-chloro-9-(3',4'-di-O-acetyl-2'-deoxy-α-D-ribopyranosyl)purine (V), and therefore firmly established the anomeric configuration of IX and X as β and α, respectively. Other nucleoside material was detected by chromatography in the reaction mixtures from fusions involving both 6-chloropurine (I) and 2,6-dichloropurine (II). The isolation of these minor constituents in crystalline form and elucidation of their actual structure are under present investigation.

(56) J. F. Gerster and R. K. Robins, *J. Org. Chem.*, **31**, 3258 (1966).
(57) A. G. Beaman and R. K. Robins, *ibid.*, **28**, 2310 (1963).
(58) H. G. Gassen and H. Witzel, *Biochim. Biophys. Acta*, **95**, 244 (1965).
(59) G. A. LePage and I. G. Junga, *Mol. Pharmacol.*, **3**, 37 (1967).

Experimental Section[60]

Melting points were taken on a Fischer-Johns melting point apparatus and are uncorrected. Optical rotations were determined with a Sargent polarimeter with a 2-dm path length. All pmr spectra were run on a Varian A-60 nuclear magnetic resonance spectrometer and spin decoupling was accomplished with a Varian V-6058A spin decoupler. Coupling constants were measured from 3 to 11 traces to an accuracy of ±0.1 cps, and are represented at the 5% significance level. Chemical shifts were measured relative to an internal standard, TMS, and are recorded as δ values. Optical rotatory dispersion curves were run on a Cary Model 60 spectropolarimeter in water. Chromatograms were developed on Whatman No. 1 paper, descending. Purine and purine nucleosides were detected with ultraviolet light (254 mμ). Carbohydrate components were detected with silver nitrate in acetone-alcoholic sodium hydroxide spray reagent.[61] The following solvent systems were utilized: A, 1-butanol saturated with water; B, ethyl acetate-1-propanol-water (4:1:2, v/v); C, methanol-chloroform (4:1, v/v); D, 5% aqueous ammonium bicarbonate; E, methanol-water (7:3, v/v); F, concentrated aqueous ammonia-N,N-dimethylformamide-2-propanol (10:25:65, v/v); G, isobutyl alcohol-water (86:14, v/v); H, isobutyl alcohol-pyridine-water (10:3:3, v/v); I, isobutyl alcohol-ethanol-water (49:11:19, v/v).

6-Chloro-9-(3',4'-di-O-acetyl-2'-deoxy-D-ribopyranosyl)purine (III and V). Method A. A thoroughly ground mixture of 20.0 g (0.13 mole) of 6-chloropurine[62] and 47.2 g (0.24 mole) of 3,4-di-O-acetyl-D-arabinal was heated under aspirator vacuum to an inside temperature of 120° in an oil bath. After 15 min, 30 mg of sulfanilic acid catalyst was thoroughly stirred into the mixture, and the heating was continued at the same temperature under aspirator vacuum for an additional 55 min. The dark amber colored melt was dissolved in 1500 ml of warm ethyl acetate. The resulting solution was filtered, cooled to 0°, washed with a cold saturated sodium carbonate solution (three 600-ml portions) and a cold saturated sodium chloride solution (three 600-ml portions) and finally dried over anhydrous sodium sulfate. The sodium sulfate was removed by filtration and the filtrate concentrated at room temperature under high vacuum to a point where crystalline material began to form. The solution was allowed to stand at room temperature for 24 hr, and the crystalline material which had separated was collected by filtration, yielding 8.53 g of 6-chloro-9-(3',4'-di-O-acetyl-2'-deoxy-α-D-ribopyranosyl)purine, mp 192–197°. Three recrystallizations from absolute ethanol gave pure 6-chloro-9-(3',4'-di-O-acetyl-2'-deoxy-α-D-ribopyranosyl)purine (V), mp 205–207°, $[\alpha]^{26}$D $+21.8°$ (c 0.75, acetone).

Anal. Calcd for $C_{14}H_{15}ClN_4O_5$: C, 47.40; H, 4.23; N, 15.80. Found: C, 47.30; H, 4.26; N, 15.90.

The preceding ethyl acetate filtrate was evaporated under high vacuum to a hard syrup, which was then dissolved in 300 ml of tetrahydrofuran and added dropwise to stirring normal pentane (20 ml of tetrahydrofuran solution for 2000 ml of normal pentane). The solid which had separated from solution was collected by filtration to furnish 11.83 g of a white solid. This solid was dissolved in the minimum amount of ethyl acetate and applied to a neutral alumina (Woelm, activity I) column (1.25 × 14 in.). Elution with dry ethyl acetate was continued until all the nucleoside material was recovered from the column (about 3 l.). The ethyl acetate eluent was evaporated at room temperature under high vacuum to a foam. This foam was dissolved in 50 ml of absolute methanol and cooled at −20° for 24 hr, yielding 2.43 g of 6-chloro-9-(3',4'-di-O-acetyl-2'-deoxy-D-ribopyranosyl)purine, mp 138–143°. Three recrystallizations from absolute methanol gave pure 6-chloro-9-(3',4'-di-O-acetyl-2'-deoxy-β-D-ribopyranosyl)purine (III), mp 159–160°, $[\alpha]^{26}$D $-33.6°$ (c 1.00, ethyl acetate).

Anal. Calcd for $C_{14}H_{15}ClN_4O_5$: C, 47.40; H, 4.23; N, 15.80. Found β-anomer: C, 47.19; H, 4.01; N, 16.21.

Method B. A mixture of 2.60 g (0.01 mole) of finely powdered 1,3,4-tri-O-acetyl-2-deoxy-β-D-ribopyranose[28] and 1.54 g of 6-chloropurine (0.01 mole) in a 25-ml, round-bottomed flask was heated in an oil bath (120°) until a yellow melt was obtained. To this melt was added 20 mg of p-toluenesulfonic acid; the melt was stirred to disperse the catalyst, and a water aspirator was at-

(60) The authors thank Mr. A. F. Lewis and staff for large-scale preparations of some intermediates and Mrs. D. Rousseau and staff for infrared, ultraviolet, and pmr spectra.
(61) W. E. Trevelyan, D. P. Proctor, and J. S. Harrison, *Nature*, **166**, 444 (1950).
(62) A. G. Beaman and R. K. Robins, *J. Appl. Chem.*, **12**, 432 (1962).

tached to the flask. Under vacuum, the contents of the flask bubbled violently and liberated considerable acetic acid. Heating was continued until a clear tan melt was obtained and no further evolution of acetic acid was observed (~15 min). The flask was removed from the oil bath and its contents dissolved in 125 ml of warm ethyl acetate and the solution filtered to remove unreacted 6-chloropurine. The ethyl acetate solution was cooled in an ice bath to 0°, extracted with a cold saturated solution of sodium carbonate (three 100-ml portions) and with cold water (one 100-ml portion), and dried over sodium sulfate. The sodium sulfate was removed by filtration and the filtrate concentrated under reduced pressure to a thick tan syrup. Approximately 50 ml of warm absolute ethanol was added to the syrup and the syrup triturated until a considerable amount of crystalline material was present. The crystalline material was removed by filtration, washed with cold ethanol, and air dried to give 1.28 g (30%) of 6-chloro-9-(3',4'-di-O-acetyl-2'-deoxy-D-ribopyranosyl)purine as an anomeric mixture. Recrystallization of this material from absolute ethanol gave a pure sample (0.88 g) of 6-chloro-9-(3',4'-di-O-acetyl-2'-deoxy-α-D-ribopyranosyl)purine (V), mp 205–207°, $[\alpha]^{26}$D +22.4° (c 0.75, acetone). Concentration of the ethanolic filtrate and washings gave a thick syrup. The syrup was dissolved in 40 ml of methanol and this solution stored at −10° for 5 days. The crystalline material present after that time (0.18 g) was removed by filtration and recrystallized four more times from methanol to provide a pure sample of 6-chloro-9-(3',4'-di-O-acetyl-2'-deoxy-β-D-ribopyranosyl)purine (III), mp 149–150°, $[\alpha]^{25}$D −28.3° (c 1.0, ethyl acetate).

Anal. Calcd for $C_{14}H_{15}ClN_4O_5$: C, 47.40; H, 4.23; N, 15.80. Found α-anomer: C, 47.00; H, 4.54; N, 15.50. Found β-anomer: C, 47.70; H, 4.84; N, 15.70.

6-Amino-9-(2'-deoxy-β-D-ribopyranosyl)purine (VII). 6-Chloro-9-(3',4'-di-O-acetyl-2'-deoxy-β-D-ribopyranosyl)purine (III) (400 mg) dissolved in 20 ml of methanol was added to 100 ml of methanolic ammonia (saturated at 0°) and this solution was then heated in a stainless steel bomb at 110° for 4 hr. The excess solvents were removed under reduced pressure, and the white syrupy solid that remained was slurried in a small amount of cold methanol. The material that did not dissolve was removed by filtration and gave, after air drying, 210 mg (74%) of the desired 6-amino-9-(2'-deoxy-β-D-ribopyranosyl)purine,[11] mp 255–256°. Recrystallization of this material from a mixture of methanol–water gave 120 mg of the pure product, mp 266–267°, $[\alpha]^{25}$D −17.0° (c 0.6, water).

Anal. Calcd for $C_{10}H_{13}N_5O_3$: C, 47.80; H, 5.18; N, 27.80. Found: C, 47.50; H, 5.07; N, 27.50.

6-Amino-9-(2'-deoxy-α-D-ribopyranosyl)purine (VIII). 6-Chloro-9-(3',4'-di-O-acetyl-2'-deoxy-α-D-ribopyranosyl)purine (V) (1.76 g) was added to 100 ml of ethanolic ammonia (saturated at 0°) and then heated for 72 hr at 120° in a sealed vessel. The excess solvents were removed *in vacuo*, and the residue that remained was slurried with a small amount of cold ethanol. The material that did not dissolve was removed by filtration. This solid material (~1.0 g) was dissolved in 15 ml of hot ethanol and the solution allowed to stand at −5° for 96 hr. The white crystalline material obtained was removed by filtration and recrystallized four more times from ethanol to provide 500 mg of 6-amino-9-(2'-deoxy-α-D-ribopyranosyl)purine (VIII), mp 232–235°, $[\alpha]^{25}$D +6.0° (c 1.0, water).

Anal. Calcd for $C_{10}H_{13}N_5O_3$: C, 47.80; H, 5.18; N, 27.90. Found: C, 47.40; H, 5.21; N, 28.20.

Hydrolysis of 6-Amino-9-(2'-deoxy-β-D-ribopyranosyl)purine (VII) in Acid. 6-Amino-9-(2'-deoxy-β-D-ribopyranosyl)purine (VII) (10 mg) was dissolved in 2 ml of 0.1 N HCl and this solution heated to 56° and maintained at this temperature for 1 hr. The solution was neutralized to pH 7 with sodium carbonate and then evaporated to dryness under reduced pressure. The solid residue was extracted with 1 ml of boiling methanol, and any undissolved material was removed by filtration. The methanol filtrate was concentrated to 0.5 ml, and this solution was chromatographed with known samples of adenine and 2'-deoxy-D-*erythro*-pentose.[63] Paper chromatography in six solvent systems showed that the methanol extract contained material with the mobility of adenine and 2'-deoxy-D-*erythro*-pentose. Adenine was identified in the following solvent systems: D, E, F, and G. Spots were detected by ultraviolet light. 2'-Deoxy-D-*erythro*-pentose was detected in solvent systems H and I.

(63) Purchased from Aldrich Chemical Co., Milwaukee, Wis.

Hydrolysis of 6-Amino-9-(2'-deoxy-α-D-ribopyranosyl)purine (VIII) in Acid. 6-Amino-9-(2'-deoxy-α-D-ribopyranosyl)purine (VIII) (50 mg) was dissolved in 10 ml of 0.1 N HCl, and this solution was heated to 75° and maintained at this temperature for 1 hr. The cooled solution was neutralized to pH 7 with sodium carbonate and then evaporated to dryness. The residue was extracted with 2 ml of boiling methanol; the extract was filtered to remove undissolved material, and the filtrate was then concentrated to 1 ml. Paper chromatography showed that adenine and 2-deoxy-D-*erythro*-pentose were present in the extract. Systems used for detecting adenine were D, E, F, and G. Systems for detecting 2-deoxy-D-*erythro*-pentose were H and I.

Periodate Oxidation and Sodium Borohydride Reduction of 6-Amino-9-(2'-deoxy-α- and -β-D-ribopyranosyl)purine. To 40 mg of 6-amino-9-(2'-deoxy-α-D-ribopyranosyl)purine (VIII) was added 4.0 ml of 0.08 M sodium periodate solution and the mixture stirred at room temperature for 15 min. Sodium borohydride (120 mg) was then added and the resulting solution allowed to stand at room temperature for another 30 min, after which excess reducing agent was destroyed by dropwise addition of 10% acetic acid (1.5 ml) until gas evolution ceased. The optical rotation was determined on this solution as $[\alpha]^{26}$D +10.2° based on the original weight of VIII. In a like manner, 30 mg of 6-amino-9-(2'-deoxy-β-D-ribopyranosyl)purine (VII) was treated with 3.0 ml of 0.08 M sodium periodate solution followed by 90 mg of sodium borohydride and neutralization with 1.4 ml of 10% acetic acid. The optical rotation of this solution was determined as $[\alpha]^{26}$D −10.8° based on the original weight of VII.

9-(2'-Deoxy-α-D-ribopyranosyl)purine-6-thione (X). To 40 ml of a freshly prepared solution of 2 N sodium hydrosulfide in methanol was added 1.0 g (0.003 mole) of 6-chloro-9-(3',4'-di-O-acetyl-2'-deoxy-α-D-ribopyranosyl)purine (V). The solution was gently refluxed for 20 min, filtered, and then cooled to 0° in an ice bath. The pH of the cold solution was adjusted to 7.0 with the slow addition of glacial acetic acid taking care not to allow the temperature to rise above 0°. The mixture was then allowed to stand for 30 min at 0° during which time a white solid formed. The solid was collected by filtration, washed with a small amount (10 ml) of cold methanol, and air dried, yielding 690 mg of material. Three recrystallizations from absolute ethanol gave pure 9-(2'-deoxy-α-D-ribopyranosyl)purine-6-thione (X), mp 217–218°.

Anal. Calcd for $C_{10}H_{12}N_4O_3S$: C, 44.74; H, 4.47; N, 20.89. Found: C, 44.69; H, 4.40; N, 21.00.

9-(2'-Deoxy-β-D-ribopyranosyl)purine-6-thione (IX). To 20 ml of a freshly prepared solution of 2 N sodium hydrosulfide in methanol was added 400 mg (0.001 mole) of 6-chloro-9-(3',4'-di-O-acetyl-2'-deoxy-β-D-ribopyranosyl)purine (III). The solution was gently heated at reflux temperature for 20 min, filtered, diluted to 30 ml with absolute methanol, and then cooled to 0° in an ice bath. The pH of the cold solution was adjusted to 7.0 with the slow addition of glacial acetic acid taking care not to allow the temperature to rise above 0°. The mixture was then allowed to stand for 30 min during which time a white solid slowly formed. This solid was collected by filtration, washed with a small amount (5 ml) of cold methanol, and air dried, yielding 210 mg of material (69.3%), mp 235–238°. Two recrystallizations from water gave pure 9-(2'-deoxy-β-D-ribopyranosyl)purine-6-thione (IX), mp 244–246°.

Anal. Calcd for $C_{10}H_{12}N_4O_3S$: C, 44.74; H, 4.47; N, 20.89. Found: C, 44.68; H, 4.51; N, 21.03.

2,6-Dichloro-9-(3',4'-di-O-acetyl-2'-deoxy-D-ribopyranosyl)purine (IV and VI). Method A. A throughly ground mixture containing 5.0 g (0.026 mole) of 2,6-dichloropurine and 5.26 g (0.026 mole) of 3,4-di-O-acetyl-D-arabinal was heated under an aspirator vacuum to 125° (inside temperature) in an oil bath. After 30 min, 80 mg of sulfanilic acid catalyst was thoroughly stirred into the mixture and the heating was continued at the same temperature under aspirator vacuum for an additional 80 min. The dark amber colored melt was dissolved in 300 ml of warm ethyl acetate. The resulting solution was filtered and the filtrate cooled to 0°, washed with a cold saturated NaHCO₃ solution (three 100-ml portions) and a cold saturated NaCl solution (three 100-ml portions), and finally dried over anhydrous Na₂SO₄. The Na₂SO₄ was removed by filtration and the filtrate concentrated under high vacuum to a stiff foam. This foam was dissolved in 70 ml of tetrahydrofuran and the resulting solution added dropwise to stirring normal pentane (5 ml of tetrahydrofuran solution/250 ml of normal pentane), yielding 4.37 g of a white solid.

This white solid was dissolved in 24 ml of ethyl acetate and applied to four preparative tlc plates (plate dimensions 7.8 × 15.7 in. with

a 5-mm thickness of SilicAR 7GF). The plates were developed ascending in a toluene–ethyl acetate solvent system (9:1, v/v), allowing the solvent to run the entire length (15 mm) of the plate. The plates were then air dried with the assistance of an infrared lamp, and the foregoing procedure was repeated two more times resulting in the separation of two major components which were detected by an ultraviolet mineralight source (254 mμ). The slower moving band was removed and extracted with hot ethyl acetate (400 ml). The adsorbent was removed by filtration and the filtrate concentrated *in vacuo* to a syrup. This syrup was dissolved in 10 ml of absolute acetone and refrigerated at $-25°$ for 1 week, yielding 210 mg of product. Recrystallization from absolute acetone gave an analytical sample of 2,6-dichloro-9-(3′,4′-di-O-acetyl-2′-deoxy-α-D-ribopyranosyl)purine (VI), mp 224–225°, $[\alpha]^{26}$D $+27.0°$ (c 0.5, ethyl acetate). The remaining filtrate was concentrated *in vacuo* to a small volume and refrigerated at $-25°$, yielding 250 mg of nucleoside material. Recrystallization from absolute acetone furnished an analytical sample of 2,6-dichloro-9-(3′,4′-di-O-acetyl-2′-deoxy-β-D-ribopyranosyl)purine (IV), mp 185–186°, $[\alpha]^{26}$D $-12.5°$ (c 1.0, ethanol).

Anal. Calcd for $C_{14}H_{14}Cl_2N_4O_5$: C, 43.19; H, 3.60; N, 14.40. Found α-anomer: C, 43.14; H, 3.57; N, 14.46. Found β-anomer: C, 43.08; H, 3.74; N, 14.57.

Method B. A mixture of 2.60 g of finely powdered 1,3,4-tri-O-acetyl-2-deoxy-β-D-ribopyranose and 1.90 g of 2,6-dichloropurine (II) in a 25-ml, round-bottomed flask was heated in an oil bath to 120°. To this preheated reaction mixture was added 20 mg of *p*-toluenesulfonic acid, and a water aspirator was attached to the flask. Upon addition of the acid catalyst, the mixture began to evolve acetic acid and gradually formed a clear green melt. When no further evolution of acetic acid was observed (10 min), the flask was removed from the oil bath and its contents dissolved in 200 ml of warm ethyl acetate. The ethyl acetate solution was cooled to 0° by the addition of ice, washed with cold saturated sodium carbonate (three 100-ml portions) and water (two 100-ml portions), and dried over sodium sulfate. The sodium sulfate was removed by filtration and the filtrate concentrated *in vacuo* until a thick syrup remained. This syrup was dissolved in warm acetone; the acetone solution was treated with Norit and then concentrated to 20 ml to yield 1.2 g of nucleoside material, mp 225–230°. Recrystallization of this crude product from acetone gave a pure sample of 2,6-di-

chloro-9-(3′,4′-di-O-acetyl-2′-deoxy-α-D-ribopyranosyl)purine (VI), mp 228–229°, $[\alpha]^{26}$D $+27.0°$ (c 0.5, ethyl acetate).

Anal. Calcd for $C_{14}H_{14}Cl_2N_4O_5$: C, 43.20; H, 3.60; N, 14.40. Found: C, 43.30; H, 3.90; N, 14.20.

Concentration of the original acetone filtrate to *ca.* 10 ml and allowing the solution to stand at $-10°$ for 24 hr provided an additional crystalline material, mp 170–178°. Recrystallization of this material from small amounts of acetone gave an analytical sample of 2,6-dichloro-9-(3′,4′-di-O-acetyl-2′-deoxy-β-D-ribopyranosyl)-purine (IV), mp 186–187°, $[\alpha]^{26}$D $-12.5°$ (c 1.0, ethanol).

Anal. Calcd for $C_{14}H_{14}Cl_2N_4O_5$: C, 43.20; H, 3.60; N, 14.40. Found: C, 43.29; H, 3.81; N, 14.70.

6-Amino-2-chloro-9-(2′-deoxy-α-D-ribopyranosyl)purine (XI). To 60 ml of anhydrous methanol was added 1.0 g (0.003 mole) of 2,6-dichloro-9-(3′,4′-di-O-acetyl-2′-deoxy-α-D-ribopyranosyl)-purine, and this mixture was cooled to 5° and saturated with dry ammonia for 1 hr. The resulting solution was sealed in a pressure bottle and allowed to remain at room temperature for 1 week. Excess ammonia was removed in a stream of nitrogen and the solvent removed *in vacuo* at room temperature. The residue was triturated with chloroform (500 ml) overnight and the resulting solid collected by filtration and air dried, yielding 550 mg (73.3%). Three recrystallizations from absolute ethanol gave pure 6-amino-2-chloro-9-(2′-deoxy-α-D-ribopyranosyl)purine (XI).

Anal. Calcd for $C_{10}H_{12}ClN_5O_3$: C, 42.03; H, 4.20; N, 24.50. Found: C, 42.26; H, 4.43; N, 24.26.

Dehalogenation of 6-Amino-2-chloro-9-(2′-deoxy-α-D-ribopyrano-syl)purine (XI). 6-Amino-2-chloro-9-(2′-deoxy-α-D-ribopyranosyl)-purine (10 mg) together with 10 mg of 5% palladium-on-carbon catalyst was added to 50 ml of water. The pH of the mixture was adjusted to 8.0 with dilute NaOH and then hydrogenated at 45 psi hydrogen pressure for 2.5 hr at room temperatue. The catalyst was removed by filtration and the filtrate concentrated *in vacuo* to a small volume. The ultraviolet spectrum of the filtrate was identical with that of 6-amino-9-(2′-deoxy-α-D-ribopyranosyl)-purine. Paper chromatograms of the filtrate in three solvent systems revealed R_{Ad} values (A,B,C) which were identical with those observed for 6-amino-9-(2′-deoxy-α-D-ribopyranosyl)purine (VIII), but were significantly different from the R_{Ad} values (A,B,C) observed for 6-amino-9-(2′-deoxy-β-D-ribopyranosyl)purine (VII).

The Absolute Configuration of Caldariomycin

Suzanne M. Johnson, Iain C. Paul, Kenneth L. Rinehart, Jr., and Rangaswamy Srinivasan

Contribution from the Department of Chemistry and Chemical Engineering, University of Illinois, Urbana, Illinois 61801, Received August 21, 1967

Abstract: The absolute configuration of the chlorine-containing mold metabolite caldariomycin has been established as *S*, both by X-ray single crystal structure analysis of the bis[(+)-3-bromocamphor-9-sulfonate] and by application of the α-phenylbutyric anhydride method.

The mold metabolite caldariomycin, from *Caldariomyces fumago*, was first isolated by Raistrick, *et al.*,[1] who assigned it the structure 2,2-dichlorocyclopentane-1,3-diol. This has now been confirmed twice by synthesis,[2,3] and the biosynthesis of the compound has also been studied.[4] Caldariomycin is optically active, but the absolute configuration of the two asymmetric centers, which must be the same, has not been reported.

A simple empirical procedure for determining the absolute configuration is the method of Horeau,[5] employing the anhydride of racemic α-phenylbutyric acid. This method appeared to be well suited to the problem of caldariomycin, since the two groups flanking the asymmetric carbon atoms, $-CCl_2-$ and $-CH_2-$, differ considerably in size.

On the other hand, the instability of caldariomycin to alkali required a modification of the usual Horeau procedure, in which the alcohol and anhydride react in pyridine solution, the excess anhydride is decomposed

(1) P. W. Clutterbuck, S. L. Mukhopadhyay, A. E. Oxford, and H. Raistrick, *Biochem. J.*, **34**, 663 (1940).

(2) J. R. Beckwith and L. P. Hager, *J. Org. Chem.*, **26**, 5206 (1961).

(3) A. W. Burgstahler, T. B. Lewis, and M. O. Abdel-Rahman, *ibid.*, **31**, 3516 (1966).

(4) P. D. Shaw, J. R. Beckwith, and L. P. Hager, *J. Biol. Chem.*, **234**, 2560 (1959).

(5) (a) A. Horeau, *Tetrahedron Letters*, 506 (1961); (b) *ibid.*, 965 (1962).