Purine Nucleosides. XI. The Synthesis of 2′-Deoxy-9-α- and -β-D-ribofuranosylpurines and the Correlation of Their Anomeric Structure with Proton Magnetic Resonance Spectra[1]

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A simple general procedure has been developed for the synthesis of 9-(2′-deoxy-D-ribofuranosyl)purines utilizing the requisite purine and 1,3,5-tri-O-acetyl-2-deoxy-D-ribofuranose (II) in a fusion coupling process. The synthesis of II has been achieved from a commercially available natural source, 2′-deoxyadenosine. In most instances, separation of anomers was achieved by fractional crystallization. The application of nuclear magnetic resonance spectroscopy for the assignment of absolute configuration of 2′-deoxyribofuranosyl nucleosides has been investigated and found to be empirically valid for the purine 2′-deoxy-D-ribofuranosides. A "pseudo-triplet" with $J_{H_1'} \cong 7$ c.p.s. and peak width $\cong 14$ c.p.s. was observed for the anomeric proton of the β-anomer. The corresponding peak for the α-anomer appeared as a multiplet of four with $J_{H_1'} \cong 3$ and 7 c.p.s. and peak width $\cong 10$ c.p.s. This method of anomeric assignment for 2′-deoxy-D-ribofuranosyl nucleosides appears to be general irrespective of the heterocyclic base.

It has been recently noted that the biological effect of certain purine nucleosides was due to contaminating mercuric ions in concentrations as low as 10^{-8} *M*,[3,4] introduced during the preparation of the purine nucleosides *via* the mercury salt procedure. The binding of purine nucleosides with mercuric ions has recently been studied[5,6] in some detail. The problems involved in freeing nucleosides from heavy metal salts has focused attention on recent synthetic methods which avoid their use. The present study is a report on the fusion procedure[7] utilizing 1,3,5-tri-*O*-acetyl-2-deoxy-D-ribofuranose to prepare 2′-deoxyribofuranosylpurine nucleosides. Some of the inherent advantages of this method have been discussed in a preliminary report[8] describing the present work. Previous syntheses of purine-2′-deoxynucleosides have been adequately discussed in several recent reviews.[9-11] The present availability of commercial 2′-deoxyadenosine suggested this nucleoside as a good source of 2-deoxy-D-ribose. Acetylation of 2′-deoxyadenosine monohydrate with acetic anhydride in the presence of pyridine gave an excellent yield of 6-acetamido-9-(3′,5′-di-*O*-acetyl-2′-deoxy-β-D-ribofuranosyl)purine (I). When I was treated directly with acetic acid and acetic anhydride at 100°, acetolysis of the glycosidic linkage was effected to give 6-acetamidopurine (III, R = CH_3) and 1,3,5-tri-*O*-acetyl-2-deoxy-D-ribofuranose (II) which was isolated and purified by vacuum distillation to give pure II in 78% yield from 2′-deoxyadenosine. 1,3,5-Tri-*O*-acetyl-2-deoxy-D-ribofuranose (II) was characterized by elemental analysis, infrared and p.m.r. spectroscopy, and deacetylation with methanolic ammonia to give 2-deoxy-D-ribose. The rotation $[\alpha]^{25}D$ +25.0° (*c* 0.66, methanol) of the colorless moderately viscous liquid II is significantly more positive than that of the crystalline 1,3,5-tri-*O*-acetyl-2-deoxy-β-D-ribofuranose, $[\alpha]^{20}D$ −161.2° (*c* 0.60, methanol) isolated in 2% yield[12] from 2-deoxy-D-ribose. This suggests II is predominantly, if not exclusively, 1,3,5-tri-*O*-acetyl-2-deoxy-α-D-ribofuranose. The ready availability of II provides an excellent starting point for the synthesis of 2′-deoxy-D-ribofuranosyl nucleosides. Acid-catalyzed fusion of 6-chloropurine[13,14] and 1,3,5-tri-*O*-acetyl-2-deoxy-D-ribofuranose (II) gave a 38% yield of a crystalline mixture of anomers of 6-chloro-9-(2′-deoxy-α- and -β-D-ribofuranosyl)purines after deacetylation with methanolic ammonia. These anomers were readily separated by fractional crystallization. Similar fusion of purine and II gave a 31% yield of crystalline 9-(2′-deoxy-α- and -β-D-ribofuranosyl)-purines, which were separated by fractional crystallization from methanol. 6-Benzamidopurine[15] (III, R = C_6H_5) and II were fused at 160° in the presence of dichloroacetic acid to give a 20% yield of pure 6-amino-9-(2′-deoxy-α-D-ribofuranosyl)purine (III). An equivalent amount of nucleoside (largely the β-anomer) remained in the filtrate and could not be readily crystallized. A small amount of recovered 6-benzamidopurine in this reaction raised the yield to 26% for the pure α-isomer III. This constitutes an interesting group of

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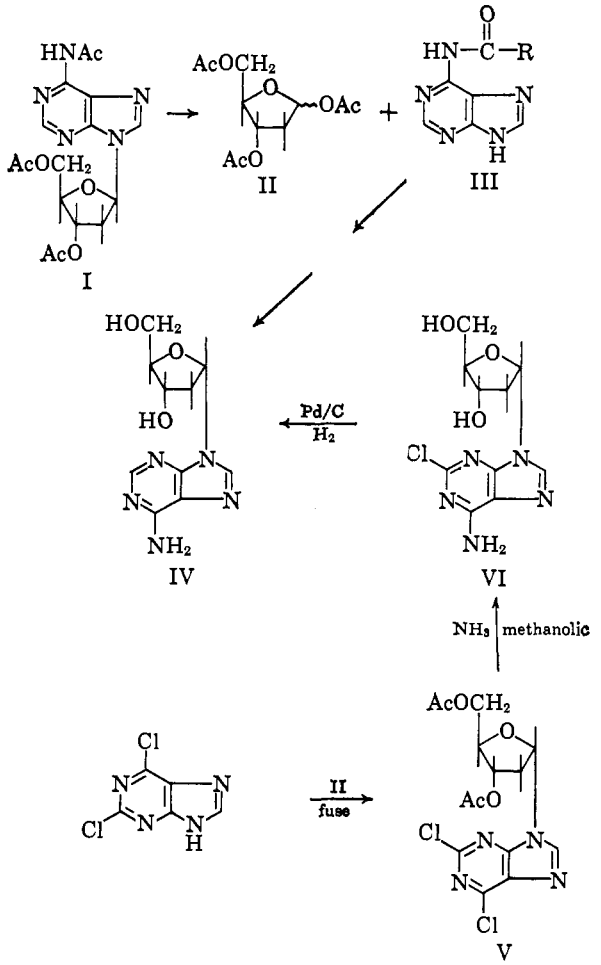
(15) M. W. Bullock, J. J. Hand, and E. L. R. Stokstad, *J. Org. Chem.*, **22**, 568 (1957).

Scheme I



chemical reactions which in effect begin with the natural 2′-deoxyadenosine (β-anomer) and in a series of steps convert the product to the corresponding α-anomer (IV). These reactions are summarized in Scheme I.

6-Chloro-9-(2′-deoxy-α- and -β-D-ribofuranosyl)-purines, 9-(2′-deoxy-α- and -β-D-ribofuranosyl)-purines, and 6-amino-9-(2′-deoxy-α-D-ribofuranosyl)-purine have previously been prepared[16] *via* the mercury salt procedure and have been adequately characterized as to anomeric configuration and position of glycosidation. These derivatives prepared from 1,3,5-tri-*O*-acetyl-2-deoxy-D-ribofuranose were found to possess physical properties in good agreement with those previously reported.[16]

Extension of the fusion procedure to the synthesis of new 2′-deoxyribofuranosylpurines involved the problem of anomeric assignment as well as position of glycosidation. The latter problem is of some concern since it has recently been shown[17] that in certain instances of purine nucleoside syntheses the glycosyl moiety became attached to position 3 of the purine ring.

2,6-Dichloropurine[14] and II fused to give a 65% yield of crude 2,6-dichloro-9-(3′,5′-di-*O*-acetyl-2′-deoxy-α- and -β-D-ribofuranosyl)purines, from which the isomer, 2,6-dichloro-9-(3′,5′-di-*O*-acetyl-2′-α-D-ribofuranosyl)purine (V), was obtained as a pure crystalline product in 32% yield. Assignment of the α-configuration was based on treatment of V with methanolic ammonia which resulted in simultaneous deacetylation and amination[18] to yield 6-amino-2-chloro-9-(2′-deoxy-α-D-ribofuranosyl)purine (VI). Catalytic dehalogenation[19] of VI gave 6-amino-9-(2′-deoxy-α-D-ribofuranosyl)purine (IV), establishing the α-configuration of the crystalline derivative IV as well as the position of glycosidation. 2,6,8-Trichloropurine[20] and 1,3,5-tri-*O*-acetyl-2-deoxy-D-ribofuranose (II) readily fused at 105° in the presence of chloroacetic acid to give a 30% yield of 2,6,8-trichloro-9-(3′,5′-di-*O*-acetyl-2′-deoxy-β-D-ribofuranosyl)purine. The ultraviolet absorption maximum at 279 mμ suggested 9-substitution.[21] An essentially equivalent amount of crude nucleoside material (probably the α-anomer) remained in the filtrate.

6-Methylpurine fused with II to give crystalline 6-methyl-9-(2′-deoxy-α-D-ribofuranosyl)purine obtained in 25% yield after deacetylation. The position of attachment of the sugar is tentatively assigned. The ultraviolet absorption data agree with those reported[22] for a 6-methyl-D-ribofuranosylpurine prepared by the chloromercuri method.

The α-configuration for 6-methyl-9-(2′-deoxy-α-D-ribofuranosyl)purine was suggested by the similarity of the large positive rotation, $[\alpha]^{26}D$ +73.1° (*c* 1.0, H_2O), to that of 9-(2′-deoxy-α-D-ribofuranosyl)purine, $[\alpha]^{26}D$ +73.4° (*c* 1.1, H_2O).

Anomeric Configuration and Proton Magnetic Resonance Spectra

It has become widespread practice to assign anomeric configuration on the basis of Hudson's rules of isorotation[23] which correlate optical rotation and anomeric configuration. However, recent examples of 2′-deoxy-D-ribofuranose nucleosides[24-26] have been observed to violate Hudson's rules. Lemieux and Hoffer[26] noted that the specific rotation of certain pyrimidine 2′-deoxy-α-D-ribofuranosides is more levorotatory than that of the corresponding β-anomers. Lemieux[27] determined the configuration of thymidine and its α-anomer by a theoretical consideration of the proton splitting patterns in the 2′-deoxyribofuranosyl portion of the molecules. In each case the splitting constants for the $H_{1'}$ protons were in reasonable agreement with the values predicted by the Karplus equation,[28] assuming a likely conformation for the deoxyribofuranose ring. It has been observed that the coupling constants between neighboring hydrogens is not only dependent on the dihedral angle which they define, but also on the substituents other than hydrogens on the vicinal carbons[29,30] and can also depend upon

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Table I. Proton Magnetic Resonance Splitting Patterns for the $H_{1'}$ Proton of Various 2'-Deoxy-α- and -β-D-ribofuranosyl Nucleosides[a]

2'-Deoxy-D-ribofuranosyl deriv.	Absorptions	α-Anomer $J_{H_{1'}}$, c.p.s.	α-Anomer Peak width, c.p.s.	β-Anomer $J_{H_{1'}}$, c.p.s.	β-Anomer Peak width, c.p.s.	Solvent
2'-Deoxyadenosine	T			7.0	14.0	DMSO
6-Amino-9-(2'-deoxy-α-D-ribofuranosyl)purine (IV)	Q	3.3, 7.5	10.8			DMSO
2'-Deoxyguanosine	T			7.0	14.0	DMSO
2'-Deoxyinosine	T			6.8	13.6	DMSO
5-Bromo-2'-deoxyuridine	T			6.6	13.2	DMSO
5-Iodo-2'-deoxyuridine	T			6.8	13.7	DMSO
6-Chloro-9-(2'-deoxy-β-D-ribofuranosyl)purine	T			6.7	13.5	D_2O
6-Chloro-9-(2'-deoxy-α-D-ribofuranosyl)purine	Q	3.3, 7.0	10.3			D_2O
9-(2'-Deoxy-α-D-ribofuranosyl)-purine	Q	3.5, 7.1	10.6			D_2O
9-(2'-Deoxy-β-D-ribofuranosyl)purine	T			6.7	13.5	DMSO
9-(2'-Deoxy-α-D-ribofuranosyl)purine-(1H)-6-thione[c]	Q	3.3, 7.0	10.3			d_6-DMSO
9-(2'-Deoxy-β-D-ribofuranosyl)purine-(1H)-6-thione[c]	T			6.7	13.5	d_6-DMSO
6-Amino-2-chloro-9-(2'-deoxy-α-D-ribofuranosyl)purine	Q	2.8, 7.2	10.0			d_6-DMSO
9-(2-Deoxy-α-D-ribofuranosylpurine-6-trimethylammonium chloride[c]	Q	3.2, 7.0	10.2			d_6-DMSO
	Q	3.2, 7.0	10.2			d_6-DMSO
6-Acetamido-9-(3',5'-di-*O*-acetyl-2'-deoxy-β-D-ribofuranosyl)purine	T			7.0	14.0	$CDCl_3$
2,6,8-Trichloro-9-(3',5'-di-*O*-acetyl-2'-deoxy-β-D-ribofuranosyl)purine	T			7.1	14.2	$CDCl_3$
6-Methyl-9-(2'-deoxy-α-D-ribofuranosyl)purine	Q	3.0, 7.0	10.0			D_2O

[a] All spectra were determined with a Varian A-60 n.m.r. spectrometer. [b] T = pseudo-triplet; Q = multiplet of four. [c] The authors wish to thank Dr. Robert R. Engle of the Cancer Chemotherapy National Service Center for samples of these compounds.

other effects.[31] Jardetzky[32] has reported the p.m.r. spectra for a variety of 2'-deoxy-β-D-ribofuranosyl nucleosides. In every case the anomeric proton $H_{1'}$ gives rise to a "pseudo-triplet"[33] with a peak width of 13.0 ± 1 c.p.s. and an apparent splitting constant of 6.5 ± 0.5 c.p.s. The latter value is in good agreement with that predicted by the Karplus equation (6.0 c.p.s.).[32] Moreover, the consistancy of this value shows that the relative orientation of the $C_{1'}$ proton to the $C_{2'}$ protons in the 2'-deoxy-β-D-ribose ring and the electronic environment of the anomeric proton are not affected to any appreciable extent by changing the heterocyclic base of the nucleoside. Other investigators[26,27,34] have obtained apparent $J_{H_{1'}}$ values of about 7.0 c.p.s. for 2'-deoxy-β-D-ribofuranosyl nucleosides.

The p.m.r. spectra of only four 2'-deoxy-α-D-ribofuranosyl nucleosides have been reported,[26,27] and in three of these spectra the resolution of the anomeric proton is not good enough to allow measurement of apparent $J_{H_{1'}}$ values. In the other case,[27] the anomeric proton gives rise to a multiplet of four[35] and the coupling constants of the $H_{1'}$ proton with the $H_{2'}$ and $H_{2''}$ protons were determined to be 7.2 ± 0.2 and 3.8 ± 0.2 c.p.s.,[27] respectively.

The present series of 2'-deoxy-α- and -β-D-ribofuranosylpurines prepared in this laboratory allow a further comparison of the proton magnetic resonance spectra of the anomers of 2'-deoxyribofuranosyl nucleosides. Inspection of Table I reveals that the β-anomers are characterized by a pseudo-triplet for the $H_{1'}$ proton with an apparent coupling constant of $J_{H_{1'}}$ of 6.8 ± 0.3 c.p.s. and a peak width of 13.7 ± 0.5 c.p.s. (for example, see Figure 1). Similarly, the α-anomers show a multiplet of four[35] with coupling constants $J_{H_{1'}}$ of 3.1 ± 0.4 and 7.2 ± 0.3 c.p.s. and a peak width of 10.4 ± 0.4 c.p.s. (for example, see Figure 2). All compounds in Table I are of established configuration except the last two whose anomeric configuration was assigned on the basis of p.m.r. spectra only. Most of the purine 2'-deoxyribonucleosides in Table I (except the last four) have been checked by Ulbricht,[36,37] and the α-anomers were found to exhibit a positive Cotton effect while the β-anomers gave a negative Cotton effect.

It has recently been suggested[37] that purine N^9 nucleosides obey Hudson's isorotation rules, while for all pyrimidine N^3 nucleosides these rules are reversed.

On the basis of the present data, it is clear that with the purine 2'-deoxy-D-ribofuranosyl nucleosides one may assign the anomeric configuration with reasonable confidence on the basis of the p.m.r. absorption band for the $H_{1'}$ proton. From the data available it is quite likely that this rather empirical assignment can be made for 2'-deoxyribofuranosyl nucleosides which

(31) M. Karplus, *J. Am. Chem. Soc.*, **85**, 2870 (1963).
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(35) For example, see ref. 33, p. 84.
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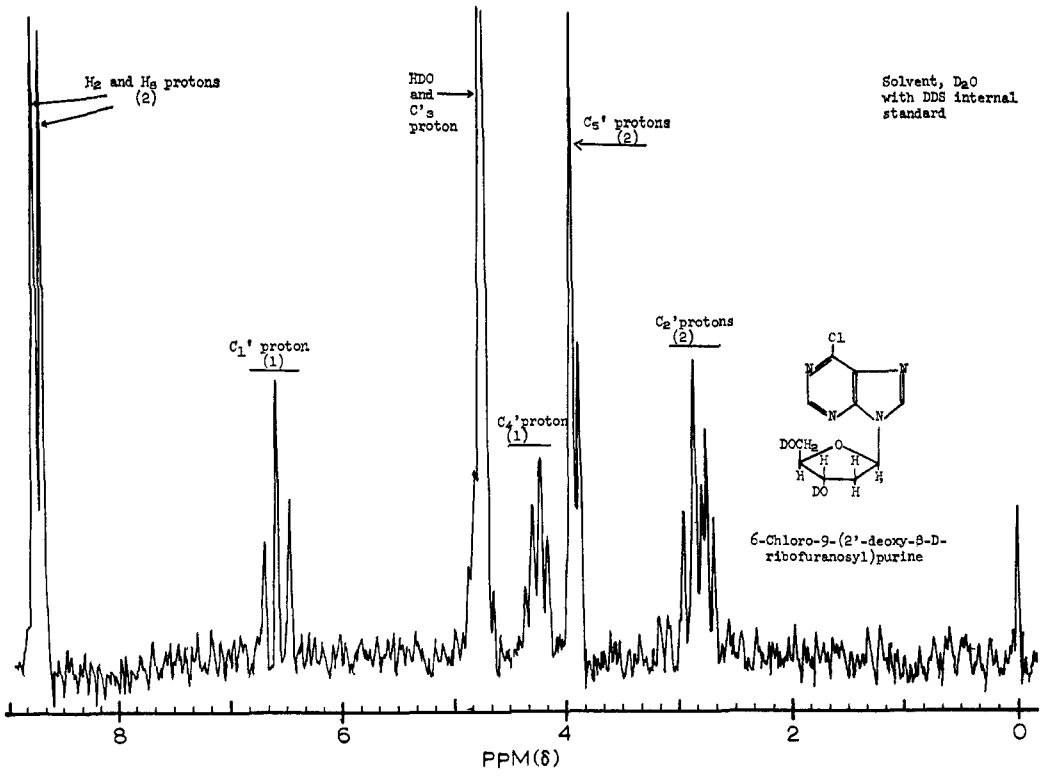

Figure 1.

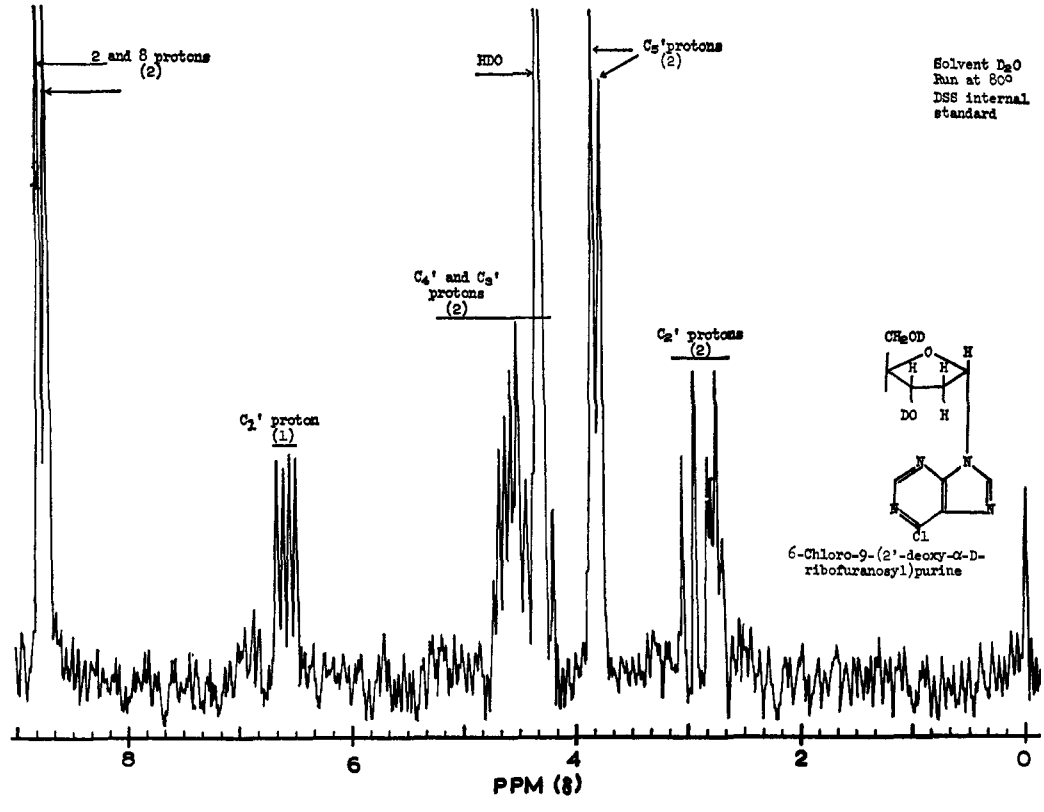

Figure 2.

possess other heterocyclic bases which do not greatly alter the conformation of the furanose ring. This method provides a valuable new physical constant for anomeric identification of these derivatives and an easily determined criterion of anomeric purity. It should be pointed out, however, that this assignment is based on particular conformational restrictions of the furanose ring[27,32,38] and that anomeric assignments made on 2′-deoxyribofuranosylpurines possessing large

(38) See M. Sundaralingam, *J. Am. Chem. Soc.*, 87, 599 (1965), for a discussion of the conformation of 2′-deoxyribofuranose in nucleosides.

functional groups attached to the hydroxyl functions should be made with extreme caution.[31] Bulky groups attached to the heterocyclic moiety which might influence conformation of the sugar should also be carefully considered. The anomeric peak splitting patterns were found to be nearly independent of temperature or solvent effects in those cases examined.

All nucleosides prepared in the present study were found to be chromatographically homogeneous in four solvent systems.

Experimental Section[39]

6-Acetamido-9-(3′,5′-di-O-acetyl-2′-deoxy-β-D-ribofuranosyl)purine (I). Deoxyadenosine monohydrate[40] (269 g., 1 mole) was added to 570 ml. of pyridine and 566 ml. of acetic anhydride in a 2-l. round-bottom flask. The suspension was stirred mechanically and the temperature was kept below 80° by occasional cooling in ice–water. The temperature fell after approximately 20 min., and the clear solution was allowed to stand for 15 hr. at room temperature. Solvent was removed *in vacuo* (oil pump) at 60° (bath temperature). The amorphous white solid was dissolved in 200 ml. of hot ethanol–toluene (5:1) and the solvent was again removed *in vacuo*. A quantitative crude yield of white solid was obtained. A small quantity was crystallized from 2-propanol giving pure 6-acetamido-9-(3′,5′-*O*-acetyl-2′-deoxy-β-D-ribofuranosyl)purine (I), m.p. 125–127°. Spectral data showed $\lambda_{max}^{pH\ 11}$ 282 mμ (ε 20,100), $\lambda_{max}^{pH\ 11}$ 273 mμ (ε 14,100), $\lambda_{max}^{1N\ NaOH}$ 291 mμ (ε 12,300).

Anal. Calcd. for $C_{16}H_{19}N_5O_6$: C, 51.0; H, 5.05; N, 18.6. Found: C, 51.0; H, 5.13; N, 18.6.

1,3,5-Tri-O-acetyl-2-deoxy-D-ribofuranose (II). The total crude 6-acetamido-9-(3′,5′-di-*O*-acetyl-2′-deoxy-β-D-ribofuranosyl)purine (I) obtained above was dissolved in 600 ml. of glacial acetic acid and 150 ml. of acetic anhydride and heated at 100° (inside temperature) in an oil bath for 4 hr. with stirring. Colorless crystals began separating after 20 min. The reaction mixture was cooled in ice and 170 g. (96%) of colorless 6-acetamidopurine (III, R = CH_3) was removed by filtration, and the filter cake was washed with 500 ml. of chloroform. Solvent was removed from the combined filtrate *in vacuo* (oil pump) at 60° leaving a brown oil. Chloroform (500 ml.) was added and an additional 2 g. of 6-acetamidopurine (III, R = CH_3) was removed by filtration. The filtrate was washed with ice-cold 3 *N* H_2SO_4, ice–water, ice-cold sodium bicarbonate solution, and then ice–water to pH 7. The organic phase was dried over sodium sulfate and then was filtered through a thin layer of charcoal and Celite mixture. The yellow solution was evaporated to a moderately viscous sirup *in vacuo* at 60°. High vacuum distillation using an insulated 5-in. Vigreux column and a fraction collector gave 203 g. (78%) of 1,3,5-tri-*O*-acetyl-2-deoxy-D-ribose (II), b.p. 124–125° (0.08 mm.), $[\alpha]^{25}D$ +25.0° (*c* 0.66, MeOH). Strong infrared absorption occurred at 1750 cm.$^{-1}$ (OAc). An n.m.r. peak at δ 2.05 corresponding to nine protons (three OAc), multiplet at δ 2.17 to 2.55 corresponding to two protons (2′ protons), multiplet at δ 4.09 to 4.52 corresponding to three protons [5′-(two) and 4′ (one) protons], multiplet at δ 5.00 to 5.41 (3′ proton), and multiplet at δ 6.28 to 6.47 (1′ proton) were observed with TMS as an internal reference in carbon tetrachloride.

Anal. Calcd. for $C_{11}H_{16}O_7$: C, 50.8; H, 6.19. Found: C, 51.0; H, 6.21.

6-Chloro-9-(2′-deoxy-α-D-ribofuranosyl)purine[16] *and 6-Chloro-9-(2′-deoxy-β-D-ribofuranosyl)purine.*[16] 6-Chloropurine[14] (4 g., 0.026 mole) was powdered finely and mixed with 1,3,5-tri-*O*-acetyl-2-deoxy-D-ribofuranose (II) (10 g., 0.039 mole) in a small round-bottom flask. The mixture was heated to 125° in a preheated oil bath and 75 mg. of chloroacetic acid was added. The mixture was stirred while heating at 125–127°; this was continued for approximately 4 min. An efficient aspirator was then attached and the acetic acid rapidly removed from the amber melt (about 1 min.). The flask was removed from the bath and allowed to cool to 100°. The melt was dissolved in ethyl acetate with the accompanying precipitation of a green solid which was removed by filtration. The sticky filter cake was washed with hot ethyl acetate and the combined filtrate was washed with two 30-ml. portions of ice-cold saturated aqueous sodium carbonate and then with ice–water to pH 7. The organic phase was dried over sodium sulfate and then passed through a charcoal Celite pad. The solvent was removed *in vacuo*, and the brown sirup was dissolved in 10 ml. of absolute ethanol and treated with 80 ml. of absolute ethanol saturated with ammonia at −10°. The ammoniacal solution was allowed to stand at 0° for 15 hr. and then was evaporated to dryness *in vacuo* at 5°. The resulting sirup was dissolved in 30 ml. of warm ethanol and the solution was again evaporated to dryness. This process was repeated and the resulting yellow solid was dissolved in a minimum volume of hot ethyl acetate–methanol (5:1) and cooled at −18° for 72 hr. A tan solid (2.6 g., 38%) was collected after filtration. Fractional crystallization from ethyl acetate–methanol (5:1) gave 1.5 g. (21%) of the more insoluble 6-chloro-9-(2′-deoxy-α-D-ribofuranosyl)purine and 0.45 g. (6.4%) of 6-chloro-9-(2′-deoxy-β-D-ribofuranosyl)purine. Separation of the 6-chloropurine deoxyribofuranosides from an impurity detected by paper chromatography and not removable by recrystallization was easily effected by alumina column chromatography using methanol. The respective deoxynucleoside was eluted rapidly in chromatographic as well as analytical purity. 6-Chloro-9-(2′-deoxy-α-D-ribofuranosyl)purine had m.p. 150.5–151.5°, $[\alpha]^{25}D$ +61.1° (*c* 1.22, H_2O). Spectral data showed $\lambda_{max}^{H_2O}$ 264 mμ (ε 9300).

Anal. Calcd. for $C_{10}H_{11}ClN_4O_5$: C, 44.4; H, 4.10; N, 20.7. Found: C, 44.5; H, 4.23; N, 20.7.

6-Chloro-9-(2′-deoxy-β-D-ribofuranosyl)purine (XV) had m.p. 144–145°, $[\alpha]^{25}D$ −10.8° (*c* 1.04, MeOH). Spectral data showed $\lambda_{max}^{H_2O}$ 264 mμ (ε 10,000).

Anal. Found: C, 44.5; H, 3.99; N, 20.7. All data agree well with those previously reported.[16]

9-(2′-Deoxy-α-D-ribofuranosyl)purine[16] *and 9-(2′-Deoxy-β-D-ribofuranosyl)purine.*[16] Purine[41] (2.77 g.,

(39) All melting points were determined on a Fisher–Johns block and are uncorrected. Nucleosides which exhibited no pH dependence in the ultraviolet are reported only in H_2O.

(40) Purchased from International Chemical and Nuclear Corp., City of Industry, Calif.

(41) A. G. Beaman, *J. Am. Chem. Soc.*, **76**, 5633 (1954).

0.023 mole) and 1,3,5-tri-*O*-acetyl-2-deoxy-D-ribofuranose (II) (12 g., 0.046 mole) were heated to 140° and fused at 140–145° for 18 min. *in vacuo* with 75 mg. of chloroacetic acid. The clear melt dissolved in ethyl acetate and was treated as in the preparation of the 6-chloropurine 2′-deoxyribofuranosides described above. The tan sirup obtained after deacylation was dissolved in a small volume of hot methanol and cooled at −18° to effect crystallization. The tan product (1.7 g., 31%) was separated by filtration. Three recrystallizations of the product from methanol gave 1 g. (18%) of 9-(2′-deoxy-β-D-ribofuranosyl)purine, m.p. 181–182°, $[\alpha]^{25.5}D$ −29.3° (*c* 1.06, H_2O). Spectral data showed $\lambda_{max}^{H_2O}$ 262.5 mμ (ε 7320).

Anal. Calcd. for $C_{10}H_{12}N_4O_3$: C, 50.8; H, 5.12; N, 23.7. Found: C, 50.9; H, 4.88; N, 23.5.

Reported data[16] are m.p. 192–194°, $[\alpha]^{26}D$ −28.0° (H_2O), $\lambda_{max}^{H_2O}$ 263 mμ (ε 6850). An authentic sample kindly provided by Dr. L. Goodman had m.p. 189–191° on the block used throughout this work and the two samples had m.m.p. 181–183°. Infrared spectra of the two samples were superimposible in every detail. The above original filtrate was allowed to evaporate partially very slowly giving 1.1 g. of crystalline nucleoside, m.p. 120–130°. Recrystallization from acetonitrile afforded 0.8 g. (14.7%) of 9-(2′-deoxy-α-D-ribofuranosyl)purine, m.p. 135–136°, $[\alpha]^{26}D$ +73.4° (*c* 1.09, H_2O). Spectral data showed $\lambda_{max}^{H_2O}$ 262.5 mμ (ε 7800). These data agree well with published values.[16]

Anal. Found: C, 50.8; H, 5.00; N, 23.9.

6-Amino-9-(2′-deoxy-α-D-ribofuranosyl)purine[16] (*IV*). 6-Benzamidopurine[15] (4.8 g., 0.02 mole) was powdered finely and mixed with 1,3,5-tri-*O*-acetyl-2-deoxy-D-ribofuranose (II) (10.4 g., 0.04 mole). The mixture was heated to 160° and 6 drops of dichloroacetic acid was added. The mixture was stirred with continued heating for 10 min. The melt suspension was fused *in vacuo* for 15 min. at 160–165°. Ethyl acetate was added after cooling to 100° and 1.35 g. of unreacted 6-benzamidopurine was recovered by filtration and washed well with hot ethyl acetate. The ethyl acetate solution was treated as in the above preparation of 6-chloropurine deoxyribofuranosides. The ammoniacal solution was stirred for 15 hr. at room temperature and then was evaporated to a brown sirup *in vacuo*. Repeated trituration with boiling ether gave an amorphous solid which was dissolved in 15 ml. of hot methanol, treated with charcoal, and allowed to stand at −18° for 2 weeks. The tan crystalline rosettes which formed (1.5 g., 30%) were removed by filtration. Four recrystallizations of this product from methanol gave 1 g. (20%) of pure 6-amino-9-(2′-deoxy-α-D-ribofuranosyl)purine (IV), m.p. 208–210°, $[\alpha]^{25.5}D$ +68.3° (*c* 1.17, H_2O). Spectral data showed $\lambda_{max}^{H_2O}$ 259.5 mμ (ε 15,500). These data agree well with published values.[16]

Anal. Calcd. for $C_{10}H_{13}N_5O_3$: C, 47.8; H, 5.22; N, 27.9. Found: C, 47.9; H, 4.93; N, 27.8.

6-Methyl-9-(2′-deoxy-α-D-ribofuranosyl)purine. 6-Methylpurine[42] (1.34 g., 0.01 mole) and 1,3,5-tri-*O*-acetyl-2-deoxy-D-ribofuranose (II) (5.2 g., 0.02 mole) were heated to 150° and 4 drops of dichloroacetic acid was added. The mixture was stirred for 5–10 min. and the resulting clear melt was fused for 15–20 min. *in vacuo*. The contents of the flask was dissolved in ethyl acetate and the resulting solution was treated as in the above preparation of 6-chloropurine deoxyribofuranosides. The sirup obtained from the ammoniacal solution was dissolved in 15 ml. of hot methanol, treated with charcoal, and allowed to cool at −18° for 1 week. A tan semicrystalline solid formed and was collected by filtration. Three recrystallizations of this solid from ethanol gave 0.63 g. (25%) of 6-methyl-9-(2′-deoxy-α-D-ribofuranosyl)-purine, m.p. 191–192.5°, $[\alpha]^{26.5}D$ +73.1° (*c* 1.01, H_2O). Spectral data showed $\lambda_{max}^{pH\ 1}$ 264 mμ (ε 7150), $\lambda_{max}^{pH\ 11}$ 260 mμ (ε 8150), $\lambda_{sh}^{pH\ 11}$ 245 to 251 mμ (ε 7150).

Anal. Calcd. for $C_{11}H_{14}N_4O_3$: C, 52.8; H, 5.60; N, 22.4. Found: C, 53.0; H, 5.80; N, 22.6.

2,6-Dichloro-9-(3′,5′-di-O-acetyl-2′-deoxy-α-D-ribofuranosyl)purine (*V*). 2,6-Dichloropurine[14] (5.56 g., 0.029 mole) and 1,3,5-tri-*O*-acetyl-2-deoxy-D-ribofuranose (II) (7.7 g., 0.03 mole) were heated to 125° and chloroacetic acid (50 mg.) was added to the clear melt. Heating was continued for 15 min. at 127–129° *in vacuo*, and the amber melt was dissolved in ethyl acetate solution, washed with ice-cold aqueous sodium carbonate and then ice–water to pH 7, and dried over sodium sulfate. The drying agent was removed by filtration and the solvent was removed *in vacuo*. The resulting semisolid mass was crystallized from a small volume of ethanol to give 4.3 g. (37.5%) of tan needles. Recrystallization of this crystalline product from ethanol gave 3.7 g. (32%) of 2,6-dichloro-9-(3′,5′-di-*O*-acetyl-2′-deoxy-α-D-ribofuranosyl)purine (V), m.p. 123.5–124.5°, $[\alpha]^{27}D$ +0.4° (*c* 1.13, MeOH). Spectral data showed $\lambda_{max}^{H_2O}$ 275 mμ (ε 11,300).

Anal. Calcd. for $C_{14}H_{14}Cl_2N_4O_5$: C, 43.2; H, 3.60; N, 14.4. Found: C, 43.3; H, 3.84; N, 14.2.

2-Chloro-6-amino-9-(2′-deoxy-α-D-ribofuranosyl)purine (*VI*). 2,6-Dichloro-9-(3′,5′-di-*O*-acetyl-2′-deoxy-α-D-ribofuranosyl)purine (V) (1.7 g., 0.0044 mole) was stirred for 15 hr. at room temperature in 100 ml. of ethanol saturated with ammonia at −10°. Aqueous sodium hydroxide (4.5 ml. of 1 *N* NaOH) was added and the solution was evaporated to dryness *in vacuo*. Crystallization of the resulting colorless solid from 20 ml. of water gave 0.85 g. (68%) of 2-chloro-6-amino-9-(2′-deoxy-α-D-ribofuranosyl)purine (VI), $[\alpha]^{26}D$ +72.8° (*c* 1.13, H_2O–DMSO 9:1 v./v.). This compound softens near 190° and slowly decomposes over a wide range. Spectral data showed $\lambda_{max}^{pH\ 1}$ 265.5 mμ (ε 14,300), $\lambda_{max}^{pH\ 11}$ 264 mμ (ε 15,700).

Anal. Calcd. for $C_{10}H_{12}ClN_5O_3$: C, 42.1; H, 4.21; N, 24.5. Found: C, 41.8; H, 4.18; N, 24.3.

6-Amino-9-(2′-deoxy-α-D-ribofuranosyl)purine[16] (*IV*). 2-Chloro-6-amino-9-(2′-deoxy-α-D-ribofuranosyl)purine (VI) (0.29 g., 0.001 mole) was hydrogenated at 47 p.s.i. for 15 hr. with 0.3 g. of 5% palladium on carbon in 75 ml. of water containing 1.02 ml. of 1 *N* NaOH. The catalyst was removed by filtration and the filtrate was evaporated to dryness. The resulting colorless solid was crystallized from methanol to give 6-amino-9-(2′-deoxy-α-D-ribofuranosyl)purine (IV) (0.16 g., 63%), m.p. 212–213.5°, $[\alpha]^{27}D$ +69.8° (*c* 0.9, H_2O). A mixture of this sample and the one prepared *via* 6-benzamidopurine fusion had m.p. 209–211°. The two samples also exhibited identical paper chromatographic

(42) S. Gabriel and J. Colman, *Ber.*, **34**, 1234 (1901). Purchased from Cyclo Chemical Corp., Los Angeles, Calif.

behavior in four different systems. Spectral data showed $\lambda_{max}^{H_2O}$ 259.5 mμ (ε 14,300). A mixture of this sample and dried 2′-deoxyadenosine[36] had m.p. 170–185°.

2,6,8-Trichloro-9-(3′,5′-di-O-acetyl-2′-deoxy-β-D-ribofuranosyl)purine. 2,6,8-Trichloropurine[20] (5.6 g., 0.025 mole) and 1,3,5-tri-*O*-acetyl-2-deoxy-D-ribofuranose (II) (6.5 g., 0.025 mole) were fused *in vacuo* at 105° for 5 min. with chloroacetic acid (50 mg.). The melt was dissolved in ethyl acetate and was treated as in the preparation of 2,6-dichloro-9-(3′,5′-di-*O*-acetyl-2′-deoxy-α-D-ribofuranosyl)purine. The resulting semisolid was crystallized from absolute methanol to give 3.9 g. (37%) of pink crystals. Recrystallization of the product from absolute methanol gave 3.2 g. (30%) of 2,6,8-trichloro-9-(3′,5′-di-*O*-acetyl-2′-deoxy-β-D-ribofuranosyl)purine, m.p. 141–142°, $[\alpha]^{25}D$ −2.7° (*c* 1.02, EtOAc). Spectral data showed $\lambda_{max}^{pH\ 1}$ 278 and 246 mμ (ε 12,700 and 8050), $\lambda_{max}^{pH\ 11}$ 278 mμ (broad) (ε 15,200).

Anal. Calcd. for $C_{14}H_{13}Cl_3N_4O_5$: C, 39.7; H, 3.07; N, 13.2. Found: C, 39.5; H, 3.28; N, 13.2.

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The Synthesis of 1-(2′-Deoxy-α- and -β-D-ribofuranosyl)benzimidazoles Related to the Naturally Occurring Nucleosides of Vitamin B_{12}[1]

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Substituted 1-(2′-deoxy-α- and -β-D-ribofuranosyl)benzimidazoles have been prepared for the first time by a simple fusion of the requisite benzimidazole and 1,3,5-tri-O-acetyl-2-deoxy-D-ribofuranose in the presence of chloroacetic acid as a catalyst. The α- and β-anomers have been separated by fractional crystallization and column chromatography. Anomeric configuration has been assigned on the basis of p.m.r. spectra, and the nucleosides have been found to obey Hudson's rules of isorotation. The excellent yield of glycosides obtained by the present synthesis suggest the fusion procedure has wide application for the preparation of 2′-deoxy-D-ribofuranosyl nucleosides.

Renewed interest in the synthesis of benzimidazole ribonucleosides has been stimulated by the recent findings that 5,6-dimethyl-1-(α-D-ribofuranosyl)benzimidazole is incorporated into vitamin B_{12} in various microbiological systems, without cleavage of the nucleoside linkage.[3,4] The importance of vitamin B_{12} as a cofactor in many biochemical reactions is well established.[5–7] It has recently been demonstrated that vitamin B_{12} plays a significant role in the conversion of ribonucleotides to 2′-deoxyribonucleotides without glycosidic cleavage in the microorganism *L. leichmannii*.[8,9] Certain 1-(β-D-ribofuranosyl)benzimidazoles have been shown to exhibit antiviral activity.[10,11] It is quite possible that these compounds are simulating purine nucleoside analogs since the inhibition of influenza B virus by 5,6-dichloro-1-(β-D-ribofuranosyl)-benzimidazole (DRB) is reversed by adenosine.[12] It has been suggested that 5,6-dichloro-1-(β-D-ribofuranosyl)benzimidazole (DRB) interferes with preliminary synthesis of ribonucleic acid.[13] Recent work[14] has confirmed this suggestion and has shown DRB exhibits specific inhibition of chromosomal RNA synthesis. Evidence has recently been obtained for a benzimidazole nucleoside as a component of an enzyme isolated from wheat embryos.[15] Although biochemical interest in 2′-deoxy-D-ribofuranosylbenzimidazoles was expressed as early as 1956 by Tamm,[11] there is until the present work no report of their synthesis. Cooley and co-workers[16] in 1950 recognized the desirability of obtaining benzimidazole 2′-deoxynucleosides related to the naturally occurring purine 2′-deoxynucleosides. By using the silver salt of 5,6-dimethylbenzimidazole and 1-chloro-3,4-diacetyl-2-deoxy-D-ribopyranose, these authors obtained 5,6-dimethylbenzimidazole-1-

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