7-Glycosylpurines. I. The Synthesis of the Anomeric 7-D-Ribofuranosyladenines and the Identification of the Nucleoside from Pseudovitamin B_{12}[1,2]

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The utility of a removable blocking group at N-3 of adenine in the preparation of 7-glycosyladenines is demonstrated by the preparation of 7α- and 7β-D-ribofuranosyladenine (XVIII and IX), and the nucleoside from pseudovitamin B_{12} is conclusively identified as 7α-D-ribofuranosyladenine (XVIII). The physical, spectral, and chemical properties of XVIII and IX are described.

There are a number of naturally occurring analogs of vitamin B_{12} that contain a purine in place of 5,6-dimethylbenzimidazole.[3] Early work on the structure of one of these analogs, pseudovitamin B_{12}, showed that in contrast to B_{12} itself, pseudovitamin B_{12} is cleaved to the purine base under mild conditions; thus 1 *N* HCl at 100° gives adenine, which was identified by chromatography and spectroscopy.[4] Later, it was found that cleavage with cerous hydroxide gives cobinamide, phosphate, and a crystalline nucleoside, the ultraviolet spectrum of which resembled that of 7-methyladenine rather than 9-methyladenine.[5] This nucleoside is cleaved in dilute hydrochloric acid to adenine and D-ribose and it consumes 1 mole of periodate per mole in 5–20 min. These data indicate that the nucleoside is 7-D-ribofuranosyladenine. The α-D configuration, established for α-ribozole isolated from vitamin B_{12},[6] although indicated,[7] has not been established in this case by chemical synthesis.

Recently we attacked the problem of the synthesis of 7-glycosylpurines and found that substitution of a purine at N-3 by a removable blocking group allowed the preparation of this type of nucleoside.[9] The only other proven 7-glycosylpurines[10-14] that have been synthesized are theophylline nucleosides, which are also substituted at N-3.[16] Although not established, it appears that substitution at N-3 may by steric hindrance prevent the attachment of the chloromercuri (or mercuri as the case may be) group to N-9 and thereby force its attachment at N-7.

In our previous work we employed the benzyl group to block N-3 of hypoxanthine and purine-6(1H)-thione, but we found that removal of the benzyl group from N-3 by catalytic hydrogenolysis or with sodium and liquid ammonia was difficult.[9] For this reason we investigated the use of other groups that might be more readily removed by methods that would not cleave the sugar moiety from N-7. 3-Benzhydryladenine (I) was readily prepared,[17] but an attempt to benzoylate it by fusion with benzoic anhydride to give II, a procedure that was successful for 3-benzyladenine,[17] gave N^6-benzoyladenine (IV) as the only isolable product. Acetylation of I with acetic anhydride, a milder reaction, gave the desired N^6-acetyl-3-benzhydryladenine (III) but in low yield.[18] Reaction of N^6-benzoyladenine (IV) and N^6-acetyladenine (V) with benzhydryl chloride in N,N-dimethylacetamide gave, in both instances, an intractable mixture of adenine, 3-benzhydryladenine (I), and some of the desired product (II and III). The use of another blocking group, the benzyloxymethyl group, was also investigated and 3-(benzyloxymethyl)adenine was prepared, but again the yield obtained was prohibitively low. These negative results caused us to return to the benzyl blocking group in spite of the difficulty of removing it, and the mercury derivative of N^6-benzoyl-3-benzyladenine (VI)[17] was prepared. Despite the relatively poor yield of mercury derivative obtained,[20] it was possible to prepare analyt-

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(2) A preliminary account of this work has appeared: J. A. Montgomery and H. J. Thomas, *J. Am. Chem. Soc.*, **85**, 2672 (1963).

(3) For a recent review of the chemistry of the B_{12} vitamins, see R. Bonnett, *Chem. Rev.*, **63**, 573 (1963).

(4) H. W. Diehl, D. G. Calkins, and J. J. Pfiffner, *J. Am. Chem. Soc.*, 74, 1108, (1952); F. B. Brown and L. E. Smith, *Biochem. J.*, **56**, xxxiv (1954).

(5) W. Friedrich and K. Bernhauer, *Chem. Ber.*, **89**, 2507 (1956).

(6) F. W. Holley, C. H. Shunk, E. W. Peel, J. J. Cahill, J. B. Lavigue, and K. Folkers, *J. Am. Chem. Soc.*, **74**, 4521 (1952).

(7) X-Ray analysis of the structure of the B_{12} vitamins suggests the α-configuration as well as attachment at N-7.[8]

(8) D. C. Hodgkin, *Biochem. Soc. Symp.* (Cambridge, Engl.), **13**, 28 (1955, (footnote).

(9) J. A. Montgomery and H. J. Thomas, *J. Org. Chem.*, **28**, 2304 (1963).

(10) Although Baker and co-workers[11] claimed the preparation of several 7-glycosylpurines from N^6,N^6-dimethyladenine and from N^6,N^6-dimethyl-2,8-di(methylthio)adenine, a recent study[12] has shown that these nucleosides are actually *3-glycosylpurines*. The 7-glycosylpurine claimed by Blackburn and Johnson[13] is probably also a 3-glycosylpurine.[12]

(11) B. R. Baker, R. E. Schaub, and J. P. Joseph, *J. Org. Chem.*, **19**, 638 (1954); B. R. Baker, J. P. Joseph, R. E. Schaub, and J. H. Williams, *ibid.*, **19**, 1780 (1954); H. M. Kissman, C. Pidacks, and B. R. Baker, *J. Am. Chem. Soc.*, 77, 18 (1955); B. R. Baker and R. E. Schaub, *ibid.*, 77, 5900 (1955); B. R. Baker, J. P. Joseph, and R. E. Schaub, *ibid.*, 77, 5905 (1955).

(12) L. B. Townsend, R. K. Robins, R. N. Loeppky, and N. J. Leonard, *ibid.*, **86**, 5320 (1964).

(13) G. M. Blackburn and A. W. Johnson, *J. Chem. Soc.*, 4347 (1960).

(14) Baddiley, *et al.*, reported the detection by chromatography of 7-glycosylpurines prepared from glycosylimidazoles but these compounds were not isolated.[15]

(15) J. Baddiley, J. G. Buchanan, and G. O. Osborne, *J. Chem. Soc.*, 3606 (1958); J. Baddiley, J. G. Buchanan, F. E. Hardy, and J. Stewart, *ibid.*, 2893 (1959).

(16) J. A. Montgomery and H. J. Thomas, *Advan. Carbohydrate Chem.*, **17**, 301 (1962).

(17) J. A. Montgomery and H. J. Thomas, *J. Heterocyclic Chem.*, **1**, 115 (1964).

(18) Since these reactions were carried out we have found that the benzyhydryl group is readily removed from N-3 of purines under acidic conditions.[19]

(19) J. A. Montgomery and H. J. Thomas, unpublished observations.

(20) Fox, *et al.*,[21] have discussed the necessity for employing *pure* mercury derivatives in condensation reactions with poly-O-acylglycosyl halides and have pointed out that pure mercury derivatives are not usually obtained unless the yield is high.

Table I. Ultraviolet Spectral Data[a]

Compd.	0.1 N HCl				pH 7 buffer				0.1 N NaOH			
	λ_{max}	$\epsilon \times 10^{-3}$	λ_{min}	$\epsilon \times 10^{-3}$	λ_{max}	$\epsilon \times 10^{-3}$	λ_{min}	$\epsilon \times 10^{-3}$	λ_{max}	$\epsilon \times 10^{-3}$	λ_{min}	$\epsilon \times 10^{-3}$
Adenosine	257	14.6	230	3.5	260	14.4	227	2.1	260	14.9	227	2.2
3β-D-Ribofuranosyladenine[b]	220	s[c]	237	3.1	215	16.7	245	2.8			247	3.8
	275	18.2			277	12.9			278	13.1		
XVIII	220	s[c]	238.5	4.5	245	s[c]	232	4.2	245	s[c]	235	6.3
	273	13.3			270	9.6			271	9.9		
IX	220	12.0	238	4.4	246	s[c]	231.5	3.9	245	s[c]	234	4.8
	272	13.6			270	9.5			270	9.8		
Nucleoside from ψB_{12}[d]	273	13.6	238	4.7					245	s[c]		
									271	9.8	234	4.4

[a] λ_{max} and λ_{min} values are given in mμ. [b] From ref. 28. [c] Shoulder. [d] From ref. 5.

ically pure material by recrystallization of the crude product from N,N-dimethylformamide. Elemental analyses established the identity of the mercury derivative as bis[N⁶-benzoyl-3-benzyladen-7(or 9)-yl]mercury (VII).

Reaction of VII with tri-O-acetyl-D-ribofuranosyl chloride in refluxing xylene in the usual manner gave the blocked nucleoside (VIII)[22] from which the benzoyl and acetyl groups were removed with methanolic sodium methoxide. The benzyl group of X was removed by hydrogenolysis using 5% palladium-on-charcoal catalyst at 80° and 47 p.s.i. of hydrogen, although the reaction was slow and incomplete as had been anticipated. The identity of IX, obtained in an over-all yield of 15% from VII, was established at this point as 7β-D-ribofuranosyladenine (IX) by its ultraviolet spectrum and by application of the *trans* rule.[24] The optical rotation of this nucleoside was $-84.9 \pm 2°$.

The other part of the problem to be solved, the production of the α-linkage at N-7 of adenine, was approached by using a sugar containing a nonparticipating group at C-1. For this purpose we selected 5-O-benzoyl-D-ribofuranosyl bromide 2,3-cyclic carbonate (XIV), the ribose derivative used by Khorana[25] to prepare 9α-D-ribofuranosyladenine. We prepared this compound from methyl 2,3-O-isopropylidene-β-D-ribofuranoside (XI)[26] in four steps (XI → XII → XIII → XV → XIV) rather than use the lengthy and time-consuming procedure previously employed.[25] Reaction of XIV with bis[N⁶-benzoyl-3-benzyladen-7(or 9)-yl]-mercury (VII) gave a mixture of blocked nucleosides which could not be separated. Deblocking was carried out as described above, and the crude mixture was separated into adenine and two nucleosides by means of a cellulose column. The first nucleoside eluted proved to be identical with the product of VII and tri-O-acetylribofuranosyl chloride and, therefore, was assigned the β-configuration (IX). Thus the second nucleoside, the optical rotation of which was 0°,[27] must be 7α-D-ribofuranosyladenine (XVIII). The optical rotations of the two anomers confirm the assignment of α- and β-configurations and agree well with the relative values for adenosine and its α-anomer (−60.4°, + 24°).

These assignments were further confirmed by eliminating from this anomeric pair all the asymmetric carbon atoms but C-1′ by metaperiodate oxidation followed by sodium borohydride reduction of the intermediate dialdehyde.[25] The product from XVIII had a rotation of $-117.9 \pm 0.2°$ and the product from IX had $+118.1 \pm 0.2°$, thus establishing that these products are optical antipodes and confirming the anomeric assignments.

Spectral Data. The ultraviolet spectral maxima and minima of 7α- and 7β-D-ribofuranosyladenine (XVIII and IX) are given in Table I along with those of the nucleoside of pseudovitamin B_{12}, 3β-D-ribofuranosyladenine,[28] and adenosine. The maxima of XVIII and IX are close to those of the 3-isomer and quite different from those of the 9-isomer in keeping with observations made on N-alkyladenines.[29,30] Furthermore the minimum of the spectrum of XVIII and of IX at pH 1 is 6.5 mμ higher than the minimum at pH 7, whereas in the case of the 3-isomer the shift is 9 mμ in the opposite direction.[31] Thus the ultraviolet spectral data confirm the fact that the ribose moiety is attached to N-7. Furthermore, the spectrum of XVIII is identical with that of the nucleoside of pseudovitamin B_{12}.

The infrared spectra of XVIII and IX and of a sample of the nucleoside from pseudovitamin B_{12}[32] are reproduced in Figure 1. These spectra clearly show the identity of XVIII and the nucleoside from pseudovitamin B_{12} and the definitive differences of the spectra of these two from that of the β-anomer.

The proton magnetic resonance spectra of the two anomers were determined and these data are recorded in the Experimental Section. Although application of the Karplus equation would lead to the prediction that the coupling constants for the proton at C-1′ in the α-anomer should be equal to or larger than that for the proton at C-1′ in the β-anomer,[33] the difference in the

(21) J. J. Fox, N. Yung, I. Wempen, and I. L. Doerr, *J. Am. Chem. Soc.*, 79, 5060 (1957).

(22) Fusion of N⁶-benzoyl-3-benzyladenine with tetra-*O*-acetylribose using *p*-toluenesulfonic acid as a catalyst[23] failed. Acid lability of the benzyl group at N-3 under these conditions may be a factor here.[18]

(23) T. Sato, T. Simadate, and Y. Ishido, *Nippon Kagaku Ryohogakukai Zasshi*, 81, 1440 (1960).

(24) B. R. Baker, *Ciba Found. Symp. Chem. Biol. Purines*, 120 (1957).

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

(26) J. A. Montgomery and K. Hewson, *J. Org. Chem.*, 29, 3436 (1964).

(27) The reported optical rotation of the nucleoside from pseudovitamin B_{12} is 0°.[5]

(28) N. J. Leonard and R. A. Laursen, *Biochemistry*, 4, 354 (1965).

(29) G. B. Elion, *Ciba Found. Symp. Chem. Biol. Purines*, 39 (1957).

(30) J. M. Gulland and E. R. Holiday, *J. Chem. Soc.*, 765 (1936).

(31) See the discussion of the ultraviolet spectra of 3- and 7-substituted adenines: N. J. Leonard and J. A. Deyrup, *J. Am. Chem. Soc.*, 84, 2148 (1962).

(32) A small sample of this nucleoside was kindly supplied by Dr. Wilhelm Friedrich.

(33) For a discussion of the uses of p.m.r. spectrometry in the assignment of anomeric configurations, see L. Goldman, J. W. Marsico, and M. J. Weiss, *J. Med. Chem.*, 6, 410 (1963); and N. J. Leonard and R. A. Laursen, *J. Am. Chem. Soc.*, 85, 2026 (1963).

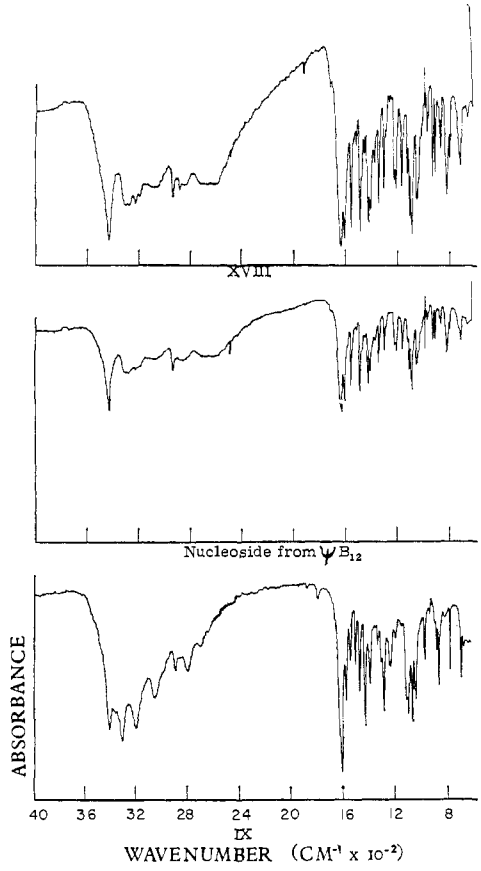


Figure 1. The infrared spectra of 7α- and 7β-D-ribofuranosyl adenine, and the nucleoside from pseudovitamin B_{12}.

observed coupling constants (IX, 5.7 c.p.s.; XVIII, 3.6 c.p.s.) is too small to provide any reliable information concerning the anomeric assignments.[34] However, the well-defined triplet resulting from the splitting of the hydrogen of the hydroxyl at C-5′ by the hydrogens at C-5′ in the spectrum of XVIII does not appear in the spectrum of IX. Instead, the absorption due to this hydrogen appears at τ 4.67, coinciding with that due to the hydroxyl proton on C-2′ or C-3′. This downfield chemical shift may be due to interaction between the purine moiety and the 5′-hydroxyl in the case of IX in which they are on the same side of the ribofuranose ring. Such interaction cannot occur in the case of XVIII, since the 5′-hydroxyl and the purine moiety are on opposite sides of the ring.

Chromatographic Data. The chromatographic data given in Table II show that the anomers XVIII and IX are readily separated by three of the four solvent systems tested. Separation is also possible on thin layer chromatograms using a mixture of chloroform and methanol as eluent. The data also show the identical travel of XVIII and the nucleoside from pseudovitamin B_{12}.

Ionization Constants. The apparent ionization constants for XVIII, IX, 3β-D-ribofuranosyladenine, and adenosine are given in Table III. The basicity of XVIII and IX is the same as that of adenosine, whereas 3β-D-ribofuranosyladenine is somewhat more basic than the other three.

(34) See M. Karplus, *J. Am. Chem. Soc.*, **85**, 2870 (1963); and also R. J. Abraham, L. D. Hall, L. Hough, and K. A. McLauchlan, *J. Chem. Soc.*, 3699 (1962).

Table II. Chromatographic Data

Compd.	R_f values in solvent system[a] A	B	C	D
Adenosine	0.32	0.56	0.57	0.58
IX	0.21	0.47	0.42	0.54
XVIII	0.16	0.43	0.34	0.64
Nucleoside from ψB_{12}	0.16	0.43	0.34	0.64

[a] Run on Whatman No. 1 paper by the descending technique. Solvent systems: A, water-saturated butanol; B, butanol-acetic acid-water (5:2:3); C, isopropyl alcohol-ammonium hydroxide-water (14:1:5); D, sodium acetate buffer, pH 6.7.

Table III. Ionization Constants[a]

Compd.	pK_a'
Adenosine	3.62
IX	3.68
XVIII	3.84
3β-D-Ribofuranosyladenine	5.5[b]

[a] Determined in 0.15 *M* sodium chloride solution. [b] From ref. 28.

Stability Studies. Further synthetic work with 7-α-D-ribofuranosyladenine (XVIII) will require the use and removal of protective groups, and for this reason the stability of this nucleoside to aqueous acid and base was compared to the stability of the β-isomer and to the anomeric 9-nucleosides. The results given in Table IV show clearly the relatively *instability* of XVIII.

Table IV. Stability Studies

Compd.	Complete disappearance of nucleoside, hr.[a] 0.1 *N* HCl, 56°	1 *N* NaOH, 100°
Adenosine	>25	2
9α-D-Ribofuranosyladenine[b]	>25	>6
IX	4	1
XVIII	<1	0.5

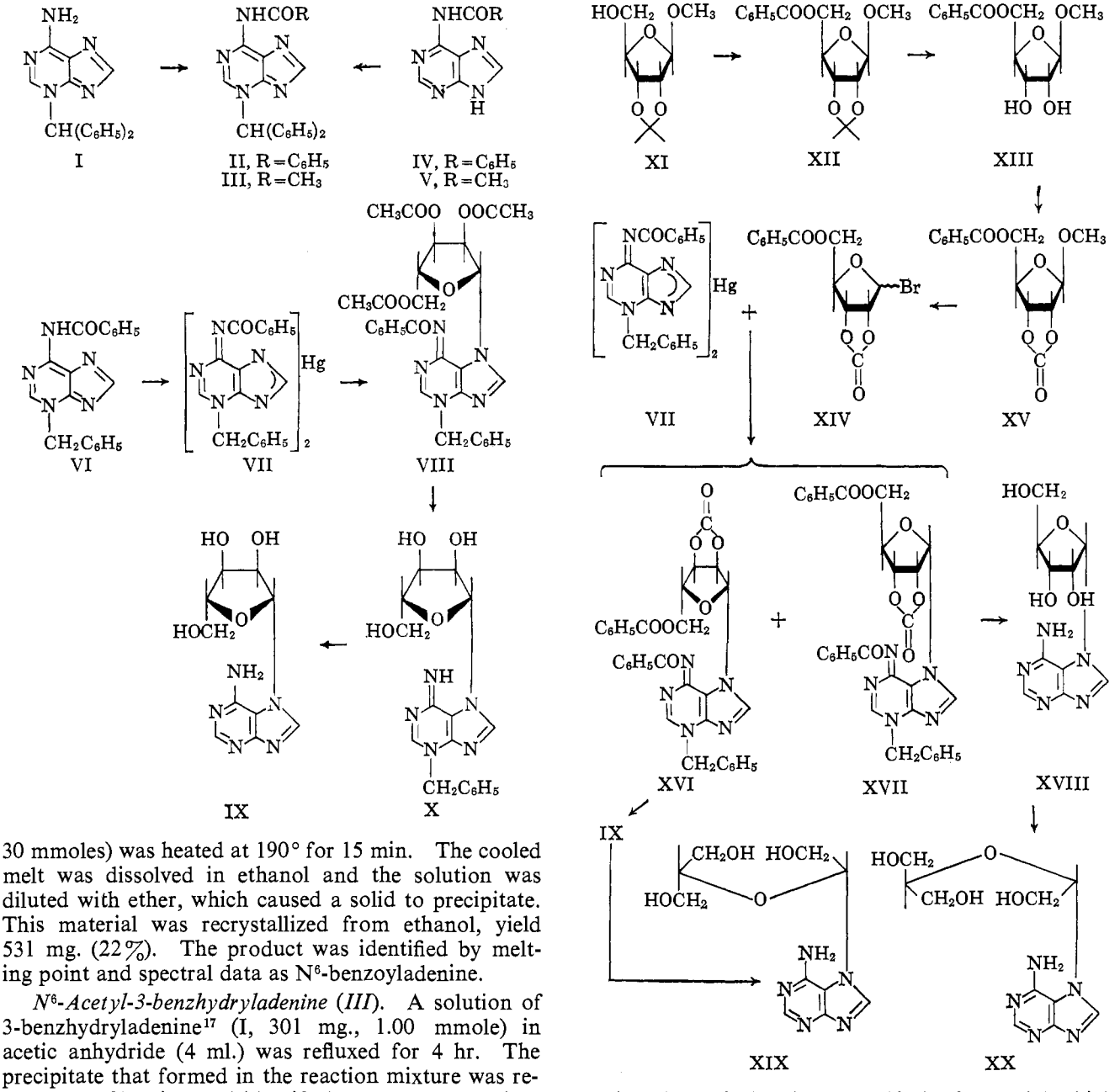
[a] Detected by chromatography. [b] Kindly supplied by Dr. H. G. Khorana (see ref. 25).

This in itself is not too surprising, but in contrast, the α-anomer of adenosine is at least as stable as adenosine. Both of the 7-anomers are much less stable than the 9-anomers; 3β-D-ribofuranosyladenine is also relatively unstable.[28]

Experimental Section

Unless stated otherwise the melting points reported were determined on a Kofler Heizbank and are corrected. The ultraviolet spectra were determined in aqueous solution with a Cary Model 14 spectrophotometer. The infrared spectra were determined in potassium bromide disks with a Perkin-Elmer Model 221-G spectrophotometer. The proton magnetic resonance spectra were determined on 10% (w./v.) solutions in DMSO-d_6 with a Varian A-60 spectrometer. The optical rotations were determined in water with a Rudolph polarimeter, Model 80.

The Reaction of 3-Benzhydryladenine (I) with Benzoic Anhydride. A mixture of 3-benzhydryladenine[17] (I, 3.01 g., 10 mmoles) and benzoic anhydride (6.78 g.,



30 mmoles) was heated at 190° for 15 min. The cooled melt was dissolved in ethanol and the solution was diluted with ether, which caused a solid to precipitate. This material was recrystallized from ethanol, yield 531 mg. (22%). The product was identified by melting point and spectral data as N^6-benzoyladenine.

N^6-Acetyl-3-benzhydryladenine (III). A solution of 3-benzhydryladenine[17] (I, 301 mg., 1.00 mmole) in acetic anhydride (4 ml.) was refluxed for 4 hr. The precipitate that formed in the reaction mixture was removed by filtration and identified as N^6-acetyladenine. The filtrate was evaporated to dryness *in vacuo* and the residue was crystallized from acetone, yield 54 mg. Recrystallization of this material from methanol gave the analytical sample: 18 mg. (5%); λ_{max} at pH 1, 282 mμ ($\epsilon \times 10^{-3}$ 16.6), at pH 7, 274 (16.7), at pH 13, 267 and 292 (10.9 and 10.0); $\bar{\nu}$ 3245, 3190, 3130, 3030 (NH, CH), 1700 (ester C=O), 1605, 1580 (C=C, C=N), and 1450 (CH) cm.$^{-1}$.

Anal. Calcd. for $C_{20}H_{17}N_5O$: C, 69.92; H, 4.99; N, 20.40. Found: C, 69.84; H, 4.94; N, 20.39.

3-(Benzyloxymethyl)adenine. To a suspension of adenine (500 mg., 3.7 mmoles) in 50 ml. of N,N-dimethylacetamide was added benzyl chloromethyl ether (0.54 ml., 3.7 mmoles). The suspension was stirred overnight with the addition of another 0.1 ml. of benzyl chloromethyl ether before adding 0.7 ml. (5.0 mmoles) of triethylamine. The precipitate of triethylamine hydrochloride was removed by filtration and the solution was evaporated to dryness *in vacuo*. The residue was triturated several times with methanol. From the methanol was isolated 60 mg. (6%) of material which was recrystallized from ethanol–water to give the analytical sample: λ_{max} at pH 1, 276 mμ ($\epsilon \times 10^{-3}$ 15.1), at pH 7, 276 (11.1), at pH 13, 276 (11.0); $\bar{\nu}$ 3360, 3295 (NH), 3150–2800 (CH, acidic H), 1665 (NH), 1615, 1570, 1515 (C=C, C=N), and 1450 (CH) cm.$^{-1}$.

Anal. Calcd. for $C_{13}H_{13}N_5O$: C, 61.17; H, 5.13; N, 27.44. Found: C, 61.32; H, 5.25; N, 27.28.

Bis[N^6-benzoyl-3-benzyladen-7(or 9)-yl]mercury (VII). To a hot solution of 3-benzyl-6-benzamidopurine[17] (VI, 10.0 g., 30.0 mmoles) in 200 ml. of ethanol was added 4.07 g. (15.0 mmoles) of mercuric chloride. A gummy precipitate immediately formed, and the solution was decanted from it. The solution was then stirred vigorously and treated dropwise with 29 ml. of 1.006 *N* sodium hydroxide. The white precipitate that formed was collected by filtration and washed with water until free of chloride ions, then with ethanol, and finally with ether. It was purified by treatment with 1400 ml. of boiling N,N-dimethylformamide, yield 4.66 g. (36%), m.p. 278°. This material

was dried at 78° (0.07 mm.) over phosphorus pentoxide for 16 hr. for analysis.

Anal. Calcd. for $C_{38}H_{28}HgN_{10}O_2$: C, 53.23; H, 3.29; N, 16.34. Found: C, 53.37; H, 3.96; N, 16.40.

7β-D-Ribofuranosyladenine (IX). A solution of 1.87 g. (5.24 mmoles) of 3-benzyl-7β-D-ribofuranosyladenine (X) in 100 ml. of ethanol and 150 ml. of water containing a suspension of 890 mg. of 5% palladium-on-charcoal catalyst was hydrogenated in the Parr shaker at 80° and 47 p.s.i. for 12 hr. The catalyst was filtered off and washed thoroughly with water. The filtrate and washings were combined and evaporated to dryness *in vacuo*. The residue was dissolved in 15 ml. of water; the solution was treated with Norite and filtered. The filtrate, upon cooling, deposited a gel which upon longer cooling crystallized, yield 430 mg. (31%), m.p. 245°.

The analytical sample was obtained by recrystallization from water. It was dried at 100° (0.07 mm.) over phosphorus pentoxide for 16 hr.: m.p. 246°; $[\alpha]^{25}D$ −84.9 ± 0.2° (0.351 g./100 ml. of water); τ (p.p.m.) 6.27 (H-5′), 5.85 m (H-2′, H-3′, H-4′), 4.67 and 4.37 (OH), 4.11 (H-1′), 3.01 (NH_2), 1.79 (H-8), and 1.45 (H-2).

Anal. Calcd. for $C_{10}H_{13}N_4O_4$: C, 44.98; H, 4.93; N, 26.23. Found: C, 45.33; H, 5.02; N, 26.32.

3-Benzyl-7β-D-ribofuranosyladenine (X). A solution of 2,3,5-tri-O-acetyl-D-ribofuranosyl chloride, obtained from an ethereal hydrogen chloride solution of 4.00 g. (12.5 mmoles) of 1,2,3,5-tetra-O-acetyl-D-ribofuranose, in 50 ml. of dry xylene was added to an azeotropically dried suspension of 5.36 g. (6.25 mmoles) of bis[N⁶-benzoyl-3-benzyladen-7(or 9-)-yl]mercury (VII) and 5.5 g. of Celite in 200 ml. of xylene. The suspension was refluxed with vigorous stirring for 1 hr. The mixture was then filtered, the xylene filtrate was evaporated to dryness *in vacuo*, and the residue was dissolved in 100 ml. of chloroform. The original xylene-insoluble material was extracted with boiling chloroform (three 100-ml. portions). The combined chloroform solutions were washed with 30% potassium iodide (two 200-ml. portions), then water (200 ml.), dried over magnesium sulfate, and evaporated to dryness *in vacuo*.

The residue was dissolved in 150 ml. of absolute methanol, and 16 ml. of 1 *N* sodium methoxide solution was added. After 30 min. of reflux, the solution was chilled in an ice bath and poured into 100 ml. of cold water, and enough Amberlite IR-120 (H) ion-exchange resin was added to neutralize the solution to pH 7. The resin was filtered off and washed with both methanol and water. The filtrate and washings were combined and evaporated to dryness *in vacuo*. The residue was dissolved in 50 ml. of ethanol; the solution was treated with Norit, then filtered, and evaporated to dryness. A light brown glass was obtained: yield 2.27 g. (51%); R_f (water-saturated butanol), 0.31; λ_{max} at pH 1, 278 mμ ($\epsilon \times 10^{-3}$ 14.3), at pH 7, 277 (13.3), at pH 13, 281 (11.5).

Methyl 5-O-Benzoyl-2,3-O-isopropylidene-β-D-ribofuranoside (XII).[35] To a solution of methyl 2,3-O-isopropylidene-β-D-ribofuranoside[26] (XI, 20.0 g., 98 mmoles) in 48 ml. of dry pyridine was added dropwise 48 ml. of benzoyl chloride. The mixture was stirred overnight and then heated to 50° for 1 hr. Ice was added to the chilled solution, which was then extracted twice with 100 ml. of chloroform. The chloroform extract was washed twice with 100 ml. of saturated sodium bicarbonate solution, once with water, and then dried over anhydrous magnesium sulfate. The chloroform was removed *in vacuo* to give a red syrup (59.2 g.). This material, which was found to be chromatographically homogenous in two solvent systems, was used in the next step without further purification.

Methyl 5-O-Benzoyl-β-D-ribofuranoside (XIII). A solution of the methyl 5-O-benzoyl-2,3-O-isopropylidene-β-D-ribofuranoside (XII) in 272 ml. of methanol containing 40 ml. of 1 *N* sulfuric acid was refluxed for 4 hr. The solution was then cooled and neutralized with solid sodium bicarbonate. The thick syrup resulting from concentration of the solution *in vacuo* was triturated with ether (3 l.) and the ether solution was dried over anhydrous magnesium sulfate. The ether was removed, the semisolid residue was dissolved in 500 ml. of ether, and the solution was diluted with 400 ml. of petroleum ether (b.p. 30–60°). The crystals that formed were removed by filtration and dried *in vacuo* over phosphorus pentoxide: yield 9.5 g.; m.p. 103–105° (Mel-Temp.); $\bar{\nu}$ 3500 and 3380 (OH), 3010, 2945, 2920 (CH), 1712 (ester C=O), 1600, 1580 (phenyl), 1450 (CH), 1275, 1125, and 1050 (COC) cm.$^{-1}$.

Anal. Calcd. for $C_{13}H_{16}O_6$: C, 58.20; H, 6.02. Found: C, 58.19; H, 6.05.

Methyl 5-O-Benzoyl-β-D-ribofuranoside 2,3-Cyclic Carbonate (XV). To a solution of methyl 5-O-benzoyl-β-D-ribofuranoside (XIII, 9.5 g., 35.5 mmoles) in 142 ml. of dry pyridine was added dropwise a solution of phosgene (17.2 g., 73.4 mmoles) in 72 ml. of dry toluene. The mixture was stirred for 1 hr. at 30° before pouring it into 850 ml. of ice water. The aqueous mixture was extracted five times with 475 ml. of ether, and the combined ether extracts were dried over anhydrous magnesium sulfate before removal of the ether *in vacuo*. The residue was dissolved in 25 ml. of benzene and the solution was diluted with petroleum ether (b.p. 30–60°). The material that crystallized weighed 8.9 g., m.p. 84–86° (Mel-Temp.). Recrystallization from methanol (50 ml.) raised the melting point to 86–88° (lit.[25] m.p. 88°): yield 7.7 g. (74%); $\bar{\nu}$ 3080, 3010, 2950, 2830 (CH), 1810 (cyclic carbonate), 1730 (ester), 1605, 1595 (phenyl), 1450 (CH), 1280, 1180, and 1100 (COC) cm.$^{-1}$. This infrared spectrum was identical with that of XV prepared by the method of Khorana.[25]

7α- and 7β-D-Ribofuranosyladenine (XVIII and IX). To a vigorously stirred, refluxing suspension of bis[N⁶-benzoyl-3-benzyladen-7(or 9)-yl]mercury (VII, 7.30 g., 8.50 mmoles) and Celite (7.30 g.) in 300 ml. of xylene was added 5-O-benzoyl-D-ribofuranosylbromide 2,3-cyclic carbonate (5.0 g., 17.0 mmoles) in 75 ml. of xylene, and the reaction mixture was refluxed for 1 hr. before it was filtered and then evaporated to dryness *in vacuo*. The xylene-insoluble material was extracted with chloroform and this extract was added to a chloroform solution of the residue that resulted from evaporation of the xylene solution. The chloroform solution was ex-

(35) The anomeric mixture of XII has been reported.[36]

(36) G. W. Kenner, B. Lythgoe, and A. R. Todd, *J. Chem. Soc.*, 957 (1948).

Table V

Fraction	Volume, ml.	Compd.	Amount, mg.
A	260	None	
B	168	Adenine	264
C	48	Adenine + IX	
D	40	IX	7
E	68	IX + XVIII	
F	112	XVIII	100
G	160	Impure XVIII	

tracted twice with 30% potassium iodide solution (400 ml.), then with water (1600 ml.), dried over anhydrous magnesium sulfate, treated with Norite, filtered, and evaporated to dryness *in vacuo*. The resulting dark brown syrup was dissolved in methanol (60 ml.) containing 1 *N* methanolic sodium methoxide (4 ml.), and the solution was refluxed for 0.5 hr. Water (20 ml.) was added to the cold solution, which was then treated batchwise with Rexyn RG50 resin (pyridinium form) until neutral. The neutral solution plus the water–methanol wash of the resin was evaporated to dryness and the residue was extracted three times with boiling water (100-ml. portions). The cold, aqueous solution was extracted several times with ether and the residual ether was removed by aspiration. The aqueous solution was then added to a suspension of 5% palladium on charcoal (2.8 g.) in 20 ml. of water. Hydrogenation was carried out at 50 p.s.i. and 80° for about 16 hr. The catalyst was removed by filtration before the solution was evaporated to dryness *in vacuo*. The residue was transferred to the top of a Whatman cellulose column (4 × 45 cm.) with 140 ml. of water-saturated butanol and the fractions obtained are reported in Table V.

Fraction F was evaporated to dryness *in vacuo* and, since the residue could not be induced to crystallize, it was dissolved in water and a saturated solution of picric acid added. The crystalline picrate that formed was removed by filtration and dissolved in water. Treatment of this solution with Dowex 1 (CO_3^{2-} form) regenerated the free nucleoside (XVIII), which crystallized on concentration of the aqueous solution: yield 21 mg.; m.p. 220–222°[37]; $[\alpha]^{25}D$ 0° (0.397 g./100 ml. of H_2O); τ (p.p.m.), 6.38 m (H-5′), 5.82 m (H-2′, H-3′, H-4′), 5.08 t (O-5′H), 4.78 and 4.38 (O-2′H and O-3′H), 3.66 (H-1′), 3.17 (NH_2), 1.81 (H-8), and 1.59 (H-2).

Anal. Calcd. for $C_{10}H_{13}N_4O_4$: C, 44.98; H, 4.93; N, 26.23. Found: C, 45.06; H, 5.13; N, 26.62.

A second reaction using 21.9 g. of VII gave a mixture that was chromatographed on a longer cellulose column (4 × 90 cm.), which gave a clean separation of the isomers. From this reaction there was obtained 236 mg. of the β-anomer (IX), 400 mg. of the α-anomer (XVIII), and 2.00 g. of adenine. These amounts indicate a 8% conversion of VII to the β-anomer and 14% conversion to the α-anomer.

Following the procedure of Khorana[25] both the α- and β-anomers were oxidized in aqueous solution with metaperiodate and the resulting dialdehydes reduced, without isolation, with sodium borohydride. The rotation ($[\alpha]^{21}D$) of the product from the α-anomer (XVIII) was −117.9 ± 0.2° (0.4330 g./100 ml. of original sample) and that of the product from the β-anomer (IX) was +118.1 ± 0.2° (0.4197 g./100 ml. of original sample).

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(37) The reported melting point of the nucleoside from pseudovitamin B_{12} is 218–222°.[5]

Thermal Methyl Transfer. The Mass Spectrum of Voacamine-d_3[1]

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The thermally induced intermolecular methyl transfer which can lead to the appearance of higher homologs in the mass spectrum of voacamine (I) and related compounds has been investigated using voacamine-d_3 (IV). The mass spectrum of IV demonstrates that the methyl group of the voacangine carbomethoxy group is transferred to the basic nitrogen of the vobasinol moiety. The structural requirements for this reaction, which could lead to a misassignment of a molecular weight, are shown to be the simultaneous presence of a methylating group (such as carbomethoxy) and an alkylatable group (such as a basic nitrogen) in a molecule of very low volatility. A method was developed for the preparation of trideuteriomethyl esters with high deuterium content.

Mass spectrometry is generally considered the best method for the determination of molecular weights because if the molecular ion is sufficiently stable, it will be detected as the peak of highest mass in the spectrum (disregarding isotope peaks). However, two situations are known in which the peak of highest mass does not correspond to the molecular weight. When the molecular ion lacks sufficient stability to give rise to a detectable peak, the ion of highest mass will originate by

(1) Part XXXIII of the series "Application of Mass Spectrometry to Structure Problems." For part XXXII see H. Achenbach and K. Biemann, *J. Am. Chem. Soc.*, **87**, 4944 (1965).