(103 mg), and pyrrolidine (110 mg) was heated under reflux in a Soxhlet extraction apparatus containing molecular sieves (5A) for 2 days. The cooled reaction mixture was stirred for 20 min in 5% sulfuric acid, after which the layers were separated and the benzene layer was extracted twice with 20-ml portions of 5% sulfuric acid. The sulfuric acid solution was basified and extracted with methylene chloride. The methylene chloride was evaporated and the residue was subjected to preparative tlc (silica gel, 10% methanol–chloroform) to yield 28 mg (19%) of ketone 11 identical in all respects with the material obtained above. The other materials from preparative tlc showed no maxima at 1745 cm^{-1} in their infrared spectra.

Deuterium Exchange of Ketone 11.—A solution of the tetracyclic ketone 11 (12.0 mg, 0.045 mmol) in 1:1 deuterium oxide–dioxane (0.5 ml) containing a small amount of anhydrous potassium carbonate was heated at 80° (oil bath) for 5 hr. Additional deuterium oxide was added (0.25 ml) and the solution was allowed to stand at room temperature for 24 hr. The solution was extracted with dry methylene chloride and the organic phase was washed with a small volume of deuterium oxide. The organic solution was concentrated under reduced pressure and the residue was dried under high vacuum for 15 hr to give the deuterated ketone (11.6 mg, 95%) as a colorless, crystalline solid. The pmr spectrum showed a disappearance of the AB quartet assigned to the methylene protons of C-1 in the protio compound. The mass spectrum showed *m/e* 289, 244, and 228.

11,12-Methylenedioxy-2,3,3a,4,5,6,8,9-octahydro-1*H*-benzo[*a*]cyclopenta[*i*]quinolizine.—A solution of ketone 11 (18 mg), 1,3-propanedithiol (180 mg), and *p*-toluenesulfonic acid hydrate (25 mg) in benzene (15 ml) was placed in a Soxhlet extractor containing molecular sieve (5A) and heated under reflux for 4.5 hr. The reaction mixture was extracted with 5% sulfuric acid and the aqueous extracts were basified and extracted with methylene chloride to yield the crude thioketal, which showed no carbonyl absorption in its infrared spectrum. The crude thioketal was dissolved in 10 ml of 95% ethanol and heated under reflux overnight with *ca.* 100 mg of Raney nickel. The reaction mixture was filtered, concentrated, and subjected to preparative tlc (5% methanol–chloroform on silica gel) to afford 10 mg of the title compound. The mass spectrum of this material was identical with that of an authentic sample[8] obtained from the hydrochloride in the usual manner. The picrates[8] of the two samples were identical by melting point behavior and their pmr spectra were identical.

Registry No.—**1**, 35667-11-9; **2**, 35667-12-0; **3**, 35667-13-1; **4**, 35667-14-2; **4** (HCl), 35667-15-3; **5**, 35667-16-4; **6**, 35667-17-5; **7**, 35667-18-6; **7** (HCl), 35667-19-7; **10**, 35667-20-0; **11**, 35667-21-1.

The Direct Utilization of Unsaturated Sugars in Nucleoside Syntheses. The Synthesis, Configuration, and Conformation of Certain Hex-1-enitol-3-yl-, Hex-2-enopyranosyl-, and Hexopyranosylpurines. The Preparation of 9-(1,5-Anhydro-2,3-dideoxy-D-*arabino*-hex-1-enitol-3-yl)adenine and 9-(2,3-Dideoxy-β-D-*erythro*-hex-2-enopyranosyl)adenine from D-Glucal[1]

ELDON E. LEUTZINGER,[2] TAKASHI MEGURO, AND LEROY B. TOWNSEND

Department of Chemistry and Department of Biopharmaceutical Sciences, University of Utah, Salt Lake City, Utah 84112

DENNIS A. SHUMAN,* MARTIN P. SCHWEIZER, CHARLES M. STEWART, AND ROLAND K. ROBINS

ICN Nucleic Acid Research Institute, Irvine, California 92664

Received December 21, 1971

The acid-catalyzed fusion of 3,4,6-tri-*O*-acetyl-D-glucal (I) and 2-acetamido-6-chloropurine has furnished the α and β anomers of 2-acetamido-6-chloro-9-(4,6-di-*O*-acetyl-2,3-dideoxy-D-*erythro*-hex-2-enopyranosyl)-9*H*-purine (III) and 2-acetamido-6-chloro-9-(1,5-anhydro-2,3-dideoxy-D-*arabino*-hex-1-enitol-3-yl)-9*H*-purine (IX). A facile conversion of III to 2-amino-9-(2,3-dideoxy-D-*erythro*-hex-2-enopyranosyl)-9*H*-purine-6-thiol (VI) and 9-(2,3-dideoxy-D-*erythro*-hex-2-enopyranosyl)guanine (VII) was effected by the appropriate functional group transformation. Cis dihydroxylation of VII furnished the 2′,3′-dihydroxyhexopyranoside, which hydrolyzed to give D-mannose, D-allose, and guanine and firmly established the position of the endocyclic double bond of III as C-2′–C-3′. The direct fusion of I with either 6-chloro-2-methylthiopurine or 6-benzamidopurine furnished a mixture of the corresponding diastereoisomeric 9-(1,5-anhydro-2,3-dideoxy-D-*erythro*-hex-1-enitol-3-yl)-9*H*-purines and 9-(2,3-dideoxy-D-*erythro*-2-enopyranosyl)-9*H*-purines. The conformation and anomeric configuration of these nucleosides was assigned with the aid of pmr spectroscopy. 9-(2,3-Dideoxy-β-D-*erythro*-hexopyranosyl)-adenine (XXIII) and 9-(1,5-anhydro-2,3-dideoxy-D-*arabino*-hexitol-3-yl)adenine (XXII) were obtained by hydrogenation of XIX and XX, respectively. Compound XXIII has a 2′,3′-dideoxypyranosyl structure similar to that found in amicetin.

The direct utilization of glycals in the "acid-catalyzed" fusion reaction has been the subject of preliminary reports from our laboratories[3,4,5] as a new and general synthetic approach to the preparation of 2′,3′-unsaturated pyranosyl nucleosides structurally related to Blasticidin S.[6] The structural elucidation[7,8] of Blasticidin S has established this nucleoside antibiotic to be a pyranosyl derivative of cytosine possessing an endocyclic double bond in the 2,3 position of the carbohydrate moiety. Blasticidin S has been shown to inhibit several transplantable animal tumors[9] and to inhibit protein synthesis.[10]

(1) This research was supported in part by Research Contract #PH43-65-1041 with the Chemotherapy National Cancer Institutes, National Institutes of Health, Public Health Service.

(2) In partial fulfillment of the requirements for the Ph.D. Thesis, Eldon E. Leutzinger, University of Utah, 1971.

(3) W. A. Bowles and R. K. Robins, *J. Amer. Chem. Soc.*, **86**, 1252 (1964).

(4) E. E. Leutzinger, R. K. Robins, and L. B. Townsend, *Tetrahedron Lett.*, 4475 (1968).

(5) E. E. Leutzinger, R. K. Robins, and L. B. Townsend, *ibid.*, 3751 (1970).

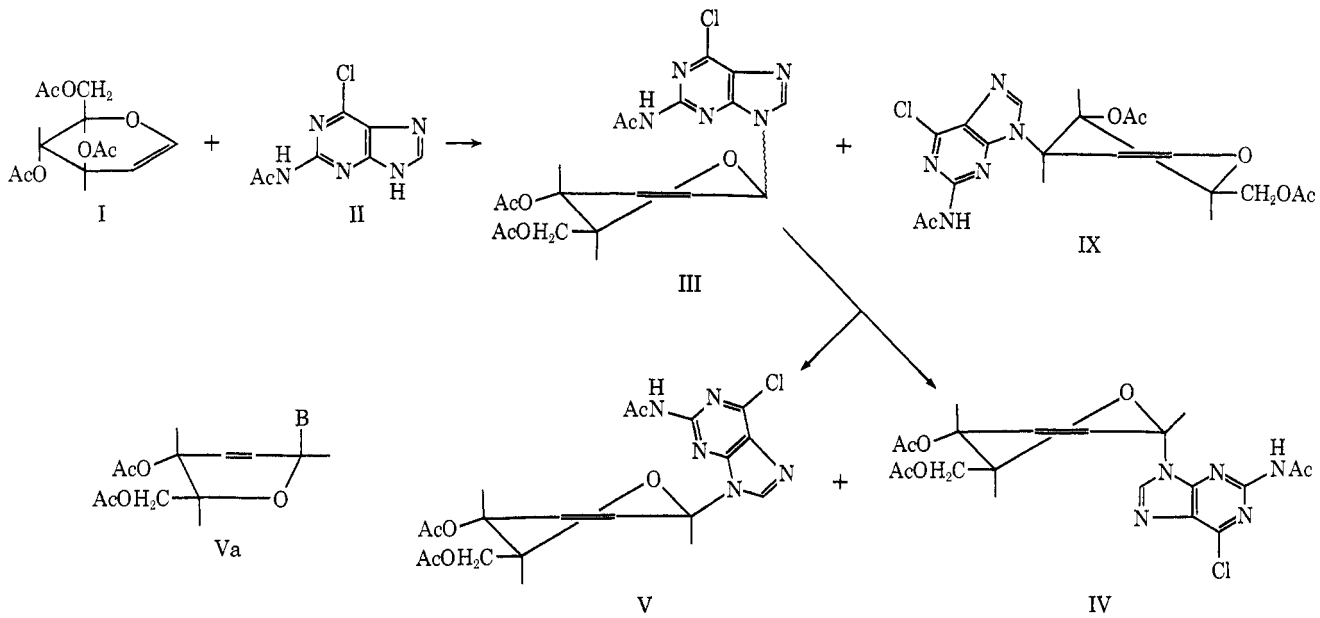
(6) S. Takeuchi, K. Hirayama, K. Ueda, H. Sakai, and H. Yonehara, *J. Antibiot. (Tokyo)*, **11A**, 1 (1958).

(7) J. J. Fox and K. A. Watanabe, *Tetrahedron Lett.*, 897 (1966); K. A. Watanabe, I. Wempen, and J. J. Fox, *Chem. Pharm. Bull.*, **18**, 2368 (1970).

(8) H. Yonehara and N. Otake, *Tetrahedron Lett.*, 3785 (1966).

(9) N. Tanaka, Y. Sakagami, H. Yamaki, and H. Umezawa, *J. Antibiot. (Tokyo)*, **14A**, 123 (1961).

(10) H. Yamaguchi and N. Tanaka, *J. Biochem.*, **60**, 632 (1966).



Another nucleoside antibiotic, amicetin,[11,12] has been shown to possess a 2,3-dideoxypyranose moiety attached directly to the heterocyclic base, cytosine. Since amicetin has likewise shown biological activity as a selective inhibitor of protein synthesis,[12] it seemed worthwhile to investigate the synthesis of similar 2′,3′-unsaturated and 2′,3′-dideoxypyranosyl nucleosides of purine bases. The use of D-glucal for syntheses of nucleosides of this type was first reported by Bowles and Robins[3] in 1964. It was discovered during the course of these studies that fusion of the requisite purine base with D-glucal gave in addition to the desired 2′,3′-unsaturated nucleoside also a 1′,2′-unsaturated pyranosyl nucleoside with purine attachment at position 3 of the pyranose ring. A preliminary report of this interesting observation has been reported from our laboratory.[5] Since our first report,[5] Ferrier, *et al.*,[13] and Kondo, *et al.*,[14] have recently noted the isolation of the 3′-deoxyglycal nucleosides of 2,6-dichloropurine and uracil. The present work describes the characterization of the various nucleoside products obtained from our studies in this area. In particular, 3,4,6-tri-*O*-acetyl-D-glucal and 6-benzamidopurine gave an excellent yield (total 76%) of the four isomeric nucleosides, XVIII, XIX, XX, and XXI. The 9-(1,5-anhydro-2,3-dideoxy-D-*arabino*- (or *ribo*-) hex-1-enitol-3-yl)-9*H*-purines represent a type of nucleoside which should be resistant to enzymatic degradation and chemical hydrolysis. It should be noted that the presence of a double bond at the 2′,3′ position such as in Blasticidin S or in 9-(2,3-dideoxy-β-D-*erythro*-hex-2-enopyranosyl)adenine (XIX) and similarly at the 1′,2′ position as in 9-(1,5-anhydro-2,3-dideoxy-D-*arabino*-hex-1-enitol-3-yl)adenine (XX), gives the pyranose ring rigidity and conformation not totally unlike the furanose ring of the naturally occurring nucleosides. The importance of this observation will be determined by further research in various biological systems.

A mixture of 3,4,6-tri-*O*-acetyl-D-glucal (I) and 2-acetamido-6-chloropurine (II) was fused in the presence of a catalytic amount of *p*-toluenesulfonic acid at 120° for 2 hr under vacuum. Preparative thick layer chromatography separated the nucleosidic mixture into two nucleoside types, III and IX. Nucleoside III was assigned the structure 2-acetamido-6-chloro-9-(4,6-di-*O*-acetyl-2,3-dideoxy-D-*erythro*-hex-2-enopyranosyl)-9*H*-purine.

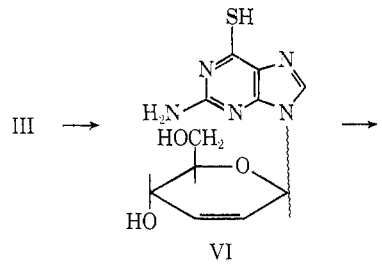
The absorption peaks in the δ 6.1–6.3 region of the pmr spectrum of III were assigned to H-2′ and H-3′ of a 2′,3′-unsaturated pyranosyl nucleoside derivative by comparison with similar absorption patterns observed previously[3,15,16] for certain 2,3-dideoxy-D-*erythro*-hex-2-enosides. The signals at δ 2.05–2.15 were assigned to the acetyl groups at C-4′ and C-6′ of the carbohydrate. The presence of only two acetyl groups for the carbohydrate moiety of III indicated that loss of one acetoxy group[17] from 3,4,6-tri-*O*-acetyl-D-glucal (I) had occurred and was accompanied by a rearrangement of the 1,2 endocyclic double bond to the 2,3 position during nucleoside formation. This type of rearrangement with glycals has also been observed in the reactions of certain phenols[16,19] with 3,4,6-tri-*O*-acetyl-D-glucal to furnish the corresponding 4,6-di-*O*-acetyl-2,3-dideoxy-D-*erythro*-hex-2-enopyranosides.

That nucleoside III was in fact an anomeric mixture was obtained from the pmr spectrum in deuteriochloroform, which showed two sets of resonances for the –NH of the 2-acetamido group, the H-8 proton and the vinyl protons. The separation and anomeric assignment is presented later.

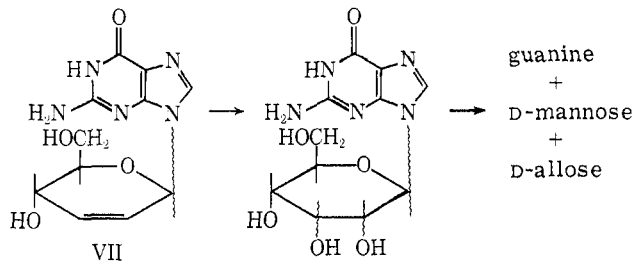
Treatment of the anomeric mixture III with a methanolic solution of sodium hydrosulfide resulted in a facile nucleophilic displacement of the 6-chloro group with a concomitant removal of the acetyl blocking groups to furnish a 41% yield of 2-amino-9-(2,3-dideoxy-D-*erythro*-hex-2-enopyranosyl)-9*H*-purine-6-thiol (VI). A comparison of the ultraviolet absorption

(11) For a review of the chemistry and biochemistry of amicetin, see R. J. Suhadolnik, "Nucleoside Antibiotics," Wiley-Interscience, New York, N. Y., 1970, p 203.
(12) A. Bloch and C. Coutsogeorgopoulos, *Biochemistry*, **5**, 3345 (1966).
(13) R. J. Ferrier and M. M. Ponipom, *J. Chem. Soc. C*, 553 (1971).
(14) T. Kondo, H. Nakai, and T. Goto, *Agr. Biol. Chem.*, **35**, 1970 (1971).
(15) D. M. Ciment and R. J. Ferrier, *J. Chem. Soc.*, 441 (1966).
(16) R. J. Ferrier, W. G. Overend, and G. H. Sankey, *ibid.*, 2830 (1965).
(17) Presumed to be the C-3′ acetoxy group.[18]
(18) See R. J. Ferrier, *ibid.*, 5443 (1964).
(19) R. J. Ferrier, W. G. Overend, and A. E. Ryan, *ibid.*, 3667 (1962).

spectrum of VI with the uv spectra of 1-methyl-,[20] 3-methyl-,[21] 7-methyl-,[22] and 9-methyl-2-amino-6-thiopurine[23] established N-9 as the site of glycosylation for VI, and consequently for III and IX.



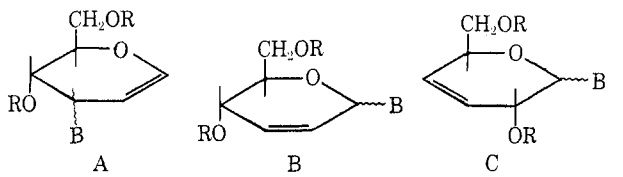
VI



VII VIII

Treatment of VI with hydrogen peroxide in a 20% aqueous ammonia solution effected a smooth conversion of the sulfur atom at position six to an oxygen atom and afforded a 78% yield of 9-(2,3-dideoxy-D-*erythro*-hex-2-enopyranosyl)guanine (VII). The pmr spectrum of VII revealed a retention of the absorption peaks assigned to the olefinic protons (H-2′ and H-3′, δ 6.0–6.35, multiplet) and established that the oxidation step had occurred without effect at the 2′,3′ endocyclic double bond.

The endocyclic double bond of the carbohydrate moiety for the above nucleosides was tentatively assigned to the 2′,3′ positions; however, this double bond could theoretically be located in one of several possible positions, 1′,2′ (A), 2′,3′ (B), and 3′,4′ (C). Structure



A B C

R = H, Ac

A was eliminated on the basis of the significant differences observed in the pattern of absorption peaks assigned to the carbohydrate moiety in the pmr spectra of I and III. Elimination of structure C was possible on the basis of the following study. Treatment of 9-(2,3-dideoxy-D-*erythro*-hex-2-enopyranosyl)guanine (VII) with 30% hydrogen peroxide containing a catalytic amount of osmium tetroxide furnished a cis glycol derivative which was subsequently assigned structure VIII. The stereospecific mode of addition for osmium tetroxide should furnish a nucleoside with the carbohydrate moiety in the manno and/or allo configuration. The hydrogen peroxide–osmium tetroxide reagent has been reported[24] to effect a facile conversion of an ethyl 2,3-dideoxy-D-*erythro*-hex-2-enopyranoside to the corresponding cis glycol. Acidic hydrolysis of VIII furnished a mixture of D-mannose, D-allose, and guanine as judged by paper chromatography against authentic samples of D-mannose, D-allose,[25] and guanine. The presence of D-mannose and D-allose in the hydrolysate of VIII indicated that VIII must be a mixture of 9-(D-*manno*-hexopyranosyl)-guanine and 9-(D-*allo*-hexopyranosyl)guanine. These results firmly established the 2′,3′ position as the site of the endocyclic double bond in VII, and consequently in VI, III, IV, and V.

The nucleoside designated as III was successfully separated into two components (IV and V) by thick layer chromatography. Pmr techniques were employed to characterize IV and V (Table I). The signals at δ 5.44 in the 100-MHz pmr spectrum for IV and at δ 5.50 in the 100-MHz pmr spectrum for V were assigned to H-4′. These assignments for H-4′ were made on the basis of the similar chemical shifts observed for the signal at δ 5.42 for III (which was established as H-4′ by decoupling from H-5′). The AB patterns for the protons in the δ 6.15–6.34 region for IV and in the δ 6.04–6.26 region for V were assigned to the C-2′ and C-3′ protons. The fine splitting seen in these patterns can be attributed to the additional coupling of the C-2′ and the C-3′ protons with H-1′ and H-4′. On the basis of these assignments, the downfield signals at δ 6.48 for IV and δ 6.60 for V were assigned to the anomeric protons.

Ferrier[13] has determined the anomeric configuration at C-1′ of the corresponding 2,6-dichloro derivatives by analysis of the various coupling constants. The magnitudes of these couplings for the two anomers were quite similar except for $J_{1'-2'}$. The results of our analysis of anomers IV and V in $CDCl_3$ are generally in accord with those of Ferrier,[13] though we consider other conformational possibilities.

The $J_{1'-2'}$ coupling for anomer IV was 2.8 Hz compared with 1.8 Hz for anomer V. Large $J_{4'-5'}$ values were found, 8.7 and 8.4 Hz, respectively, suggesting the half-chair *H1* conformation for the carbohydrate moiety. However, the alternate half-boat[26,27] conformation (Va) will also fit the $J_{4'-5'}$ data and cannot be readily eliminated, since the anomeric configuration is not known. In the discussion below regarding the adenine analogs XVIII and XIX, the conformation and the α,β anomeric configuration dilemma were solved by synthesizing the $N^3 \rightarrow$ C-6′ cyclonucleoside of the β anomer XIX. The spectra of XVIII and XIX with respect to the deblocked carbohydrate were essentially comparable to those of IV and V, and it is not expected that the conformation would change from *H1* for the adenine compounds to alternate half-boat for the 2-acetamido-6-chloro derivatives. On the basis of

(20) C. W. Noell, D. W. Smith, and R. K. Robins, *J. Med. Pharm. Chem.*, **5**, 996 (1962).
(21) L. B. Townsend and R. K. Robins, *J. Amer. Chem. Soc.*, **84**, 3008 (1962).
(22) R. N. Prasad and R. K. Robins, *ibid.*, **79**, 6401 (1957).
(23) C. W. Noell and R. K. Robins, *J. Med. Pharm. Chem.*, **5**, 558 (1962).
(24) C. L. Stevens, J. B. Fillipi, and K. G. Taylor, *J. Org. Chem.*, **31**, 1292 (1966).
(25) The authors wish to thank Dr. Bernice Kohn and Dr. Leon Lerner for a gift of D-allose.
(26) K. A. Watanabe, R. S. Goody, and J. J. Fox, *Tetrahedron*, **26**, 3883 (1970).
(27) We acknowledge one of the referees for this suggestion.

TABLE I

Compd	Chemical shifts, ppm H-8	H-2	H-1′	H-2′	H-3′	H-4′	H-5′	H-6′,6″
IV[a]	8.18		6.48	6.15	6.34	5.44	~3.94	~4.16
V[a]	8.12		6.60	6.04	6.26	5.50	~4.2	~4.24
XVIII[b]	8.61	8.61	6.82	6.44	6.67	4.45	3.91	3.91
XIX[b]	8.50	8.57	6.83	6.32	6.56	4.54	4.05	4.05
IX[c]	8.18		6.89	5.00	5.50			
XI[c]	8.50		6.63	4.80	5.20	4.28		
XII[c]	8.33		6.75	4.88	5.23	4.00		
XX[b]	8.49	8.49	6.94	5.10	5.47	4.62	4.27	4.12
XXI[b]	8.48	8.60	7.15	5.27	5.65	4.43	4.43	4.10

[a] Spectra were determined in $CDCl_3$ with TMS as internal standard at 100 MHz on a Varian XL-100 spectrometer. [b] Spectra were determined in DMSO-d_6/D_2O with TMS as external standard at 90 MHz on a Bruker HFX-90 spectrometer. [c] Spectra were determined in DMSO-d_6/D_2O with TMS as internal standard at 60 MHz on a Jeolco C60H spectrometer.

these considerations, IV and V were assigned the α and β configurations, and *H1* conformations, respectively.

The formation of 9-(1,5-anhydro-2,3-dideoxy-D-*erythro*-hex-1-enitol-3-yl)-9*H*-purines has been previously reported from the fusion of 3,4,6-tri-*O*-acetyl-D-glucal with purines.[5,13,14] The pmr spectra of the only other nucleoside IX isolated from the fusion of the D-glucal (I) with 2-acetamido-6-chloropurine (II) was similar to that of 6-chloro-9-(4,6-di-*O*-acetyl-1,5-anhydro-2,3-dideoxy-D-*arabino*-hex-1-enitol-3-yl)-2-methylthio-9*H*-purine (XIII) and revealed a pair of doublets at δ 6.89 and a pair of overlapping doublets at δ 5.00, which are characteristic of a hex-1-enitol derivative, and which were assigned to H-1′ and H-2′, respectively. The doublet at δ 5.50 in the pmr spectrum of IX was assigned to H-3′ and the large coupling constant ($J_{3'-4'}$ = 7.5 Hz) suggested that the 2-acetamido-6-chloropurinyl substituent was in approximately the same orientation as the 6-chloro-2-methylthiopurinyl substituent in XIII. Thus, based on the similarities between the pmr spectra of XIII and IX and the large $J_{3'-4'}$ observed, the nucleoside IX was tentatively assigned the structure 2-acetamido-6-chloro-9-(4,6-di-*O*-acetyl-1,5-anhydro-2,3-dideoxy-D-*arabino*-hex-1-enitol-3-yl)-9*H*-purine.

The direct isolation and characterization of a minor nucleoside from certain transfer RNAs as a 2-methylthiopurine nucleoside derivative[28,29] has created interest in the synthesis of other 2-methylthiopurine nucleosides. The fusion of 3,4,6-tri-*O*-acetyl-D-glucal and 2-methylthio-6-chloropurine as reported in a preliminary communication[5] gave the three nucleosides XI, XII, and XV after treatment with methanolic ammonia followed by fractional crystallization.

The assignment of the position of the endocyclic double bond in XI and XII was previously firmly established by a comparison of the pmr spectra of XI and XII with that of 3,4,6-tri-*O*-acetyl-D-glycal (I) (in particular, $J_{1'-2'} \cong 6$ Hz was indicative of a vinyl ether[30]) and by the utilization of the double resonance technique at 100 MHz.[5] Acetylation of XI and XII to XIII and XIV caused a significant downfield shift of the C-4′ proton which was in agreement with the assignment of purinyl substitution at C-3′.[5] The configuration at C-3′ of XI and XII has now been determined by pmr studies in analogy to those reported by Ferrier[13] for the 3′-substituted 2,6-dichloro derivatives. Large $J_{4'-5'}$ (9.0 Hz) and $J_{3'-4'}$ (8.0 Hz) couplings suggest essentially diaxial orientations for H-3′, H-4′, and H-5′, which is consistent with the *H1* conformation and pseudoequatorial orientation of the substituent at C-3′. Thus XI is 9-(1,5-anhydro-2,3-dideoxy-D-*arabino*-hex-1-enitol-3-yl)-6-chloro-2-methylthio-9*H*-purine and XII is the corresponding ribo derivative.

The other nucleosides XV showed absorption peaks in the δ 5.9–6.3 region, which was characteristic for H-2′ and H-3′ of a 2′,3′-unsaturated pyranosyl derivative. On the basis of the similarities in the pmr spectra in the region attributed to the carbohydrate moiety of XV and III, this nucleoside was assigned the structure 6-chloro-9-(2,3-dideoxy-D-*erythro*-hex-2-enopyranosyl)-2-methylthio-9*H*-purine.

Treatment of XV with methanolic ammonia in a sealed vessel at room temperature for 4 days furnished a 40% yield of 6-amino-9-(2,3-dideoxy-D-*erythro*-hex-2-enopyranosyl)-2-methylthio-9*H*-purine (XVI). The subsequent desulfurization of XVI with Raney nickel furnished 9-(2,3-dideoxy-D-*erythro*-hex-2-enopyranosyl)-adenine (XVII), mp 241–242°. A pmr spectrum of XVII revealed an absorption pattern in the δ 5.8–6.2 region attributed to H-2′ and H-3′ of a 2′,3′-unsaturated pyranosyl derivative and indicated a retention of the endocyclic double bond. The synthesis of 9-(2,3-dideoxy-D-*erythro*-hex-2-enopyranosyl)adenine, mp 241–242°, has been previously prepared by a different procedure, although no attempt was made to establish the anomeric configuration of the product.[31] In fact, this structural assignment has been recently questioned.[13]

In view of the claimed antitumor activity of the latter product,[32] a detailed investigation and synthesis of the four possible isomeric adenine nucleosides XVIII, XIX, XX, and XXI was undertaken. The fusion of 2,4,6-tri-*O*-acetyl-D-glucal (I) with 6-benzamidopurine in the presence of *p*-toluenesulfonic acid catalyst at 165° for 3 hr gave a 76% yield of nucleosidic material. Deacylation of the crude nucleoside mixture with methanolic ammonia was followed by separation of the nucleosides by column chromatography and fractional crystallization to give the isomeric nucleosides XVIII, XIX, XX, and XXI. A

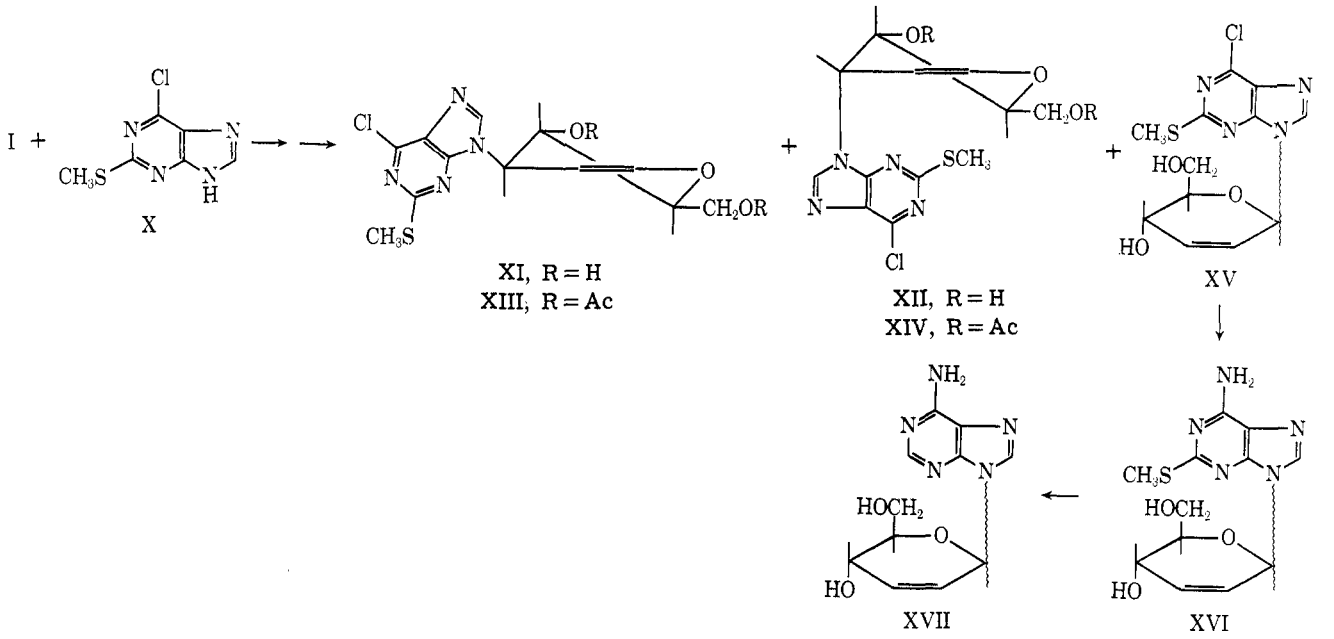
(28) S. M. Hecht, N. J. Leonard, W. J. Burrows, D. J. Armstrong, F. Skoog, and J. Occolowitz, *Science*, **166**, 1272 (1969).

(29) S. M. Hecht, L. H. Kirkegaard, and R. M. Bock, *Proc. Nat. Acad. Sci. U. S.*, **68**, 48 (1971).

(30) R. J. Ferrier, *Advan. Carbohyd. Chem.*, **24**, 199 (1969).

(31) J. J. K. Novak and F. Sorm, *Collect. Czech. Chem. Commun.*, **27**, 902 (1962).

(32) J. J. K. Novak and F. Sorm, *Experientia*, **17**, 213 (1962).



comparison of the ultraviolet absorption spectra of these nucleosides with the ultraviolet spectra of the possible *N*-methyladenines[22,33,34] established N-9 as the site of glycosylation.

The α and β anomers of 9-(2,3-dideoxy-D-*erythro*-hex-2-enopyranosyl)adenine were identified readily due to the presence of the typical AB spin patterns for H-2′ and H-3′, with ~10 Hz coupling. Large $J_{4'-5'}$ couplings of 8–9 Hz for each isomer suggested diaxial arrangements of H-4′ and H-5′, consistent not only with *H1*, but also with the alternate half-boat.[26] A large $J_{1'-2}$ of 2.9 Hz was observed for XVIII, whereas for XIX the coupling was about 1.5 Hz.[35] Examination of models revealed that an α anomer in the *H1* conformation and a β anomer in the alternate half-boat (Va) were both consistent with diaxial H-4′ and H-5′ and equatorial-vinylic H-1′ and H-2′, the latter arrangement leading to an expected vicinal $J_{1'-2'}$ of about 3 Hz (in an axial-vinylic case the $J_{1'-2'}$ would be about 1.5 Hz).[30]

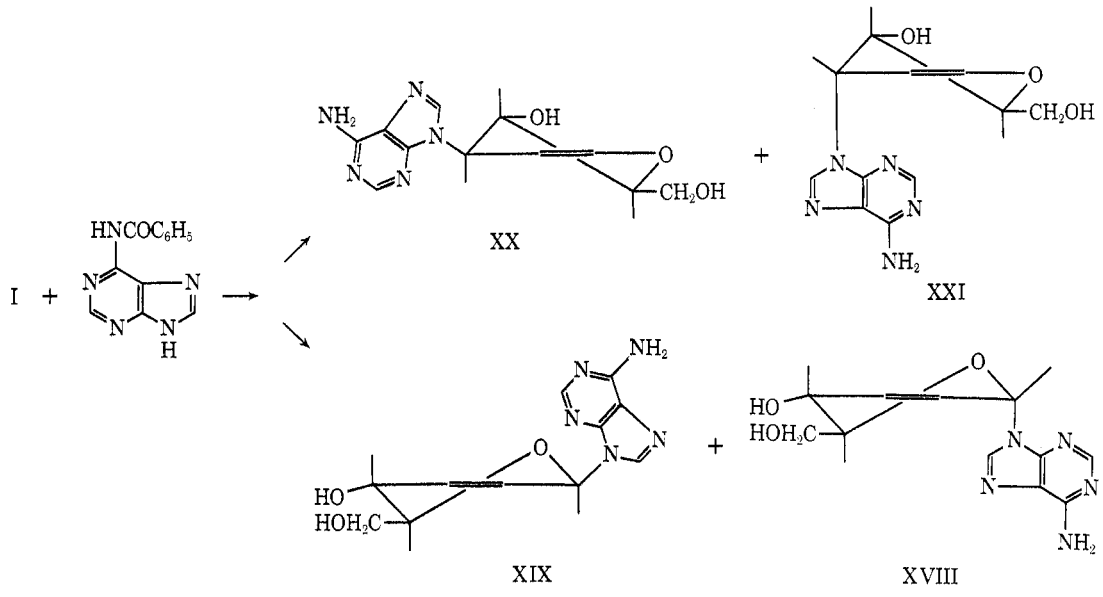
At this point it was not possible to proceed with an assignment based upon pmr, since both conformation and anomeric configuration were unknown. Thus, a chemical assignment was attempted. Further study of Drieding's models showed that in the case of the β anomer it might be possible to form a $N^3 \rightarrow$ C-6′ cyclonucleoside; however, $N^3 \rightarrow$ C-6′-cyclonucleoside formation from the α anomer would be difficult, if not impossible, no matter what the carbohydrate conformation because of distance considerations. Accordingly, the 6′-*O*-tosyl derivatives of both XVIII and XIX were synthesized by treatment with *p*-toluenesulfonyl chloride in pyridine–chloroform. Selective tosylation at the 6′ position was shown in the pmr spectra in DMSO-d_6 by the absence of the 6′-OH (triplet signal δ 5.09 ppm from TMS-capillary). Upon heating, the tosylated XIX reacted to form the $N^3 \rightarrow$ C-6′ cyclonucleoside as determined by thin layer chromatography[36] and uv[37] ($\lambda_{max}^{pH\,1}$ 271 nm) which established XIX as the β anomer. The conformation could then be assigned as *H1*, since the β anomer XIX exhibited a small $J_{1'-2'}$ and XVIII, the α anomer, exhibited a 2.9 Hz coupling. It is of interest to note that this assignment of C-1 configuration is consistent with other reported pmr data on anomeric pairs of 2′,3′-unsaturated glycosides and nucleosides where the H-1′ signal for the α anomer resonates at higher field than the β anomer.[13,27,38–40]

Compound XX was assigned the structure 9-(1,5-anhydro-2,3-dideoxy-D-*arabino*-hex-1-enitol-3-yl)adenine and compound XXI the structure 9-(1,5-anhydro-2,3-dideoxy-D-*ribo*-hex-1-enitol-3-yl)adenine based on the similarities in the pmr spectra of these compounds with 9-(1,5-anhydro-2,3-dideoxy-D-*arabino*-hex-1-enitol-3-yl)-6-chloro-2-methylthio-9*H*-purine (XI) and 9-(1,5-anhydro-2,3-dideoxy-D-*ribo*-hex-1-enitol-3-yl)-6-chloro-2-methylthio-9*H*-purine (XII), respectively.

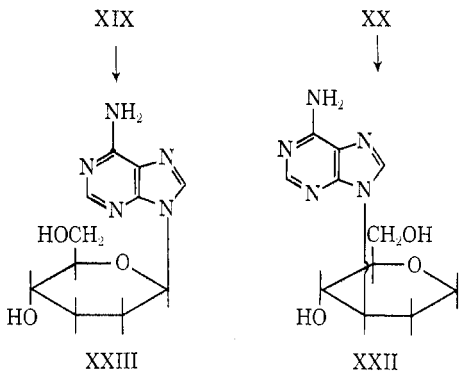
Chemical shift data for the various nucleosides are presented in Table I. In the case of XIX and XXI, the H-8 proton was assigned by incorporation of deuterium at C-8.[41]

Reduction of the endocyclic double bond of 9-(2,3-dideoxy-β-D-*erythro*-hex-2-enopyranosyl)adenine (XIX) with hydrogen in the presence of palladium/charcoal catalyst gave 9-(2,3-dideoxy-β-D-*erythro*-hexopyranosyl)-adenine (XXIII) in a 60% yield. The synthesis of compound XXIII is of interest since it represents a route to 2′,3′-dideoxypyranosylpurines related to the nucleoside antibiotic, amicetin.[11,12] Hydrogenation of 9-(1,5-anhydro-2,3-dideoxy-D-*arabino*-hex-1-enitol-3-yl)adenine (XX) and 9-(2,3-dideoxy-α-D-

(33) R. K. Robins and H. H. Lin, *J. Amer. Chem. Soc.*, **79**, 490 (1957).
(34) L. B. Townsend, R. K. Robins, R. N. Loeppky, and N. J. Leonard, *ibid.*, **86**, 5320 (1964).
(35) Because of solubility considerations, DMSO-d_6 was used as solvent. Somewhat poorer resolution is observed in this solvent than is seen in $CDCl_3$ for the acyl derivatives.
(36) D. A. Shuman, R. K. Robins, and M. J. Robins, *J. Amer. Chem. Soc.*, **91**, 3391 (1969).
(37) U. M. Clark, A. R. Todd, and J. Zussman, *J. Chem. Soc.*, 2952 (1951).
(38) R. U. Lemieux, E. Fraga, and K. A. Watanabe, *Can. J. Chem.*, **46**, 61 (1968).
(39) R. J. Ferrier and M. M. Ponpipom, *J. Chem. Soc. C*, 560 (1971).
(40) M. Fuertes, G. Garcia-Muñoz, R. Madroñero, and M. Stud, *Tetrahedron*, **26**, 4823 (1970).
(41) M. P. Schweizer, S. I. Chan, P. O. P. Ts'o, and G. K. Kelmkamp, *J. Amer. Chem. Soc.*, **86**, 696 (1964).



erythro-hex-2-enopyranosyl)adenine (XVIII) gave 9-(1,5-anhydro-2,3-dideoxy-D-*arabino*-hexitol-3-yl)adenine (XXII) and 9-(2,3-dideoxy-α-D-*erythro*-hexopyranosyl)adenine (XXIV), respectively. The structure of the hydrogenated compounds XXIII, XXII, and XXIV was confirmed by ultraviolet and pmr spectra, elemental analysis and by a negative color test for carbohydrate unsaturation with Hanes–Isherwood reagent.[42]



Experimental Section

Pmr spectra at 60 MHz were obtained on a C6OH Jeolco instrument using TMS as an internal standard. A Varian HA-100 and a Bruker HFX were used to obtain pmr spectra at 100 and 90 MHz, respectively. Chemical shifts were measured to within an accuracy of ±0.01 ppm. Coupling constants were measured to within an accuracy of ±0.1 Hz. Ultraviolet spectra were obtained on a DK-2 absorption spectrometer. Melting points were observed on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were performed by Heterocyclic Chemical Corp., Harrisonville, Mo.

2-Acetamido-6-chloropurine.—Chlorine gas was passed into 150 ml of cold (−5°) absolute ethanol for 5 min. The flow of chlorine was decreased to a moderate rate and to this cold solution was added 2-acetamido-6-benzylthiopurine[43] (6.0 g, 21 mmol) over a period of 1 hr. During this addition, the 2-acetamido-6-benzylthiopurine slowly dissolved and a white solid gradually separated from solution. The flow of chlorine was continued for an additional 10 min, then discontinued, and the mixture was allowed to stand at −5° with stirring for 1 hr. The solid was then collected by filtration, washed with cold absolute ethanol (200 ml), and slurried in 600 ml of anhydrous ether and the solid was collected by filtration. The solid was washed with an additional 600 ml of anhydrous ether and air dried to furnish 3.5 g of 2-acetamido-6-chloropurine. Recrystallization from a dimethylacetamide–water mixture (1:10, v/v) gave 2.0 g (47%) of pure 2-acetamido-6-chloropurine (II), mp >300° dec, which was found to be identical in all respects with an authentic sample of 2-acetamido-6-chloropurine:[44] uv $\lambda_{max}^{pH\ 1}$ 249 nm (ε 11,000), 285 (10,600); $\lambda_{max}^{pH\ 11}$ 236 nm (ε 26,200), 284 (9930); $\lambda_{max}^{H_2O}$ 253.5 nm (ε 10,150), 284.5 (10,800).

2-Acetamido-6-chloro-9-(4,6-di-*O*-acetyl-2,3-dideoxy-D-*erythro*-hex-2-enopyranosyl)-9*H*-purine (III).—A finely powdered mixture of 2-acetamido-6-chloropurine (II) (0.8 g, 4 mmol) and 3,4,6-tri-*O*-acetyl-D-glucal (I) (3.2 g, 0.012 mol) was heated to an inside temperature of 120° in an oil bath. To this hot mixture was added 50 mg of *p*-toluenesulfonic acid with thorough stirring, and heating was then continued at an inside temperature of 140° under aspirator vacuum for 2 hr. The dark melt was dissolved in warm ethyl acetate (300 ml) and the insoluble material was removed by filtration. The filtrate was cooled to 0° and washed with cold saturated sodium bicarbonate solution (3 × 75 ml) and cold water (3 × 75 ml), and the solution was then dried over anhydrous sodium sulfate. The sodium sulfate was removed by filtration and the filtrate was concentrated under high vacuum and at room temperature to 5 ml. This solution was applied to four preparative thick layer chromatography plates (7.75 × 15.75 in., 2 mm thickness of absorbent) of SilicAR 7GF (Mallinckrodt Chemical Co.). The plates were developed the full length (14 in., measured from the base line) with a *n*-heptane–tetrahydrofuran–acetone (7:3:1, v/v/v) solvent system and then air dried. The plates were developed in this solvent system three additional times, which resulted in the separation of two major bands [detected by a short-wave (254 nm) ultraviolet light]. The slower moving ultraviolet-absorbing band was removed and extracted with 400 ml of warm absolute ethyl alcohol. The ethyl alcohol was evaporated under high vacuum and at room temperature to afford a residue which was dissolved in 10 ml of bromoethane. This was allowed to stand at −20° for 2 days to afford 90 mg of III: mp 125–127°; uv $\lambda_{max}^{pH\ 1}$ 227 nm (ε 27,300), 257.5 (13,000), 283 (12,400); $\lambda_{max}^{pH\ 11}$ 229 nm (ε 22,900), 257 (12,000), 283.5 (11,900); λ_{max}^{EtOH} 228.5 nm (ε 27,700), 257.5 (12,000), 286.5 (11,500).

Anal. Calcd for $C_{17}H_{18}ClN_5O_6$: C, 48.17; H, 4.25; N, 16.53. Found: C, 48.03; H, 4.20; N, 16.45.

2-Amino-9-(2,3-dideoxy-D-*erythro*-hex-2-enopyranosyl)-9*H*-purine-6-thiol (VI).—Metallic sodium (4.7 g) was dissolved in 100 ml of absolute methanol, and the solution was cooled to 0° and then saturated with H_2S gas. To 40 ml of this solution (2 *N* NaSH) was added 420 mg (0.991 mmol) of 2-acetamido-6-chloro-9-(4,6-di-*O*-acetyl-2,3-dideoxy-D-*erythro*-hex-2-enopyranosyl)-9*H*-purine (III). This solution was heated at reflux temperature for 3 hr and cooled to 0°, and the pH was adjusted to 7.0 by the slow addition of glacial acetic acid. Excess H_2S and

(42) T. A. Khwaja and C. Heidelberger, *J. Med. Chem.*, **10**, 1066 (1967).

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

(44) R. H. Iwamoto, E. M. Acton, and L. Goodman, *ibid.*, **6**, 684 (1963).

solvent were removed under aspirator vacuum at room temperature. The residue was extracted with anhydrous acetone (2 × 200 ml), insoluble material was removed by filtration, and the filtrate was evaporated under high vacuum at room temperature to afford a white solid. This solid was then slurried in 10 ml of cold methyl alcohol. The solid was collected by filtration, washed with cold methyl alcohol (2 × 2 ml), and air dried to yield 120 mg (41%) of product. Recrystallization from absolute methyl alcohol gave 60 mg of an analytical sample of 2-amino-9-(2,3-dideoxy-D-*erythro*-hex-2-enopyranosyl)-9*H*-purine-6-thiol (VI): mp 187–188°; uv $\lambda_{max}^{pH\ 1}$ 345 nm (ϵ 12,000), 361.5 (4600); $\lambda_{max}^{pH\ 11}$ 251.5 nm (ϵ 7500), 318 (11,000).

Anal. Calcd for $C_{11}H_{13}N_5O_3S$: C, 44.75; H, 4.41; N, 23.73. Found: C, 44.81; H, 4.45; N, 23.64.

9-(2,3-Dideoxy-D-*erythro*-hex-2-enopyranosyl)guanine (VII).—To 80 ml of 20% ammonia solution was added 340 mg (1.20 mmol) of 2-amino-9-(2,3-dideoxy-D-*erythro*-hex-2-enopyranosyl)-9*H*-purine-6-thiol (VI). To this solution was added 4% hydrogen peroxide (12 ml) and the solution was stirred at room temperature for 15 min. The excess hydrogen peroxide was destroyed by the addition of small amounts of platinum black and then evaporated to a residue under water aspirator vacuum with a water bath at 50°. The residue was extracted with hot absolute ethyl alcohol (2 × 200 ml) and filtered through a 4-mm-thick Celite pad. The Celite pad was washed with hot absolute ethyl alcohol (50 ml) and the combined filtrates were evaporated to a residue under high vacuum at room temperature. This residue was slurried in 20 ml of acetone and the solid was collected by filtration to yield 270 mg (84%) of product. Recrystallization from 10 ml of water gave an analytical sample of 9-(2,3-dideoxy-D-*erythro*-hex-2-enopyranosyl)guanine (VII): mp >220° dec; uv $\lambda_{mxx}^{pH\ 1}$ 253 nm (ϵ 11,500), 275 (8200); $\lambda_{max}^{pH\ 11}$ 262 nm (ϵ 12,000).

Anal. Calcd for $C_{11}H_{13}N_5O_4 \cdot 1.50H_2O$: C, 43.13; H, 5.26; N, 22.86. Found: C, 42.75; H, 5.19; N, 22.58.

The pmr spectrum of VII revealed an absorption peak at δ 4.0 which integrated for three protons and was assigned to the 1.5 mol of water.

9-(D-*manno*,D-*allo*-Hexopyranosyl)guanine (VIII).—9-(2,3-Dideoxy-D-*erythro*-hex-2-enopyranosyl)guanine (VII) (50 mg, 0.16 mmol) was dissolved in a 1:1 mixture of water and *tert*-butyl alcohol (10 ml) and to this solution was added 0.5 mg (0.002 mmol) of osmium tetroxide and 1 ml of 30% hydrogen peroxide. The solution was allowed to stand at room temperature for 2 days, after which the excess hydrogen peroxide was decomposed by the addition of a small amount of platinum black. The mixture was filtered through a 4-mm-thick Celite bed, the Celite bed was washed with 20 ml of hot water, and the combined filtrates were evaporated to a residue under high vacuum at room temperature. The residue was recrystallized from water (3 ml) to furnish 6 mg of VIII: mp >160° dec; uv $\lambda_{max}^{pH\ 1}$ 253 nm (ϵ 10,000), 275 (7000); $\lambda_{max}^{pH\ 11}$ 263 nm (ϵ 10,000).

Anal. Calcd for $C_{11}H_{15}N_5O_6$: C, 42.17; H, 4.79; N, 22.36. Found: C, 42.21; H, 4.83; N, 22.50.

Hydrolysis of 9-(D-*manno*,D-*allo*-Hexopyranosyl)guanine (VIII).—9-(D-*manno*,D-*allo*-Hexopyranosyl)guanine (VIII) (50 mg, 0.16 mmol) was dissolved in 5 ml of water. To this solution was added 10 g of Amberlite IR-120 resin (H^+ form) and the mixture was allowed to stand at room temperature for 24 hr. The resin was then removed by filtration and washed with 50 ml of water at room temperature. The filtrates were combined and concentrated to 1 ml under high vacuum at room temperature. An ultraviolet absorption spectrum and a positive Fehling's test indicated that hydrolysis had occurred. The hydrolysate was applied to Whatman No. 1 chromatography paper, and the paper was developed by the descending technique with a cyclohexane–pyridine–water (40:23:19.5, v/v/v) solvent system,[45] air dried, and then sprayed with a silver nitrate spray reagent.[46] A final spray with ethanolic sodium hydroxide solution revealed two components (detected as black spots) present in the hydrolysate. These two components were identified as D-mannose and D-allose by comparison of the $R_{galactose}$ values (1.45 and 1.25, respectively) with those observed for authentic D-mannose (1.45) and D-allose (1.25).[25]

2-Acetamido-6-chloro-9-(4,6-di-*O*-acetyl-2,3-dideoxy-α- and -β-D-*erythro*-hex-2-enopyranosyl)-9*H*-purine (IV and V).—2-Acetamido-6-chloro-9-(4,6-di-*O*-acetyl-2,3-dideoxy-D-*erythro*-hex-2-enopyranosyl-9*H*-purine (III) (0.5 g) was dissolved in 10 ml of ethyl acetate and applied to three preparative layer plates (7.75 × 15.75 in., 2 mm thickness) of SilicAR 7GF (Mallinckrodt Chemical Co.). The plates were developed the full length (14 in. measured from the base line) in an isopropyl ether–*n*-propyl alcohol–acetone solvent system (8:1:1, v/v/v) and air dried. The same plates were developed two more times with the same solvent system, which resulted in the separation of two distinct ultraviolet-absorbing bands. The bands were removed and individually extracted with 500 ml of warm ethyl acetate. The slower moving component was crystallized from bromoethane to give 170 mg of pure 2-acetamido-6-chloro-9-(4,6-di-*O*-acetyl-2,3-dideoxy-β-D-*erythro*-hex-2-enopyranosyl)-9*H*-purine (V), mp 149–159°, $[\alpha]^{26}D$ +80.6° (*c* 0.5, $CHCl_3$).

Anal. Calcd for $C_{17}H_{19}ClN_5O_6$: C, 48.17; H, 4.25; N, 16.53. Found: C, 48.30; H, 4.34; N, 16.83.

The faster moving component was dissolved in 5 ml of methylene chloride and the solution was added dropwise with stirring to *n*-pentane (2 ml of CH_2Cl_2 solution per 100 ml of *n*-pentane). The solid which precipitated was collected by filtration to yield 60 mg of pure 2-acetamido-6-chloro-9-(4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*erythro*-hex-2-enopyranosyl)-9*H*-purine (IV), mp 78–80°, $[\alpha]D$ −16.4° (*c* 0.5, $CHCl_3$).

Anal. Found: C, 48.17; H, 4.41; N, 16.11.

2-Acetamido-6-chloro-9-(4,6-di-*O*-acetyl-1,5-anhydro-2,3-dideoxy-D-*arabino*-hex-1-enitol-3-yl)purine (IX).—The faster moving ultraviolet-absorbing band from the preparative plate used in the preparation and chromatography of III was extracted with 400 ml of warm ethyl alcohol. The ethyl alcohol was evaporated under high vacuum and at room temperature to furnish a syrup. This syrup was dissolved in 400 ml of hot *n*-heptane and the resulting solution was allowed to cool at room temperature for 2 days in a closed vessel. The crystalline solid which had separated was collected by filtration to yield 120 mg of an analytically pure sample of IX: mp 104–105°; uv $\lambda_{max}^{pH\ 1}$ 229 nm (ϵ 25,500), 260 (9700), 285 (11,100); $\lambda_{max}^{pH\ 11}$ 231 nm (ϵ 24,000), 260 (9700), 285 (11,100); λ_{max}^{MeOH} 230 nm (ϵ 26,100), 257 (9500), 288 (11,100).

Anal. Calcd for $C_{17}H_{18}ClN_5O_6$: C, 48.17; H, 4.25; N, 16.53. Found: C, 48.21; H, 4.40; N, 16.15.

9-(1,5-Anhydro-2,3-dideoxy-D-*arabino*-hex-1-enitol-3-yl)-6-chloro-2-methylthio-9*H*-purine (XI) and 9-(1,5-Anhydro-2,3-dideoxy-D-*ribo*-hex-1-enitol-3-yl)-6-chloro-2-methylthio-9*H*-purine (XII).—A finely powdered mixture of 6-chloro-2-methylthiopurine[47] (X) (10 g, 50 mmol) and 3,4,6-tri-*O*-acetyl-D-glucal (I) (20 g, 73 mmol) was heated to an inside temperature of 120° in an oil bath. To the hot mixture was added, with thorough mixing, 50 mg of *p*-toluenesulfonic acid and heating was continued at 120° (inside temperature) under aspirator vacuum for 2.5 hr. The dark melt was dissolved in warm ethyl acetate (900 ml), the insoluble material was removed by filtration, and the filtrate was cooled to 0°. The filtrate was washed with cold saturated sodium bicarbonate solution (3 × 200 ml) and cold water (3 × 200 ml) and then dried over anhydrous sodium sulfate. The sodium sulfate was removed by filtration and the filtrate was concentrated to 500 ml. Silica gel (J. T. Baker powder, 40 g), Celite (Johns Manville, 20 g) and 0.05% by weight of phosphor (Du Pont # 609) was added to the ethyl acetate solution and the resulting mixture was evaporated under high vacuum and at room temperature to a dry powder. This powder was placed on top of a preformed nylon dry column (1.75 × 13 in.) of Baker silica gel powder containing 0.05% by weight of phosphor. The column was eluted with 2 l. of a *n*-pentane–ethyl acetate (9:1, v/v) solvent system and the eluent was discarded. The nucleoside band near the top of the column (dark band under ultraviolet light, 254 nm) was excised and triturated with 1 l. of warm absolute ethyl alcohol, and the silica gel was removed by filtration. The filtrate was concentrated to a stiff foam under high vacuum at 60°. This foam was dissolved at room temperature in absolute methyl alcohol (250 ml) and then cooled to −20°. The cold solution was saturated with ammonia and then allowed to stand at −20° for 12 hr. Excess ammonia and solvent was removed at room temperature under aspirator vacuum to afford a syrup. The syrup was dissolved at room temperature in absolute methanol (500 ml) and the resulting solution was allowed to stand at room temperature for 2 days. The crystalline solid which had separated was collected by filtration to furnish 1.77 g of XI, mp 196–198°. Recrystallization from absolute methanol furnished 1.24 g of XI: mp 214–215°; uv $\lambda_{max}^{pH\ 1}$ 236 nm (ϵ 11,000), 260

(45) H. Ruckert, E. Pfeil, and C. Sharf, *Chem. Ber.*, **90**, 2558 (1965).

(46) L. Hough and J. K. N. Jones in "Methods in Carbohydrate Chemistry," Vol. 1, Academic Press, New York, N. Y., 1962, p 21; L. Hough, *Nature (London)*, **165**, 400 (1950).

(47) C. Wayne Noell and R. K. Robins, *J. Org. Chem.*, **24**, 320 (1959).

(7200), 305 (5300); $\lambda_{max}^{pH\ 11}$ 238.5 nm (ε 12,800), 260 (7900), 305 (6200); $\lambda_{max}^{H_2O}$ 236 nm (ε 14,100), 260 (8500), 305 (6900).

Anal. Calcd for $C_{12}H_{13}ClN_4O_3S$: C, 43.84; H, 3.98; N, 17.04. Found: C, 43.96; H, 4.12; N, 16.98.

The filtrate from above was concentrated to 100 ml and allowed to stand at room temperature for 72 hr. The crystals which had formed were collected by filtration to yield 1.36 g of XV, mp 170–174°.

The above filtrate was then concentrated to 50 ml. After 1 week in a closed vessel at room temperature there was obtained 1.81 g of XII, mp 149–157°. Recrystallization from absolute methanol furnished 827 mg of an analytically pure sample of XII: mp 177–178°; uv $\lambda_{max}^{pH\ 1}$ 237.5 nm (ε 18,000), 261 (10,200), 306 (7700); $\lambda_{max}^{pH\ 11}$ 236 nm (ε 29,200), 261 (11,300), 305 (8200); $\lambda_{max}^{H_2O}$ 237.5 nm (ε 18,000), 261 (9500), 305 (8200).

Anal. Found: C, 43.84; H, 4.00; N, 17.20.

6-Chloro-9-(4,6-di-*O*-acetyl-1,5-anhydro-2,3-dideoxy-D-*arabino*-hex-1-enitol-3-yl)-2-methylthio-9*H*-purine (XIII) and 6-Chloro-9-(4,6-di-*O*-acetyl-1,5-anhydro-2,3-dideoxy-D-*ribo*-hex-1-enitol-3-yl)-2-methylthio-9*H*-purine (XIV).—To 25 ml of acetic anhydride and pyridine (1:4, v/v) was added 230 mg (0.70 mmol) of XI and the mixture was allowed to stand at room temperature for 12 hr with frequent shaking. The resulting solution was poured into 150 ml of crushed ice and stirred thoroughly for 15 min. This mixture was extracted with $CHCl_3$ (2 × 100 ml), the chloroform fractions were combined and washed with cold (0°) 1 *N* HCl solution (3 × 200 ml) and cold (0°) saturated sodium bicarbonate solution (3 × 200 ml), and the chloroform solution was then dried over anhydrous sodium sulfate. The sodium sulfate was removed by filtration and the chloroform filtrate was concentrated to 10 ml volume and applied to the top of a dry-packed column of Baker silica gel powder (12 × 0.5 in.). The column was eluted with ethyl acetate (500 ml) and the eluent was evaporated under high vacuum and at room temperature to afford a stiff syrup. This syrup was dissolved in 2 ml of anhydrous ether and allowed to stand at −10° for 24 hr. The crystals which had separated were collected by filtration to yield 40 mg of analytically pure XIII: mp 117–118°; uv $\lambda_{max}^{pH\ 1}$ 237 nm (ε 18,100), 260 (10,600), 305 (8400); $\lambda_{max}^{pH\ 11}$ 238 nm (ε 16,700), 260 (10,600), 304 (8800); λ_{max}^{EtOH} 237.5 nm (ε 20,400), 260 (11,900), 305 (9800).

Anal. Calcd for $C_{16}H_{17}ClN_4O_5S$: C, 46.54; H, 4.12; N, 13.58. Found: C, 46.47; H, 4.18; N, 13.43.

The same procedure as above was followed except that 260 mg (0.791 mmol) of XII was used instead of 250 mg. The dry column was eluted with ethyl acetate (700 ml) and the eluent was concentrated to a 5-ml volume. This solution was applied to two preparative layer SilicAR 7GF chromatography plates (7.75 × 15.75 in., 2 mm thickness). The plates were developed the full length (14 in., measured from the base line) in an ether–petroleum ether (bp 60–90°) (9:1, v/v) solvent system. The band was removed and extracted with 200 ml of warm absolute ethanol. The ethanol solution was evaporated under high vacuum and at room temperature to a syrup. This syrup was dissolved in 5 ml of water–methanol (9:1, v/v) and the resulting solution was lyophylized to yield 40 mg of analytically pure XIV: mp 72–73°; uv $\lambda_{max}^{pH\ 1}$ 236.5 nm (ε 18,200), 261 (9800), 305 (7800); $\lambda_{max}^{pH\ 11}$ 238 nm (ε 16,300), 261 (9600), 305 (7800); λ_{max}^{EtOH} 237 nm (ε 19,100), 261 (10,000), 306 (8700).

Anal. Calcd for $C_{16}H_{17}ClN_4O_5S \cdot H_2O$: C, 44.60; H, 4.41; N, 13.01. Found: C, 44.98; H, 4.24; N, 12.97.

A pmr spectrum of XIV in DMSO-d_6 showed a water peak at δ 4.0 which integrated for two protons or one molecule of water.

6-Chloro-9-(2,3-dideoxy-D-*erythro*-hex-2-enopyranosyl)-2-methylthio-9*H*-purine (XV).—The filtrate from the acid-catalyzed fusion of 3,4,6-tri-*O*-acetyl-D-glucal with 6-chloro-2-methylthiopurine was reported to furnish a crude nucleoside with mp 170–174°. Recrystallization of this product from absolute methanol gave 1.03 g of analytically pure 6-chloro-9-(2,3-dideoxy-D-*erythro*-hex-2-enopyranosyl)-2-methylthio-9*H*-purine (XV): mp 184–185°; uv $\lambda_{max}^{pH\ 1}$ 260 nm (ε 11,800), 305 (7200); $\lambda_{max}^{pH\ 11}$ 234 nm (ε 19,100), 263.5 (11,500), 305 (7600); $\lambda_{max}^{H_2O}$ 233 nm (ε 18,400), 263 (11,800), 305 (7800).

Anal. Calcd for $C_{12}H_{13}ClN_4O_3S$: C, 43.84; H, 3.98; N, 17.04. Found: C, 43.75; H, 4.15; N, 17.34.

6-Amino-9-(2,3-dideoxy-D-*erythro*-hex-2-enopyranosyl)-2-methylthio-9*H*-purine (XVI).—To 40 ml of methanolic ammonia was added 200 mg (0.608 mmol) of 6-chloro-9-(2,3-dideoxy-D-*erythro*-hex-2-enopyranosyl)-2-methylthio-9*H*-purine (XV). This mixture was sealed in a pressure bottle and allowed to stand at room temperature for 3 days. Excess ammonia and solvent were removed under aspirator vacuum and the residue was then triturated with four portions of 150 ml each of anhydrous ether.

The remaining solid was slurried in 10 ml of hot absolute ethyl alcohol and cooled to room temperature, and the solid was collected by filtration to yield 116 mg (62%) of product, mp 196–198°. Recrystallization from 5 ml of ethyl alcohol–water (9:1, v/v) furnished 49 mg of pure 6-amino-9-(2,3-dideoxy-D-*erythro*-hex-2-enopyranosyl)-2-methylthio-9*H*-purine (XVI): mp 236–237°; uv $\lambda_{max}^{pH\ 1}$ 269 nm (ε 13,000); $\lambda_{max}^{pH\ 11}$ 235 nm (ε 17,300), 275 (13,000); $\lambda_{max}^{H_2O}$ 260 nm (ε 17,600).

Anal. Calcd for $C_{12}H_{15}N_5O_3S$: C, 46.60; H, 4.85; N, 22.65. Found: C, 46.69; H, 4.87; N, 22.68.

9-(2,3-Dideoxy-D-*erythro*-hex-2-enopyranosyl)adenine (XVII).—6-Amino-9-(2,3-dideoxy-D-*erythro*-hex-2-enopyranosyl)-2-methylthio-9*H*-purine (XVI) (80 mg, 0.26 mmol) was dissolved in 80 ml of water, 400 mg of W-4 Raney nickel[48] was added, and the mixture was heated at reflux temperature for 3 hr. An additional 400 mg of Raney nickel was then added and the mixture was allowed to stand at room temperature for 16 hr. The Raney nickel was removed by filtration through a 3-mm-thick Celite bed, the Celite bed was washed with 50 ml of hot water, and the combined filtrates were evaporated to dryness under high vacuum and room temperature. Ultraviolet spectral analysis revealed that the 2-methylthio group had been removed. Crystallization from water furnished 12 mg of an analytically pure sample of 9-(2,3-dideoxy-D-*erythro*-hex-2-enopyranosyl)adenine (XVII): mp 241–242°; uv $\lambda_{max}^{pH\ 1}$ 257 nm (ε 14,700); $\lambda_{max}^{pH\ 11}$ 260 nm (ε 15,600); $\lambda_{max}^{H_2O}$ 259.5 nm (ε 15,300).

Anal. Calcd for $C_{11}H_{13}N_5O_3$: C, 50.19; H, 4.95; N, 26.62. Found: C, 49.99; H, 4.90; N, 26.78.

9-(2,3-Dideoxy-β-D-*erythro*-hex-2-enopyranosyl)adenine (XIX) and 9-(2,3-Dideoxy-α-D-*erythro*-hex-2-enopyranosyl)adenine (XVIII).—A finely powdered mixture of 6-benzamidopurine[49] (20.0 g, 84 mmol) and I (46.5 g, 0.171 mol) was heated in an oil bath (165°) until a melt was formed. To the melt was added *p*-toluenesulfonic acid monohydrate (100 mg) and the heating was continued under aspirator vacuum for 3 hr. The melt was poured into 500 ml of ethyl acetate and washed twice with water. The ethyl acetate layer was dried over magnesium sulfate, filtered, concentrated to a small volume, and applied to a silicic acid column (J. T. Baker, No. 3405; 18 × 2.5 in.). The column was washed with petroleum ether (bp 30–60°)–chloroform (1:4, v/v) to remove glucal and then chloroform to remove nucleosidic material (64 mmol). The nucleosidic material was dissolved in 750 ml of methanol saturated with ammonia at 0° and set at room temperature for 3 days. Concentration of the methanolic solution gave 7.9 g of crystalline material, mp 212–214°. A 500-mg portion of this was preabsorbed on 15 g of Mallinckrodt SilicAR CC7 (200–325 mesh) and applied to a SilicAR CC7 column (15 × 1.25 in., packed in chloroform). The column was successively washed with 2 l. of methanol–dichloromethane (5:95, v/v) and 2 l. of methanol–dichloromethane (6:94, v/v), followed by methanol–dichloromethane (7:93, v/v). The first main fraction was evaporated and the residue was crystallized from ethanol to give a mixture of XX and XXI (0.14 g). The second main fraction after evaporation and crystallization from ethanol gave 0.16 g of pure XVIII: mp 243–245° dec; uv $\lambda_{max}^{pH\ 1}$ 257 nm (ε 15,500); $\lambda_{max}^{pH\ 11}$ 260 nm (ε 16,200); λ_{max}^{EtOH} 260 nm (ε 16,000).

Anal. Calcd for $C_{11}H_{13}N_5O_3$: C, 50.19; H, 4.94; N, 26.62. Found: C, 50.19; H, 4.93; N, 26.80.

The filtrate which was obtained from the filtration of the nucleosidic material, mp 212–214°, in the above preparation was concentrated and applied to a silicic acid column (J. T. Baker No. 3405; 10 × 2.5 in.). Elution of the column with methanol–chloroform (8:92, v/v) removed 7 g of nucleosidic material. Fractional crystallization of the nucleosidic material from ethanol concentrated XIX in the filtrates. The filtrate was evaporated and the residue (5.8 g) was dissolved in water and applied to a Dowex AG 1X8 200–400 mesh column (OH form; 20 × 5.5 in.). Elution with 50% aqueous methanol gave, after evaporation and crystallization of the appropriate fractions, 1.4 g of XIX: mp 195–196.5° (resolidifies, mp 210–215°); uv $\lambda_{max}^{pH\ 1}$ 257 nm (ε 15,000); $\lambda_{max}^{pH\ 11}$ 259 nm (ε 16,000); λ_{max}^{EtOH} 259 nm (ε 15,600).

(48) Purchased from W. R. Grace and Co.

(49) A. Kossel, *Z. Physiol. Chem.*, **12**, 241 (1888).

Anal. Found: C, 49.98; H, 4.97; N, 26.56.

9-(1,5-Anhydro-2,3-dideoxy-D-*arabino*-hex-1-enitol-3-yl)adenine (XX) and 9-(1,5-Anhydro-2,3-dideoxy-D-*ribo*-hex-1-enitol-3-yl)-adenine (XXI).—A mixture of XX and XXI (1.6 g) obtained as described in the preparation of XVIII was applied to a Dowex AG 1X8 200–400 mesh column (OH form; 19 × 5.5 in.). Elution of the column with 50% aqueous methanol gave after concentration and crystallization from ethanol 0.67 g of XXI, mp 219–220, uv $\lambda_{max}^{pH\ 1}$ 258 nm (ϵ 15,200), $\lambda_{max}^{pH\ 11}$ 260 nm (ϵ 15,600), λ_{max}^{EtOH} 260 nm (ϵ 15,400), and 0.69 g of XX, mp 198–201°, uv $\lambda_{max}^{pH\ 1}$ 258 nm (ϵ 15,200), $\lambda_{max}^{pH\ 11}$ 260 nm (ϵ 15,600), λ_{max}^{EtOH} 260 nm (ϵ 15,400).

Anal. Found for XXI: C, 50.20; H, 5.00; N, 26.49. Found for XX: C, 49.95; H, 5.03; N, 26.63.

9-(2,3-Dideoxy-α-D-*erythro*-hexopyranosyl)adenosine (XXIV).—9-(2,3-Dideoxy-α-D-*erythro*-hex-2-enopyranosyl)adenine (XVIII) (200 mg, 0.8 mmol) was dissolved in 50 ml of water. To this solution was added 100 mg of 10% Pd/C and the mixture was then shaken with hydrogen at 45 psi and room temperature for 8 hr. The Pd/C was removed by filtration through a Celite bed, the Celite bed was washed with 50 ml of hot water, and the combined filtrates were evaporated *in vacuo* to a residue. The residue was crystallized from ethanol–water to give 100 mg of XXIV: mp 236–237°; uv $\lambda_{max}^{pH\ 1}$ 257 nm (ϵ 14,300); $\lambda_{max}^{pH\ 11}$ 260 nm (ϵ 14,900); $\lambda_{max}^{H_2O}$ 260 nm (ϵ 15,700).

Anal. Calcd for $C_{11}H_{15}N_5O_3$: C, 49.80; H, 5.69; N, 26.40. Found: C, 49.62; H, 5.61; N, 26.56.

9-(2,3-Dideoxy-β-D-*erythro*-hexopyranosyl)adenine (XXIII).—Hydrogenation of 9-(2,3-dideoxy-β-D-*erythro*-hex-2-enopyranosyl)adenine (XIX) (100 mg, 0.4 mmol) for 6 hr as in the procedure for XXIV gave after crystallization from ethanol 60 mg of XXIII: mp 218.5–219.5° dec; uv $\lambda_{max}^{pH\ 1}$ 256 nm (ϵ 11,800); $\lambda_{max}^{pH\ 11}$ 258 nm (ϵ 12,300); $\lambda_{max}^{H_2O}$ 258 nm (ϵ 12,200).

Anal. Calcd for $C_{11}H_{15}N_5O_3$: C, 49.80; H, 5.69; N, 26.40. Found: C, 49.77; H, 5.49; N, 26.55.

9-(1,5-Anhydro-2,3-dideoxy-D-*arabino*-hexitol-3-yl)adenine (XXII).—Hydrogenation of 9-(1,5-anhydro-2,3-dideoxy-D-*arabino*-hex-1-enitol-3-yl)adenine (XX) (200 mg, 0.8 mmol) as in the procedure for XIV gave after crystallization from ethanol 120 mg of XXII: mp 233–235°; uv $\lambda_{max}^{pH\ 1}$ 257 nm (ϵ 14,400); $\lambda_{max}^{pH\ 11}$ 260 nm (ϵ 14,700); $\lambda_{max}^{H_2O}$ 260 nm (ϵ 14,700).

Anal. Calcd for $C_{11}H_{15}N_5O_3 \cdot {}^1/_4H_2O$: C, 48.17; H, 5.88; N, 25.53. Found: C, 48.15; N, 5.60; N, 25.73.

Registry No.—III, 20787-44-4; IV, 35667-23-3; V, 35667-24-4; VI, 35667-25-5; VII, 20789-68-8; VIII (manno), 35667-27-7; VIII (allo), 35667-28-8; IX, 35666-84-3; XI, 30624-97-6; XII, 31654-90-7; XIII, 35666-86-5; XIV, 35666-87-6; XV, 35667-29-9; XVI, 35667-30-2; XVII, 35667-31-3; XVIII, 35666-83-2; XIX, 35737-21-4; XXI, 35657-25-1; XXII, 35657-26-2; XXIII, 35657-27-3; XXIV, 35657-28-4; 2-acetamido-6-chloropurine, 7602-01-9.

Acknowledgment.—The authors wish to thank Dr. G. D. Daves and Mr. W. Anderson for the decoupled 100-MHz pmr spectra, Mr. E. B. Banta for the 90-MHz pmr spectra, and Mr. A. F. Lewis and staff for the large-scale preparation of intermediates.

The Absolute Configuration of Methyl 3-*O*-Acetyl-2,3-dihydroxy-2-methylpropanoate by Nuclear Magnetic Resonance and Chemical Determination

FRED L. SHORE[1] AND GEORGE U. YUEN*

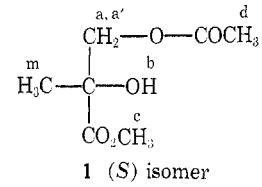
Department of Chemistry, Arizona State University, Tempe, Arizona 85281

Received April 18, 1972

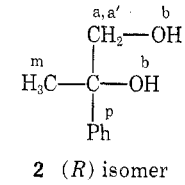
The chemical transformation of (*S*)-(+)-atrolactic acid to methyl 3-*O*-acetyl-(*R*)-2,3-dihydroxy-2-methylpropanoate (1), $[\alpha]^{23}D$ −9.5°, gives an absolute configuration in agreement with the prediction from solvate models and the sense of nonequivalence apparent in the nmr spectra of 1 in the solvent (*R*)-1-(1′-naphthyl)ethylamine.

A study of the Grignard reaction with optically active carbonyl compounds, being carried out in this laboratory, yields products of unknown stereochemistry, whose absolute configuration can be determined most conveniently by degradation to an enantiomer of α-methylglyceric acid. The resolution of α-methylglyceric acid (2,3-dihydroxy-2-methylpropanoic acid) was attempted without success by Glattfield and Sherman.[2] Preparation and assignment of the absolute configuration of methyl 3-*O*-acetyl-2,3-dihydroxy-2-methylpropanoate (1) (Table I) is described herein. Two methods of assignment were used: chemical transformation of (*S*)-(+)-atrolactic acid of known absolute configuration[3] to methyl 3-*O*-acetyl-(*R*)-2,3-dihydroxy-2-methylpropanoate, *via* reactions remote from the asymmetric center; and establishment of a consistent pattern between the sense of nonequivalence apparent in the nmr spectra of methyl 3-*O*-acetyl-(*R*)-2,3-dihydroxy-2-methylpropanoate and its enantiomer in the solvent (*R*)-1-(1′-naphthyl)ethylamine with predictions based on solvate models.

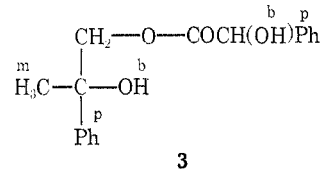
Atrolactic acid was prepared by the method of Eliel and Freeman[4] and partially resolved as the quinine salt using the procedure of McKenzie and Clough.[5] The partially resolved (*S*)-atrolactic acid was then reduced by lithium aluminum hydride to (*S*)-(+)-



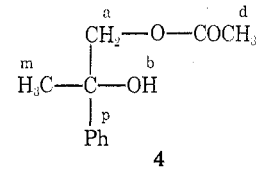
1 (*S*) isomer



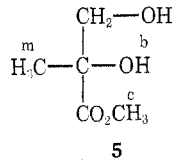
2 (*R*) isomer



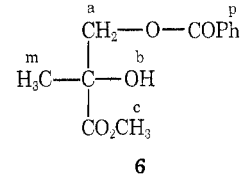
3



4



5



6

(1) Taken from the Ph.D. Dissertation of Fred L. Shore.

(2) J. W. E. Glattfeld and L. P. Sherman, *J. Amer. Chem. Soc.*, **47**, 1742 (1925).

(3) J. H. Brewster, *ibid.*, **78**, 4061 (1956).

(4) E. L. Eliel and J. P. Freeman, *Org. Syn.*, **33**, 7 (1953).

(5) A. McKenzie and G. W. Clough, *J. Chem. Soc.*, **97**, 1016 (1910).