Structure of Prumycin

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The antibiotic prumycin has been identified as 4-D-alanylamino-2-amino-2,4-dideoxy-L-arabinose (1). ^{1}H and ^{13}C N.m.r. data for prumycin and related compounds, particularly the methyl glycoside of *O*-acetyl-*NN'*-bisbenzyloxycarbonylprumycin (6β) and *NN'*-diacetylprumycin (3), were major factors in establishing the structure. The absolute configuration was derived by comparing the optical rotation of prumycin with those of other L-arabinose derivatives.

THE antifungal antibiotic prumycin, isolated by Hata and his co-workers [1] from a cultured broth of *Streptomyces sp.* strain no. 1028, was previously identified as a 5-D-alanylamino-2-amino-2,5-dideoxypentofuranose (A) on the basis of evidence obtained from colour reactions, periodate oxidation, and spectrometry.[2] Recently, however, an improved fermentation of the antibiotic has afforded us additional quantities of prumycin and has made it possible for us to continue our structural studies. We now correct our earlier report: prumycin contains a pyranose rather than a furanose ring system and is 4-D-alanylamino-2-amino-2,4-dideoxy-L-arabinopyranose (1).

Prumycin was isolated as one of the two anomeric dihydrochlorides, $C_8H_{17}N_3O_4,2HCl,H_2O$, depending on the crystallization procedures. Potentiometric titrations showed the presence of two basic groups and the antibiotic gave a positive reaction for a reducing sugar. In addition, it underwent a positive colour reaction with ninhydrin and also with the Elson–Morgan reagent,[2]

[1] T. Hata, S. Ōmura, M. Katagiri, K. Atsumi, J. Awaya, S. Higashikawa, K. Yasui, H. Terada, and S. Kuyama, *J. Antibiotics*, 1971, **24**, 900.

[2] S. Ōmura, M. Tishler, M. Katagiri, and T. Hata, *J.C.S. Chem. Comm.*, 1972, 633.

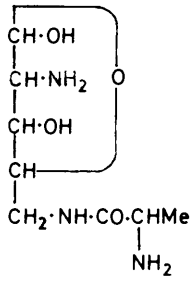
indicating the presence of a 2-amino-sugar unit. Hydrolysis of prumycin in 6N-hydrochloric acid at 100 °C for 4 h gave a ninhydrin-positive substance identified as D-alanine. The linkage of the carboxy-group of D-alanine to the amino-sugar through an amide bond was established from the i.r. spectrum of prumycin. Acetylation of prumycin with acetic anhydride and sodium acetate yielded the tetra-acetyl compound (2) (M^+ 387). Treatment of (2) with sodium methoxide in methanol at room temperature gave *NN'*-diacetylprumycin (3).

Prumycin was converted into the *NN'*-bisbenzyloxycarbonyl compound (4) by treatment with benzyl chloroformate. The product (4) reacted with methanol containing toluene-*p*-sulphonic acid to give the anomeric methyl glycosides (5). However, only the β-anomer was isolated (M^+ 501). On acetylation with acetic anhydride and pyridine, this anomer (5β) yielded the methyl β-glycoside of *O*-acetyl-*NN'*-bisbenzyloxycarbonylprumycin (6β). The α-anomer (6α) was obtained by acetylation of the methyl α-glycoside (5α) which was present in the mother liquor after separation of the more insoluble anomer (5β).

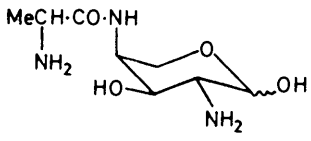
Hydrogenation of methyl *NN'*-bisbenzyloxycarbonyl-β-prumycinide (5β) over palladium black in ethanol containing a small amount of acetic acid removed the two benzyloxycarbonyl groups, yielding methyl β-prumycinide, isolated as the dihydrochloride (7). Attempts to prepare this methyl glycoside from prumycin directly by treatment with methanol containing hydrogen chloride were unsuccessful. Treatment of (7) with hydrazine caused cleavage of the peptide bond yielding methyl 2,4-diamino-2,4-dideoxy-β-L-arabinoside (8).

Much of the evidence for the structure of prumycin was obtained from the n.m.r. spectra of prumycin and its transformation products. The method used to determine the relative orientations of the hydrogen atoms is based on the evaluation of approximate bond angles from coupling constants,[3] thus allowing, at least, a distinction between diaxial and diequatorial or axial–equatorial protons.[4]

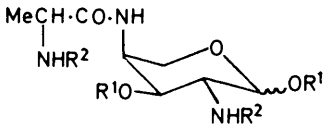
Prumycin and its derivatives in D_2O exhibit a large value (11·5 Hz) for $J_{2,3}$ in the resonances assigned to H-2 for both anomers (Figure 1), similar in magnitude to the corresponding data for known pyranose sugars.



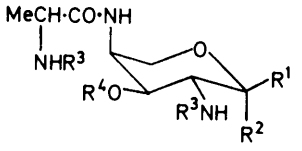
(A)



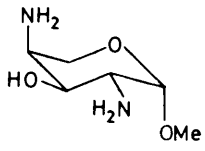
(1)



(2) $R^1 = R^2 = Ac$
(3) $R^1 = OH, R^2 = Ac$
(4) $R^1 = OH, R^2 = Z$



(5α) $R^1 = OMe, R^2 = H, R^3 = Z, R^4 = H$
(5β) $R^1 = H, R^2 = OMe, R^3 = Z, R^4 = H$
(6α) $R^1 = OMe, R^2 = H, R^3 = Z, R^4 = Ac$
(6β) $R^1 = H, R^2 = OMe, R^3 = Z, R^4 = Ac$
(7) $R^1 = H, R^2 = OMe, R^3 = R^4 = H$



(8)

$Z = PhCH_2O \cdot CO$

The n.m.r. spectrum of methyl *O*-acetyl-*NN'*-bisbenzyloxycarbonyl-β-prumycinide (6β) (Figure 2) was studied with the use of spin decoupling and deuterium exchange in D_2O. The large value of $J_{2,3}$ (11·5 Hz) established that H-2 and H-3 are *trans*-diaxial. Since the secondary couplings for H-2 and H-3 are relatively small ($J_{1,2}$ 3·5; $J_{3,4}$ 4·1 Hz), equatorial configurations are strongly favoured for H-1 and H-2. Equilibration with D_2O caused changes in the appearance of the signals for H-2, H-4, and the alanine α-proton; this effectively excludes the furanose structure (A) since in this structure, apart from the alanine α-proton, only one ring CH group and the methylene protons would exchange with deuterium. Since the amino-group was already known to be attached to C-2, the attachment of the alanine residue to C-4 was established. A pair of doublets both showing coupling to H-4 exhibited a large separation (12·0 Hz)

[3] M. Karplus, *J. Amer. Chem. Soc.*, 1963, **88**, 2871; R. U. Lemieux, R. K. Kullnig, and R. V. Moir, *ibid.*, 1958, **80**, 2237; R. U. Lemieux, R. K. Kullnig, H. J. Berstein, and W. G. Schneider, *ibid.*, p. 6098.

[4] G. Slomp and F. A. MacKeller, *J. Amer. Chem. Soc.*, 1967, **89**, 2454.

characteristic of a geminal coupling constant. These signals were assigned to the two C-5 protons. Their

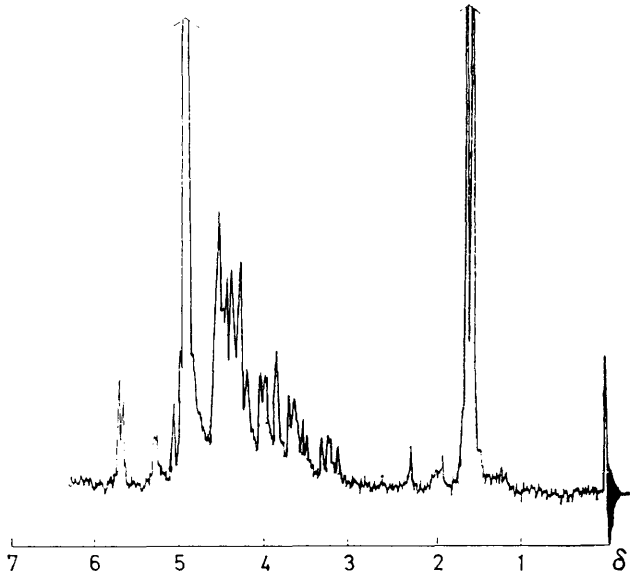


FIGURE 1 ^{1}H N.m.r. spectrum (100 MHz) of prumycin in D_2O

chemical shifts (δ 3·54 and 3·92) are consistent with the presence of an adjacent oxygen atom. These n.m.r. features are completely in accord with a pyranose structure.

TABLE 2

^{13}C Chemical shifts of anomeric carbon atoms

	δ_C	
NN'-Diacetylprumycin [a]	91·6	93·5
Glucosamine, HCl [a]	90·0	93·6
Glucose [a]	92·8	96·7
Fructose [b]	98·2 (pyranose)	
	101·8 (furanose)	
Ribose	102·1	97·4

[a] Anomeric mixture. [b] Major anomers.

The coupling patterns of the protons on the sugar unit of prumycin and its derivatives are summarized in Table 1. The spectral data for D-arabinose in D_2O [5] and triacetyl-β-L-arabinose in $CDCl_3$ [6] have been included to illustrate the similarity with prumycin.

Evidence for the pyranose structure was also obtained from the ^{13}C n.m.r. spectrum of *NN'*diacetylprumycin (3) (Figure 3). Recent studies on five- and six-membered

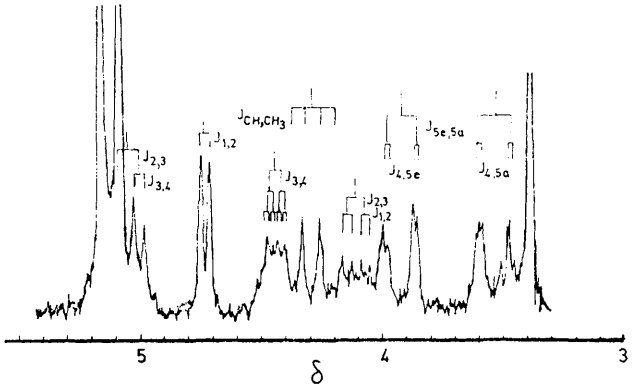


FIGURE 2 Expanded 100 MHz ^{1}H n.m.r. spectrum of the glycoside (6β) after treatment with D_2O

ring sugars [7] have shown that the resonance for the anomeric carbon atom of a pyranose appears *ca.* 5 p.p.m. to higher field than that of a furanose. The resonances assigned to C-1 in the spectrum of (3) occur at 91·6 and 93·5 p.p.m.; these values are similar to those of other pyranose sugars (Table 2).

TABLE 1

Coupling constants (Hz) of the sugar unit and of prumycinand derivatives, showing possible conformational relationships of protons

Compound (solvent)	$J_{1eq,2}$	$J_{1ax,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5ax}$	$J_{4,5eq}$	$J_{5ax\ 5eq}$
Prumycin, 2HCl (D_2O)							
β-isomer	3·5						
α-isomer		8·1	11·0				
Methyl β-prumycinide, 2HCl (7)(D_2O)	3·5		10·5				
Methyl *O*-Ac-*NN'*-Z_2-α-prumycinide (6α) [$(CD_3)_2SO$]		8·0	10·4	4·0			
Methyl *O*-Ac-*NN'*-Z_2-β-prumycinide (6β) ($CDCl_3$)	3·5		11·5	4·1	2·0	2·0	12·0
Conformational relationship	*eq, ax*		*ax, ax*	*ax, eq*	*eq, ax*	*eq, eq*	
β-D-Arabinose (D_2O) [a]	2·3			3·6			
α-D-Arabinose (D_2O) [a]		7·2		3·4			
β-L-Arabinose triacetate ($CDCl_3$) [b]	1·0				1·0		
β-D-Arabinose tetra-acetate [$(CD_3)_2CO$] [c]	2·9		11·8	3·0	1·0	1·9	13·2

[a] Ref. 5. [b] Ref. 6. [c] P. L. Durette and D. Horton, *J. Org. Chem.*, 1971, **36**, 2661.

The absolute configuration of the sugar unit of prumycin was deduced from a comparison of the optical

TABLE 3

Optical rotations of prumycin and related compounds

	$[\alpha]_D$ (°)
α-L-Arabinose	+89 (*c* 4·0, H_2O) [a]
α-Prumycin	+68·8 (*c* 0·5, MeOH)
β-L-Arabinose	+202 (*c* 4·0, H_2O) [a]
β-Prumycin	+115 (*c* 0·5, MeOH)
Me *O*-Ac-*NN'*-Z_2-α-prumycinide (6α)	+18 (*c* 0·48 MeOH)
Me *O*-Ac-*NN'*-Z_2-β-prumycinide (6β)	+123 (*c* 0·48, MeOH)
Methyl tri-*O*-acetyl-β-L-arabino-pyranoside	+182 (*c* 4·4, $CHCl_3$) [b]

[a] E. Montgomery and C. S. Hudson, *J. Amer. Chem. Soc.*, 1934, **56**, 2074. [b] C. S. Hudson and J. K. Dale, *J. Amer. Chem. Soc.*, 1918, **40**, 992.

[5] R. U. Lemieux and J. D. Stevens, *Canad. J. Chem.*, 1966, **44**, 249.

[6] R. U. Lemieux and J. D. Stevens, *Canad. J. Chem.*, 1965, **43**, 2059.

[7] L. F. Johnson and W. C. Jankowski, 'Carbon-13 NMR Spectra,' Wiley, New York, 1972; L. D. Hall and L. F. Johnson, *Chem. Comm.*, 1969, 509; D. E. Dorman and J. D. Roberts, *J. Amer. Chem. Soc.*, 1967, **93**, 4463.

rotations of prumycin, arabinose, and related compounds. On the basis of observations first made by Hudson,[8] we assign to prumycin the L-configuration because the α-anomer of prumycin and methyl *O*-acetyl-*NN'*-bisbenzyloxycarbonyl-α-prumycinide (6α) are both more laevorotatory than the corresponding β-anomers. These rotations and those for the arabinose series are summarized in Table 3.

Two observations reported in our previous paper [2] influenced us in accepting the furanose structure (A). The colour formed by *NN'*-diacetylprumycin (3) in the Morgan–Elson test suggested, on the basis of previous studies,[9] that it contained a 4-hydroxy-group and a free aldehyde group. Re-examination of the colour reaction established that the previous test was inadequately performed. The red colour we observed must have been due to some artefact since it showed an absorption maximum at 500 nm rather than two maxima at 550 and 590 nm characteristic of 2-acetamido-aldoses.[10]

The second observation concerns the periodate oxidation of prumycin. Although the 3 mol. equiv. of periodate consumed in our reported experiment favoured structure (A), further examination of the data and of data from additional experiments indicated a slow over-oxidation which is difficult to interpret. Two mol. equiv. of periodate were consumed rapidly, within 0·5 h, whereas the third mol. equiv. required 5·5 additional hours. As much as 5·5 mol. equiv. of periodate are consumed if the oxidation is allowed to proceed for 1 week.

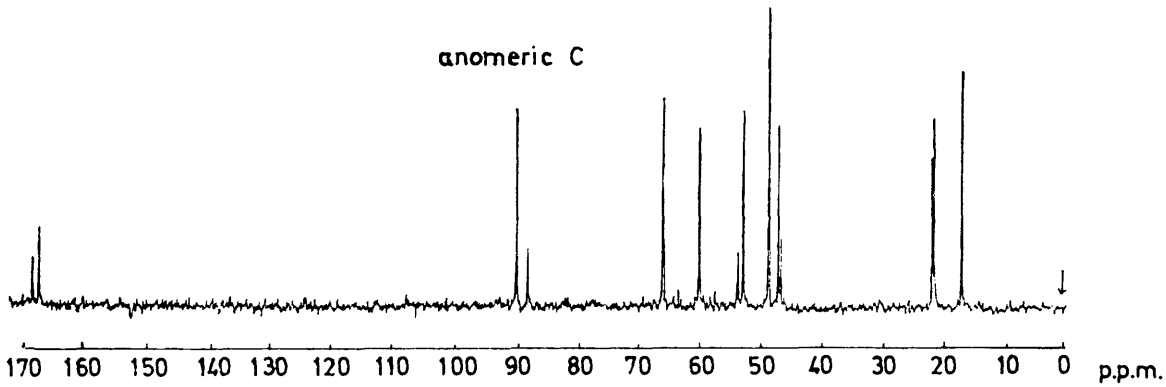


FIGURE 3 ^{13}C N.m.r. spectrum of *NN*-diacetylprumycin (arrow indicates calculated shift of Me_4Si)

EXPERIMENTAL

N.m.r. spectra were obtained with a JEOLCO 100 MHz instrument, for use of which we thank the Chemistry Department, Yale University.

Purification of Prumycin (1) *Hydrochloride.*—Crude prumycin hydrochloride prepared according to the procedure of Hata *et al.*[1] was passed in aqueous solution through a Sephadex G-10 column. Blue Dextran was used to indicate the prumycin front. The column was developed with water and those fractions which gave a positive ninhydrin test were collected and evaporated to dryness. The residue was dissolved in hot methanol and allowed to crystallize at room temperature. The crystalline β-*anomer* (1β), separated by filtration, had m.p. 198—200° (decomp.); $[\alpha]_D^{20}$ +115° (*c* 0·5 in MeOH); for n.m.r. see Table 1.

The α-*anomer* (1α) was obtained by dissolving the same residue in ethanol and adding water slowly to induce crystallization. It separated as needles, m.p. 184—185° (decomp.); $[\alpha]_D^{20}$ +68·8° (*c* 0·5 in MeOH); for n.m.r. see Table 1 [Found (α-anomer): C, 31·2; H, 6·85; Cl, 23·1; N, 13·7. Found (β-anomer): C, 30·95; H, 6·6; Cl, 23·2; N, 13·35. $C_8H_{17}N_3O_4,2HCl,H_2O$ requires C, 30·95; H, 6·75; Cl, 22·9; N, 13·55%).

Hydrolysis of Prumycin; Isolation of D-*Alanine.*—A solution of prumycin (1 g) in 6N-HCl (10 ml) was heated in a sealed Pyrex tube at 100 °C for 4 h. The solution was concentrated to dryness under vacuum and the residue, dissolved in water (2 ml), was chromatographed on Amberlite IR 120 (H^+) resin with 3N-HCl as eluant, to give D-alanine hydrochloride (40 mg; 15%), m.p. 175—176° (decomp.) (from ethanol); t.l.c. R_F 0·34 (MeCOEt–AcOH–H_2O, 3 : 1 : 1); $[\alpha]_D^{25}$ +13·5° (*c* 0·5 in H_2O).

NN'OO'-Tetra-acetylprumycin (2).—A mixture of prumycin dihydrochloride (3·2 g, 10 mmol), acetic anhydride (50 ml), and sodium acetate (3·6 g) was stirred at room temperature for 18 h. The mixture was filtered and evaporated and the residue was extracted with chloroform. Evaporation of the extract gave a syrup which was chromatographed in chloroform, on a silica gel column (100 g) with $CHCl_3$–EtOH (10 : 1 to 10 : 4) as eluant. The product was obtained as a white *powder* (1·8 g, 45%); R_F 0·7 (silica gel; EtOH–$CHCl_3$, 3 : 1); $[\alpha]_D^{25}$ +69·8° (*c* 0·48 in MeOH); $\nu_{max.}$ (KBr) 3300, 1745, 1655, 1535, 1375, 1236, 1050, and 1014 cm^{-1}; δ [$(CD_3)_2SO$] 1·35 (3H, d, *J* 7 Hz), 1·95 (3H, s), 2·07 (6H, s), and 2·13 (3H, s); *m/e* 387 (M^+) (Found: C, 49·55; H, 6·35; N, 10·9. $C_{16}H_{25}N_3O_8$ requires C, 49·6; H. 6·45; N, 10·85%).

NN'-*Diacetylprumycin* (3).—Compound (2) (500 mg, 1·3 mmol) was dissolved in methanol (30 ml) containing sodium methoxide (180 mg) and left for 1 h at room temperature. The solution was neutralized with 3% HCl in methanol. After filtration the methanolic solution was evaporated to half volume and kept overnight in a refrigerator. The

[8] C. S. Hudson, *J. Amer. Chem. Soc.*, 1909, **31**, 66; 1916, **38**, 1566; S. C. Williams and J. K. N. Jones, *Canad. J. Chem.*, 1967, **45**, 275.

[9] R. W. Jeanloz and M. Tremege, *Fed. Proc.*, 1956, **15**, 286; R. Kuhn, A. Gauhe, and H. H. Baer, *Chem. Ber.*, 1956, **89**, 1027.

[10] D. Aminoff, W. T. J. Morgan, and W. N. Watkins, *J. Biol. Chem.*, 1952, **51**, 379.

crystalline product was filtered off and recrystallized from hot methanol to yield *compound* (3) (300 mg, 30%), m.p. 200° (decomp.); $[\alpha]_D^{25}$ +48·8° (*c* 0·44 in MeOH); $\nu_{max.}$ (KBr) 3500, 3000, 1650, 1545, 1375, 1247, 1158, 1120, and 1052 cm^{-1}; δ [$(CD_3)_2SO$] 1·23 (3H, d, *J* 7·0 Hz) and 1·96 (6H, s) (Found: C, 47·6; H, 6·7; N, 13·5. $C_{12}H_{21}N_3O_6$ requires C, 47·5; H, 6·95; N, 13·8%).

NN′-*Bisbenzyloxycarbonylprumycin* (4).—To a solution of prumycin dihydrochloride (8·0 g, 26 mmol) in water (30 ml), sodium hydrogen carbonate (11 g) was added with stirring, followed by benzyl chloroformate (19 × 0·5 ml). After 3 h stirring, the mixture was refrigerated for 6 h. Chloroform (40 ml) was then added and, after shaking, the precipitate was filtered off, washed with chloroform, and dried. Recrystallization from 30% methanol gave *compound* (4) (5·6 g) as needles. The product was soluble in glacial acetic acid, acetone, pyridine, and hot ethanol, and slightly soluble in hot water; it had m.p. 180—182°; $[\alpha]_D^{25}$ +23·4° (*c* 1·0 in MeOH); $\nu_{max.}$ (KBr) 3210, 1680, 1485, 1400, 1320, 1090, 1045, 1000, and 925 cm^{-1}; δ [$(CD_3)_2SO$] 1·20 (3H, d, *J* 7·2 Hz), 5·02 (4H, s), and 7·35 (10H, s) (Found: C, 59·35; H, 5·9; N, 8·3. $C_{24}H_{29}N_3O_8$ requires C, 59·15; H, 5·9; N, 8·6%).

Methyl NN′-*Bisbenzyloxycarbonyl*-β-*prumycinide* (5β).—A solution of compound (4) (5 g, 10 mmol) and toluene-*p*-sulphonic acid (5 g) in methanol (250 ml) was boiled under reflux for 5 h. The purple mixture was cooled, neutralized (Ag_2O), and filtered. The filtrate was evaporated to dryness and the residue dissolved in chloroform (30 ml). After filtration, the solution was evaporated *in vacuo* to a syrup. The syrup in chloroform was chromatographed on a silica gel column (100 g) with $CHCl_3$–MeOH (20 : 1) as eluant. The resulting syrup afforded *compound* (5β) (500 mg, 10%), m.p. 178—179° (from ethyl acetate); $[\alpha]_D^{25}$ +91° (*c* 0·5 in MeOH); $\nu_{max.}$ (KBr) 3331, 2892, 1680, 1521, 1272, and 1054 cm^{-1}; δ [$(CD_3)_2SO$] 1·24 (3H, d, *J* 7·1 Hz), 3·25 (3H, s), 5·02 (4H, s), and 7·32 (10H, s); *m/e* 501 (M^+) (Found: C, 59·8; H, 6·3; N, 8·55. $C_{25}H_{31}N_3O_8$ requires C, 59·9; H, 6·2; N, 8·4%). The mother liquor on evaporation to dryness left a syrupy mixture of (5β) and (5α) which we were unable to crystallize.

Methyl O-*Acetyl*-NN′-*bisbenzyloxycarbonyl*-β-*prumycinide* (6β).—To a solution of compound (5β) (200 mg, 0·4 mmol) in pyridine (2 ml) was added acetic anhydride (2 ml). The solution was refluxed for 5 h and evaporated to dryness. A solution of the residue in chloroform (50 ml) was washed with water, dried ($MgSO_4$), and evaporated. The residue in chloroform was chromatographed on a silica gel column. Elution with chloroform containing 0—5% methanol gave a syrup (90 mg) which afforded *compound* (6β) (60 mg, 28%), m.p. 105—106°; $[\alpha]_D^{25}$ +123° (*c* 0·48 in MeOH); $\nu_{max.}$ (KBr) 3330, 2890, 1720, 1680, 1520, 1490, 1370, 1265, 1235, 1122, 1045, 938, 890, 733, and 698 cm^{-1}; for n.m.r. see Table 1; t.l.c. (silica gel G; MeOH–$CHCl_3$, 1 : 10) R_F 0·76; *m/e* 543 (M^+) (Found: C, 59·65; H, 6·0; N, 7·65. $C_{27}H_{33}N_3O_9$ requires C, 59·65; H, 6·1; N, 7·75%).

Methyl O-*Acetyl*-NN′-*bisbenzyloxycarbonyl*-α-*prumycinide* (6α).—The syrupy residue of (5β) and (5α) (500 mg) obtained from the mother liquor in the preparation of (5β) was treated with acetic anhydride and pyridine in the same way as was the crystalline (5β). The mixture was evaporated to dryness. The residue in chloroform was chromatographed over silica gel (30 g) and the column was developed with $CHCl_3$–MeOH (20 : 1). The eluate containing (6α) [lower spot on the t.l.c. plate (R_F 0·56; silica gel G; MeOH–$CHCl_3$, 1 : 10)] afforded a syrup (85 mg) which crystallized from carbon tetrachloride to give the *product* (45 mg), m.p. 151—154°; $[\alpha]_D^{23}$ +18° (*c* 0·48 in MeOH); $\nu_{max.}$ (KBr) 3670, 1720, 1708, 1696, 1675, 1510, 1420, 1355, 1284, 1240, 1065, and 1040 cm^{-1}; δ [$(CD_3)_2SO$] 1·22 (3H, d, *J* 7·1 Hz), 1·8 (3H, s), 3·35 (3H, s), 3·58br (2H, s), 4·80 (1H, q, *J* 10·4 and 4·0 Hz), 5·00 (3H, s), and 7·31 (10H, s); *m/e* 543 (M^+) (Found: C, 59·55; H, 6·1; N, 7·65. $C_{27}H_{33}N_3O_9$ requires C, 59·65; H, 6·1; N, 7·75%).

Methyl β-*Prumycinide* (7) *Dihydrochloride*.—To a solution of compound (5β) (310 mg, 0·6 mmol) in ethanol (20 ml) were added acetic acid (1 ml) and palladium black (20 mg). Hydrogen was bubbled through the mixture for 17 h. The mixture was then filtered and evaporated to a thick syrup. The syrup was dissolved in water and passed through a column of Amberlite 4B (OH^-) resin to remove anions. The eluate was neutralized with dilute aqueous HCl to pH 4—5 and then lyophilized to yield a white *powder* (140 mg, 70%); $[\alpha]_D^{23}$ +153·4° (*c* 0·455 in H_2O); t.l.c. R_F 0·45 (silica gel; Pr^iOH–AcOH–pyridine–H_2O, 15 : 3 : 10 : 12); $\nu_{max.}$ (KBr) 3400, 2950, 1665, 1540, 1240, 1180, 1120, and 1058 cm^{-1}; for n.m.r. see Table 1 (Found: C, 35·5; H, 6·9; N, 13·5. $C_9H_{19}N_3O_4$,2HCl requires C, 35·3; H, 6·85; N, 13·75%).

Methyl 2,4-*Diamino*-2,4-*dideoxy*-β-L-*arabinoside Dihydrochloride* (8).—Compound (7) (120 mg, 0·4 mmol) in hydrazine hydrate (4 ml) was refluxed for 64 h. The solution was evaporated to dryness and the residue dissolved in water. Three fractions were revealed by t.l.c. (MeCOEt–AcOH–H_2O, 3 : 1 : 1). The solution was chromatographed on a silica gel column with the same solvent. The first fraction (R_F 0·57) was not investigated. The second fraction (R_F 0·34) was identified by n.m.r. as alanine. The third fraction (R_F 0·13) was adjusted to pH 3 with 0·5N-HCl and lyophilized to yield *compound* (8) (13 mg, 10%) as a powder, identified by its n.m.r. and i.r. spectra; $[\alpha]_D^{25}$ +114° (*c* 0·43 in MeOH); $\nu_{max.}$ (KBr) 3380, 2900, 1590, 1492, 1295, 1135, 1060, and 1033 cm^{-1}; δ (D_2O) 2·48 (3H, s) and 5·00br (1H, s) (Found: C, 30·8; H, 6·7; N, 11·65. $C_6H_{14}N_2O_3$,2HCl requires C, 30·65; H, 6·8; N, 11·9%).

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