Structure of Prumycin

By Satoshi Ömura,* Michiko Katagiri, Kiyoo Atsumi, and Toju Hata, Kitasato Institute and Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108, Japan

Ann A. Jakubowski, E. Bleecker Springs, and Max Tishler,* Wesleyan University, Middletown, Connecticut 06457, U.S.A.

The antibiotic prumycin has been identified as 4-D-alanylamino-2-amino-2,4-dideoxy-L-arabinose (1). ¹H and ¹³C N.m.r. data for prumycin and related compounds, particularly the methyl glycoside of *O*-acetyl-*NN'*-bisbenzyl-oxycarbonylprumycin ($\beta\beta$) 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-workers¹ 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

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

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

² S. Ömura, 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 Dalanine. 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 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 axialequatorial 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.



Z=PhCH,O·CO

isolated (M^+501) . On acetylation with acetic anhydride and pyridine, this anomer (5β) yielded the methyl β glycoside of *O*-acetyl-*NN'*-bisbenzyloxycarbonylprumycin (6 β). The α -anomer (6 α) was obtained by acetylation of the methyl α -glycoside (5 α) which was present in the mother liquor after separation of the more insoluble anomer (5 β).

Hydrogenation of methyl NN'-bisbenzyloxycarbonyl- β -prumycinide (5 β) 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)

The n.m.r. spectrum of methyl O-acetyl-NN'-bisbenzyloxycarbonyl- β -prumycinide (6 β) (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)

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

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.

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



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triacetyl- β -L-arabinose in CDCl₃⁶ 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 1H n.m.r. spectrum of the glycoside (6) 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

δ

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

Compound (solvent)	J 100.2	J 142,2	J 2. 3	J 3.4	J 4. 5az	J 4.507	J bax beg
Prumvcin, 2HCl (D _o O)	0 101,1	5 100. 1	0 210	0 0,1	0 -,0	0	• • • • •
β-isomer	3.5						
α-isomer		8.1	11.0				
Methyl B-prumycinide, 2HCl (7)(D.O)	3.5		10.5				
Methyl O-Ac-NN'-Z ₂ -α-prumycinide (6a) [(CD ₂),SO]		8.0	10.4	4 ·0			
Methyl O-Ac-NN'-Z ₀ -B-prumycinide (6B) (CDCl ₂)	3.5		11.5	4.1	$2 \cdot 0$	$2 \cdot 0$	12.0
Conformational relationship	eq. ax		ax, ax	ax, eq	eq, ax	cq, eq	
β -D-Arabinose (D ₀ O) ^a	2.3			3.6	•		
α -D-Arabinose (D ₀ O) ^a		7.2		3.4			
B-L-Arabinose triacetate (CDCl.) b	1.0				1.0		
β -D-Arabinose tetra-acetate [(CD_3) ₂ CO] c	2.9		11.8	3.0	1.0	$1 \cdot 9$	$13 \cdot 2$
^a Ref. 5. ^b Ref. 6. ^c P. L. Du	rette and D.	Horton, J.	. Org. Chem.	, 1971, 36	2661.		

features are completely in accord with a pyranose structure.

TABLE 2

¹³C Chemical shifts of anomeric carbon atoms

	δ _c			
NN'-Diacetylprumycin "	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	$102 \cdot 1$. ,	97.4	
^a Anomeric mixture.	^b Major an	omers.		

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

⁵ R. U. Lemieux and J. D. Stevens, *Canad. J. Chem.*, 1966, **44**, 249. ⁶ R. U. Lemieux and L. D. Stevens, *Canad. J. Chew.* 1965, **43**

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

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

	[α] _D (°)
a-l-Arabinose	89 (c 4.0, H ₂ O) •
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 (6 α)	+18 (c 0.48 MeOH)
Me O-Ac- NN' -Z ₂ - β -prumycinide (6 β)	+123 (c 0.48, MeOH)
Methyl tri-O-acetyl-β-L-arabino-	+182 (c 4.4, CHCl ₃) b
pyranoside	
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^a E. Montgomery and C. S. Hudson, J. Amer. Chem. Soc., 1934, 56, 2074. ^bC. S. Hudson and J. K. Dale, J. Amer. Chem. Soc., 1918, 40, 992.

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.

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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 paper² 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

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 (1 β), separated by filtration, had m.p. 198—200° (decomp.); $[\alpha]_{\rm D}^{20} + 115^{\circ}$ (c 0.5 in MeOH); for n.m.r. see Table 1.

The α -anomer (1 α) 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.); $[\alpha]_{D}^{20} + 68\cdot8^{\circ}$ (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. C₈H₁₇N₃O₄.2HCl,H₂O requires C, 30·95; H, 6·75; Cl, 22·9; N, 13·55%).



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 overoxidation 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.

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

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.

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. $R_{\rm F}$ 0.34 (MeCOEt-AcOH-H₂O, 3:1:1); [α]_p²⁵ +13.5° (c 0.5 in H₂O).

 $NN'OO^{-\tilde{T}etra-acetylprumycin}$ (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 CHCl₃-EtOH (10:1 to 10:4) as eluant. The product was obtained as a white *powder* (1·8 g, 45%); $R_{\rm F}$ 0·7 (silica gel; EtOH-CHCl₃, 3:1); $[\alpha]_{\rm D}^{25}$ +69·8° (c 0·48 in MeOH); $\nu_{\rm max}$ (KBr) 3300, 1745, 1655, 1535, 1375, 1236, 1050, and 1014 cm⁻¹; δ [(CD₃)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. C₁₆H₂₅N₃O₈ 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

R. W. Jeanloz and M. Tremege, Fed. Proc., 1956, 15, 286;
R. Kuhn, A. Gauhe, and H. H. Baer, Chem. Ber., 1956, 89, 1027.
D. Aminoff, W. T. J. Morgan, and W. N. Watkins, J. Biol. Chem., 1952, 51, 379.

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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); ν_{max} (KBr) 3500, 3000, 1650, 1545, 1375, 1247, 1158, 1120, and 1052 cm⁻¹; δ [(CD₃)₂SO] 1.23 (3H, d, J 7.0 Hz) and 1.96 (6H, s) (Found: C, 47.6; H, 6.7; N, 13.5. C₁₂H₂₁N₃O₆ 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°; $[\alpha]_D^{25} + 23.4^\circ$ (c 1.0 in MeOH); ν_{max} (KBr) 3210, 1680, 1485, 1400, 1320, 1090, 1045, 1000, and 925 cm⁻¹; δ [(CD₃)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. C₂₄H₂₉N₃O₈ requires C, 59.15; H, 5.9; N, 8.6%).

Methyl NN'-Bisbenzyloxycarbonyl- β -prumycinide (5 β).—A solution of compound (4) (5 g, 10 mmol) and toluene-psulphonic acid (5 g) in methanol (250 ml) was boiled under reflux for 5 h. The purple mixture was cooled, neutralized (Ag₂O), 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); $[\alpha]_{\rm p}^{25}$ +91° (c 0.5 in MeOH); ν_{max} (KBr) 3331, 2892, 1680, 1521, 1272, and 1054 cm⁻¹; δ [(CD₃)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. C₂₅H₃₁N₃O₈ 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 (MgSO₄), 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°; [a]_p²⁵ +123° (c 0·48 in MeOH); $\gamma_{max.}$ (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–CHCl₃, 1:10) $R_{\rm F}$ 0·76; m/e 543 (M⁺) (Found: C, 59·65; H, 6·0; N, 7·65. C₂₇H₃₃N₃O₉ requires C, 59·65; H, 6·1; N, 7·75%).

Methyl O-Acetyl-NN'-bisbenzyloxycarbonyl-a-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 (6a) [lower spot on the t.l.c. plate ($R_{\rm F}$ 0.56; silica gel G; MeOH-CHCl₃, 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); ν_{max} (KBr) 3670, 1720, 1708, 1696, 1675, 1510, 1420, 1355, 1284, 1240, 1065, and 1040 cm⁻¹; § [(CD₃)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. C₂₇H₃₃N₃O₉ 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%); $[\alpha]_{0}^{23}$ +153·4° (c 0.455 in H₂O); t.l.c. $R_{\rm F}$ 0.45 (silica gel; PrⁱOH-AcOH-pyridine-H₂O, 15:3:10:12); $v_{\rm max}$ (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. C₉H₁₉N₃O₄,2HCl requires C, 35·3; H, 6·85; N, 13·75%).

Methyl 2,4-Diamino-2,4-dideoxy-3-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 $(R_{\rm F} \ 0.57)$ was not investigated. The second fraction $(R_{\rm F} \ 0.57)$ 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; $[\alpha]_D^{25} + 114^\circ$ (c 0.43 in MeOH); v_{max.} (KBr) 3380, 2900, 1590, 1492, 1295, 1135, 1060, and 1033 cm⁻¹; & (D₂O) 2.48 (3H, s) and 5.00br (1H, s) (Found: C, 30.8; H, 6.7; N, 11.65. C₆H₁₄N₂O₃,-2HCl requires C, 30.65; H, 6.8; N, 11.9%).

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