Carbohydrate Research, 92 (1981) 191–205
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

2-*epi*-FORTIMICIN B. PARTICIPATION OF 1-*N*-BENZYLOXYCARBONYLAMINO AND 1-ACETAMIDO GROUPS IN SOLVOLYSIS OF 2-*O*-(METHYLSULFONYL)FORTIMICIN B DERIVATIVES*

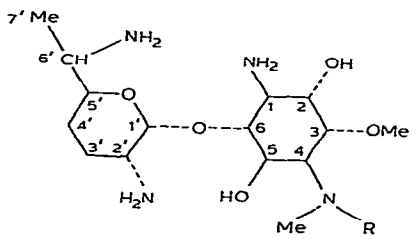
JACK TADANIER†, ROBERT HALLAS, JERRY R. MARTIN, MOMIR CIROVIC, AND RUTH S. STANASZEK
Abbott Laboratories, Department of Chemical Research, North Chicago, IL 60064 (U.S.A.)
(Received April 16th, 1980; accepted for publication in revised form, September 30th, 1980)

ABSTRACT

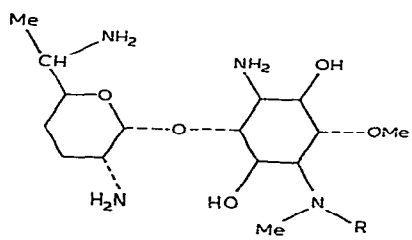
Synthesis of 2-*epi*-fortimicin B has been accomplished by processes involving solvolyses of both 1-*N*-benzyloxycarbonyl- and 1-*N*-acetyl-2-*O*-(methylsulfonyl)fortimicins B, which occur with participation of the carbonyl oxygen atoms of the 1-*N*-acyl groups. The results illustrate both the greater effectiveness of acetamido groups in neighboring-group participation relative to benzyloxycarbonylamino groups, and the sensitivity of the nature of the products to the reaction conditions.

INTRODUCTION

The aminoglycoside antibiotics fortimicin A (**2**) and fortimicin B (**1**) are pseudodisaccharides formed in fermentations by *Micromonospora olivoasterospora*[1]. Fortimicin A, which has the greater antibacterial activity[2], differs structurally from fortimicin B by the attachment of a glycyl group to the N-4 atom of the cyclitol moiety[3]. In the context of a program of chemical modification of the naturally occurring fortimicins[4], undertaken to elucidate structure–activity relationships that



1 R = H
2 R = C(=O)CH2NH2



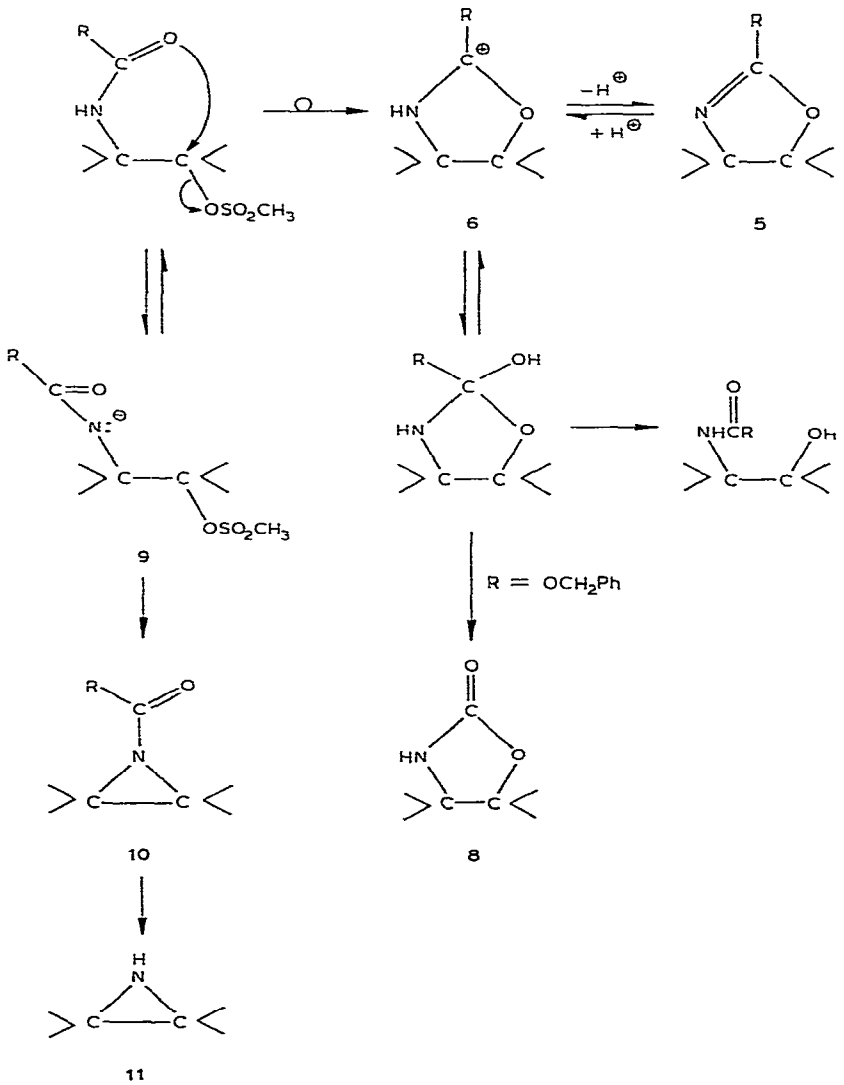
3 R = H
4 R = C(=O)CH2NH2

*Presented, in part, at the 19th Interscience Conference on Antimicrobial Agents and Chemotherapy, October 1979, Boston, Massachusetts, U.S.A.
†To whom inquiries should be addressed.

might lead to a design of modified fortimicins with enhanced therapeutic properties, we have prepared 2-*epi*-fortimicin B (3), which has been used for synthesis of 2-*epi*-fortimicin A (4) as reported in the accompanying paper[5].

DISCUSSION

Among the methods that might be employed to effect epimerization of a suitable fortimicin derivative at C-2, we considered nucleophilic substitution of a fortimicin 2-sulfonic ester. Such a process does not jeopardize the configurational in-



Scheme 1

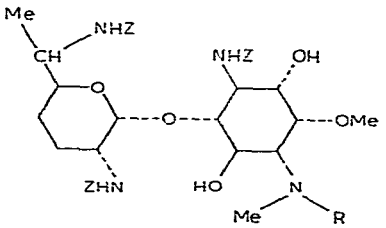
tegrity of adjacent asymmetric centers, but requires protection during solvolysis of vicinal, *trans*-amino groups such as, in the present case, the 1-amino group. Protecting groups commonly used for amino groups are such acyl groups as benzyloxycarbonyl and acetyl. Protection of the 1-amino group of a fortimicin derivative with an acyl group seemed particularly auspicious for the planned solvolytic epimerization, as β-acylamino sulfonic esters are known to be solvolyzed, under suitable conditions, with intramolecular participation of the carbonyl oxygen atom from a conformation in which the leaving and participating groups are antiperiplanar[6]. Such participation of the oxygen atom of the amide carbonyl leads to cyclic oxazolines (**5**) or cyclic oxazolinium ions (**6**), with inversion of configuration at the carbon atom undergoing substitution (Scheme I). As cyclic oxazolines and oxazolinium ions normally undergo hydrolysis to acylamino alcohols (**7**) with cleavage of the carbon–oxygen bond derived from the carbonyl group of the participating *N*-acyl group, the net result is replacement of the methanesulfonate group by hydroxyl with stereospecific inversion of configuration. Such *N*-alkoxycarbonyl groups, as benzyloxycarbonyl groups have the unique feature that the intermediate cyclic oxazolines or cyclic oxazolinium ions may cleave either to alkoxycarbonylamino alcohols (**7**) (R = $PhCH_2O$) or to cyclic carbamates (**8**), with net inversion at the carbon at which substitution occurs (Scheme I).

Examples of solvolytic epimerization effected by *trans*-secondary amide groups in the aminoglycoside field have been reported in which the participating groups have been both acetyl[7] and alkoxycarbonyl[8]. In the case of the alkoxycarbonyl groups, it was found[8] that attempted displacement of the methanesulfonate groups of *trans*-alkoxycarbonylamino methanesulfonates gave *cis*-carbamates with inversion at the carbon atom undergoing substitution.

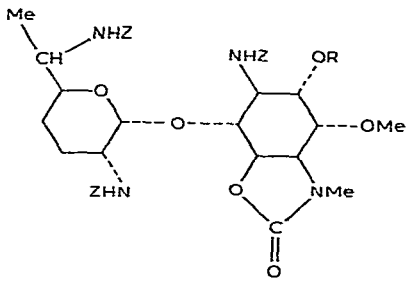
In selecting appropriate conditions for solvolytic epimerization effected by β-acylamino group participation, the ambient nature of the amide group must be considered. Examples of amide-nitrogen participation with formation of acylaziridines **10** or aziridines **11** (Scheme I) have been reported, but are more common with β-alkoxycarbonylamino groups[6b].

Studies in these laboratories with fortimicin A derivatives and various 4-*N*-acylfortimicins B have established the lability of 4-*N*-acyl groups in the presence of mild base[4b]. This is exemplified in the present work by the facile conversion of 1,4,2′,6′-tetra-*N*-acetylfortimicin B (ref. 3) (**25**) into 1,2′,6′-tri-*N*-acetylfortimicin B (**26**), as described later. To avoid the possibility that solvolysis and/or deprotection conditions involved in the epimerization sequence might cleave the 4-*N*-acyl groups of intermediate fortimicin A derivatives, our work was initiated with the object of preparing 2-*epi*-fortimicin B (**3**). It was hoped that the methodology developed[4a] for conversion of fortimicin B into fortimicin A would be applicable to the conversion of 2-*epi*-fortimicin B into 2-*epi*-fortimicin A.

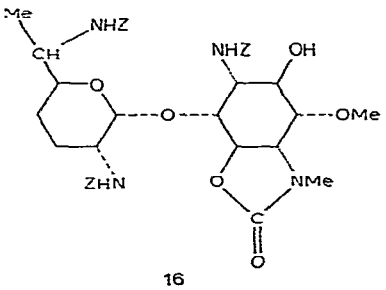
For our initial studies, we chose 1,2′,6′-tri-*N*-benzyloxycarbonyl-2-*O*-(methylsulfonyl)fortimicin B 4,5-carbamate (**15**) as the substrate for solvolytic 2-epimerization. Compound **15** was prepared in a sequence starting with treatment of 1,2′,6′-



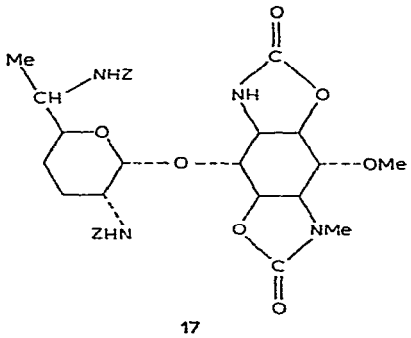
12 R = H
13 R = C(=O)OEt



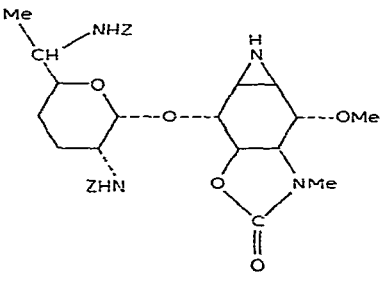
14 R = H
15 R = OMs



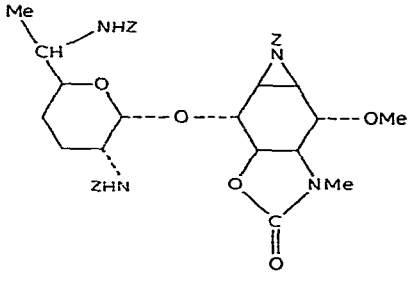
16



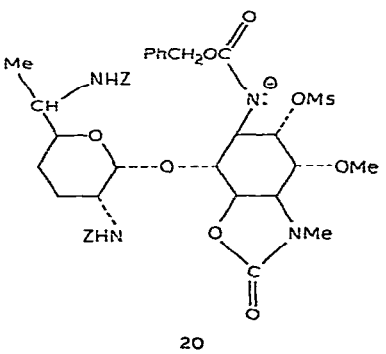
17



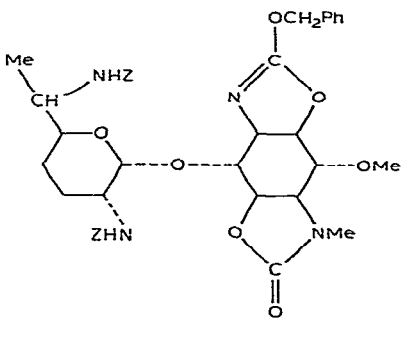
18



19



20



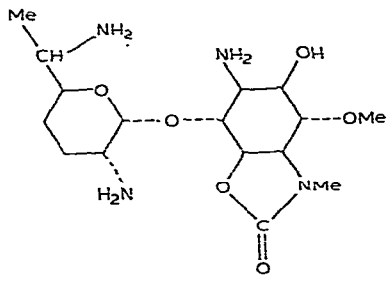
21

tri-*N*-benzyloxycarbonylfortimicin B (**12**) with ethoxycarbonyl chloride, followed by cyclization of the resulting ethoxycarbonyl derivative **13** to the 4,5-carbamate **14**. The preparation of **14** from **12** was conveniently accomplished in a one-flask sequence without isolation of the intermediate 4-*N*-ethoxycarbonyl derivative **13**. The solvolytic stability of the *cis*-4,5-carbamate ring was established by the mode of preparation of **14**. The carbamate **14** was readily converted into the 2-methanesulfonate **15**, which has the benzyloxycarbonyl group at C-1 as the potential neighboring group. It was thus hoped that conditions could be found for efficient conversion of **15** into 1,2',6'-tri-*N*-benzyloxycarbonyl-2-*epi*-fortimicin B 4,5-carbamate (**16**) and/or 2',6'-di-*N*-benzyloxycarbonyl-2-*epi*-fortimicin B 1,2;4,5-biscarbamate (**17**). As methods were available for removal of the benzyloxycarbonyl groups by hydrogenolysis, and removal of the carbamate groups by alkaline hydrolysis, we anticipated that conversion of **16** and/or **17** into 2-*epi*-fortimicin B (**3**) could be effected in good yield.

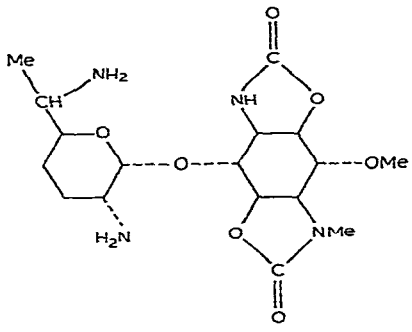
An attempt to effect C-2-epimerization by C-1-carbonyl oxygen participation by solvolysis of the methanesulfonate **15** in methanol in the presence of sodium carbonate gave instead the aziridine derivative **18**, which was characterized by the high-field absorptions attributed to the C-1 and C-2 carbon atoms in the ^{13}C-n.m.r. spectrum[9] and H-1 and H-2 in the ^{1}H-n.m.r. spectrum[10]. As alkoxycarbonylaziridines are known to be hydrolyzed to aziridines under mildly basic conditions[6b], formation of **18** is believed to occur by nitrogen participation of the ambident 1-*N*-benzyloxycarbonylamido group to form the *N*-benzyloxycarbonyl aziridine **19**, which hydrolyzes to the aziridine **18** under the reaction conditions. The exclusive participation of the nitrogen atom of the 1-*N*-benzyloxycarbonyl group under the basic conditions just described, in contrast to the oxygen participation to be discussed next, suggests that the nitrogen participation occurs via the 1-*N*-benzyloxycarbonylamide enolate anion **20** [Scheme I, 9→10→11 (R = $PhCH_2O$)].

Treatment of the methanesulfonate **15** with a two-phase mixture of sodium bicarbonate in aqueous oxolane for five days at 67° gave the 2-*epi*-1,2-benzyloxycarbonyloxazoline **21**. Although the latter was degraded on attempted column chromatography, it was characterized by a strong absorption at 1663 cm^{-1} ($CDCl_3$) in the i.r. spectrum, which is attributed to the C=N absorption, and by its conversion with a refluxing solution of ammonium acetate in aqueous dimethoxycthane to a mixture of 1,2',6'-tri-*N*-benzyloxycarbonyl-2-*epi*-fortimicin B 4,5-carbamate (**16**) and 2',6'-di-*N*-benzyloxycarbonyl-2-*epi*-fortimicin B 1,2;4,5-biscarbamate (**17**), which were separated by column chromatography. The benzyloxycarbonyl groups of the mono- and bis-carbamates (**16** and **17**) were removed by catalytic hydrogenolysis, and the i.r., ^{1}H-n.m.r. and mass spectra of the products **22** and **23**, isolated as the perhydrochloric acid salts, were compatible with their structures.

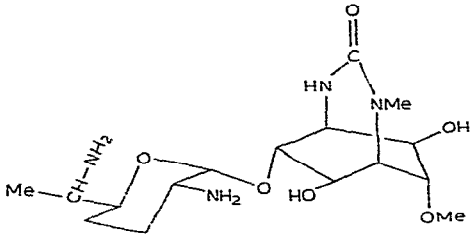
Direct conversion of the methanesulfonate **15** into a mixture of the mono- and bis-carbamates **16** and **17** was effected by solvolysis of **15** in a refluxing solution of ammonium acetate in aqueous 1,2-dimethoxyethane. The products were readily separable by column chromatography, as already described, but for convenience in the preparative sequence, the mixture of **16** and **17** was treated with sodium hydrogen-



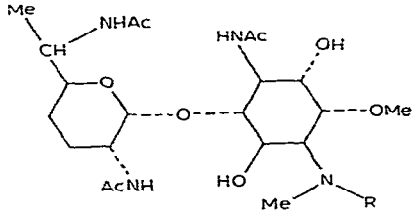
22



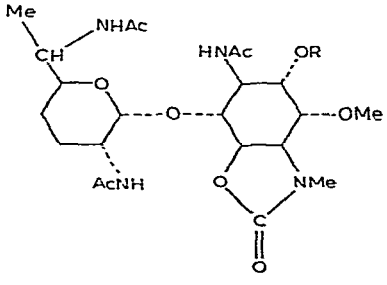
23



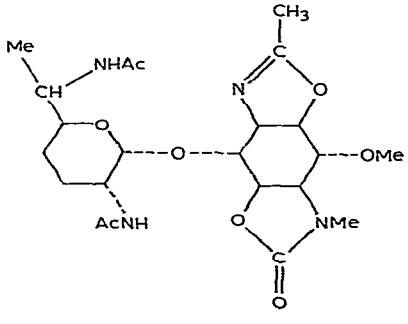
24



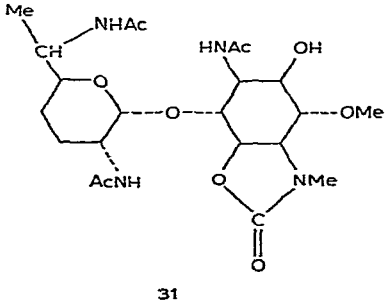
25 R = Ac
26 R = H
27 R = C(=O)OEt



28 R = H
29 R = Ms



30



31

TABLE I

^{13}C-N.M.R. DATA[a]

C	1		3[b]		18[c]	24[b]		28[c]	31[c]	30[c]
	pD 1.8	*β-Shift*	*pD 3.16*	*β-Shift*		*pD 1.8*	*β-Shift*			
1′	96.0	6.5	95.8	5.4	97.0	95.1	5.6	99.2	95.7	96.9
2′	51.9		51.9		49.8	51.6		49.1	49.3	48.3
3′	21.5	5.5	21.5	5.8	26.4	21.7	5.1	24.4	24.2	24.1
4′	26.0		26.2		24.8	26.3		24.6	26.2	26.9
5′	71.0	4.1	71.1	3.9	71.2	70.6	2.6	70.7	70.5	70.8
6′	49.4		49.3		49.5	49.4		48.7	47.6	48.1
7′	15.1	3.4	15.2	3.6	17.9	15.4	3.0	14.9	16.3	18.2
1	53.5		54.9		30.3	56.9		56.3	49.9	67.6
2	65.5	5.7	65.6	3.9	30.3	73.2		69.4	69.6	81.1
3	74.1	5.8	76.6	2.4	74.3	81.7		77.1	77.7	75.7
4	58.1		61.2		57.3	63.0		58.6	60.0	58.9
5	66.0	4.6	68.8	3.7	73.0	74.8		75.6	72.4	74.5
6	74.2	9.9	72.9	6.5	78.6	86.2		79.9	78.3	80.3
–[d]								24.2, 23.3, 23.3	23.3, 23.3, 23.3	23.4, 23.2
–[e]										13.9

[a] ^{13}C-Fourier transform spectra were recorded on a Varian XL-100-15 spectrometer at 25.16 MHz with a Nicolet TT-100 computer, or a JEOLFX90Q spectrometer at 22.50 MHz. ^{13}Dioxane was used as an internal reference in D_2O solution, where a flip angle of 60° was used. Tetramethylsilane was used as an internal reference in $CDCl_3$ solutions, where a flip angle of 40° was used. All solutions were ~ 10% (w/v). [b] D_2O solution; titrations were carried out with aqueous D_2SO_4; pD measurements were made with a pH meter and are uncorrected. [c] $CDCl_3$ solution. [d] Acetyl methyl groups. [e] Oxazoline-ring methyl group.

carbonate in refluxing methanol, which converted the monocarbamate **16** into the biscarbamate **17**.

Partial alkaline hydrolysis of 2-*epi*-fortimicin B 1,2;4,5-biscarbamate (**23**) gave a mixture of 2-*epi*-fortimicin B 1,4-urea (**24**) and 2-*epi*-fortimicin B (**3**). The structure of the urea was established by the ^{1}H-n.m.r. spectrum, which showed the chemical shift of the 4-*N*-methyl protons to be characteristic of an acylated methylamino group, by ^{13}C-n.m.r. studies described later, and the mass spectrum, which showed a molecular-ion peak. Prolonged alkaline hydrolysis completely converted the biscarbamate into 2-*epi*-fortimicin B (**3**). the ^{13}C-n.m.r. parameters of which are listed in Table I.

An alternative synthesis of 2-*epi*-fortimicin B had as its key step participation of the 1-*N*-acetyl group of the labile 1,2′,6′-tri-*N*-acetyl-2-*O*-(methylsulfonyl)fortimicin B 4,5-carbamate (**29**). This process involved preparation of 1,4,2′,6′-tetra-*N*-acetylfortimicin B (**25**) from fortimicin B as previously described[3]. Hydrolysis of the latter with sodium hydrogencarbonate in aqueous methanol gave 1,2′,6′-tri-*N*-acetylfortimicin B (**26**). Treatment of **26** with ethyl chloroformate gave 1,2′,6′-tri-*N*-acetyl-4-*N*-ethoxycarbonylfortimicin B (**27**), which was readily cyclized to the 4,5-carbamate **28** in refluxing aqueous methanol in the presence of sodium hydrogencarbonate. The conversion of (**25**) into **28** may be conveniently accomplished in a one-flask sequence without isolation of the intermediates.

Attempted preparation of the 2-methanesulfonate **29** with methanesulfonic anhydride in pyridine gave the 2-*epi*-fortimicin B 1,2-oxazoline derivative **30**. As the methanesulfonate **29** is the probable intermediate in the conversion of **28** into **30**, the contrast in the reactivity of **29** with that of the isolable 1,2′,6′-tri-*N*-benzyloxycarbonyl-2-*O*-(methylsulfonyl)fortimicin B 4,5-carbamate (**15**) provides a striking, qualitative contrast in the effectiveness of acetamido and benzyloxycarbonylamino groups as participating neighboring groups in solvolysis reactions.

Although the C=N absorption in the i.r. spectrum of the oxazoline **30** was obscured by the amide carbonyl absorption, compound **30** was characterized by its facile conversion into 1,2′,6′-tri-*N*-acetyl-2-*epi*-fortimicin B 4,5-carbamate **31** under conditions of mild, acid-catalyzed hydrolysis, by the molecular-ion peak in the high-resolution mass spectrum, and by the ^{13}C-n.m.r. spectrum as described later.

Base-catalyzed hydrolysis of **31** gave 2-*epi*-fortimicin B (**3**), identical with that prepared as already described.

Relevant ^{13}C-n.m.r. data are recorded in Table I. 2-*epi*-Fortimicin B 1,4-urea (**24**) was characterized by the β-shifts of the resonances of the C-1′, C-3′, C-5′ and C-7′ carbon atoms of the diamino sugar ring on protonation of the 2′- and 6′-amino groups. In contrast, the chemical shifts of the carbon atoms of the cyclitol ring of **24** were essentially insensitive to pH, as the cyclitol-ring nitrogen atoms of **24** were acylated.

The only appreciable difference between the ^{13}C-n.m.r. spectra of the 2-epimeric 1,2′,6′-tri-*N*-acetylfortimicin B 4,5-carbamates **28** and **31** was the difference between the chemical shifts of the C-1 atoms. The higher-field chemical shift of C-1

of the 2-*epi* derivative **31** is believed to be a consequence of intramolecular hydrogen-bonding of the 2-hydroxyl hydrogen with the *cis*-related 1-acetamido group.

The 2-*epi*-oxazoline derivative **30** is characterized by downfield shifts of the C-1 and C-2 resonances relative to those of the 2-*epi*-1-acetamido-2-hydroxy derivative **31**. In addition, the resonance of the methyl carbon atoms of the oxazoline ring lies at much higher field than those of the acetyl methyl carbon atoms.

EXPERIMENTAL

General methods. — Optical rotations were determined with a Hilger and Watts polarimeter. I.r. spectra were recorded with a Perkin–Elmer Model 521 grating spectrometer. ^{1}H-N.m.r. spectra were determined at 100 MHz with a Varian Associates HA-100 spectrometer. Chemical shifts determined with deuteriochloroform solutions are reported from internal Me_4Si. Chemical shifts determined with D_2O solutions are reported from external Me_4Si. Mass spectra were obtained with an A.E.I. MS-902 spectrometer operated at 70 eV and 100–150° with a direct-probe insert. Silica gel for column chromatography was that of Merck (Darmstadt), 70–230 mesh. Ratios for chromatography solvents are expressed by volume. Solutions were dried with magnesium sulfate. Solvents were evaporated under diminished pressure on a rotary evaporator.

1,2′,6′-Tri-N-*benzyloxycarbonyl-4*-N-*ethoxycarbonylfortimicin B* (**13**). — To a stirred solution of 1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (ref. 4) (**12**, 3.02 g) methanol (130 mL), and 60 mL of a solution prepared from sodium hydrogencarbonate (3.02 g) in water (72 mL), was added ethyl chloroformate (0.90 mL). Stirring was continued for 3 h at room temperature. The major portion of the methanol was evaporated and the residue was partitioned between chloroform and 5% aqueous sodium hydrogencarbonate. The chloroform solution was separated and washed with water. The aqueous solutions were washed in series with four, 100-mL portions of chloroform. The chloroform solutions were combined, dried, and evaporated to give 3.36 g (100%) of **13** as a white glass; $\tilde{\nu}_{max}^{CDCl_3}$ 3555, 3437, 1707, and 1658 cm^{-1}; $\delta(CDCl_3)$: 1.15 d ($J_{6',7'}$ 6.4 Hz, 6′-CH_3), 3.02 (NCH_3), and 3.43 (OCH_3).

1′,2′,6′-Tri-N-*benzyloxycarbonylfortimicin B 4,5-carbamate* (**14**). — (*a*) A solution of compound **13** (13.0 g), sodium hydrogencarbonate (5.3 g), and methanol (370 mL) was boiled for 1.5 h under reflux. The solution was evaporated, and the residue triturated with chloroform. The chloroform supernatant was filtered, and the filtrate evaporated. The residue was chromatographed on 850 g of silica gel with 9:1 benzene–ethanol to yield 10.9 g (89%) of pure **14**, identical with that prepared as described next.

(*b*) A stirred suspension of compound **12** (18.2 g), sodium hydrogencarbonate (12.6 g), ethyl chloroformate (8.1 mL), and methanol (750 mL) was kept overnight at room temperature and then boiled for 1.5 h under reflux. The solvent was evaporated and the residue triturated with chloroform. The supernatant was filtered and the filtrate evaporated leaving 16.7 g (89%) of **14**. An analytical sample was

prepared by chromatographing 1.08 g of the product on a column of 100 g of silica gel with 9 : 1 benzene–ethanol to give 0.602 g of pure **14**; $[\alpha]_D^{21}$ +2.5° (*c* 1.0, methanol); $\tilde{\nu}_{max}^{CDCl_3}$ 3562, 3438, 3320, 1759, and 1706 cm^{-1}; $\delta(CDCl_3)$: 0.98 d ($J_{6',7'}$ 6.0 Hz, 6'-CH_3), 2.83 (NCH_3), and 3.44 (OCH_3).

Anal. Calc. for $C_{40}H_{48}N_4O_{12}$: C, 61.84; H, 6.23; N, 7.21. Found: C, 61.62; H, 6.36; N, 7.16.

*1,2',6'-Tri-*N*-benzyloxycarbonyl-2-*O*-(methylsulfonyl)fortimicin B 4,5-carbamate* (**15**). — To a stirred solution of **14** (0.155 g) in pyridine (2 mL), cooled in an ice bath, was added methanesulfonic anhydride (0.42 g). Stirring was continued with cooling for 1 h and then overnight at room temperature. The resulting mixture was poured into of 5% aqueous sodium hydrogencarbonate and extracted with chloroform. The extract was washed with 5% aqueous sodium hydrogencarbonate, dried, and evaporated to give 0.169 g (100%) of **15** as a glass; $[\alpha]_D^{21}$ −4.2° (*c* 1.0, methanol); $\tilde{\nu}_{max}^{CDCl_3}$ 3440, 3300, 1760, and 1708 cm^{-1}; $\delta(CDCl_3)$: 1.00 d ($J_{6',7'}$ 6.3 Hz, 6'-CH_3), 2.83 (NCH_3), 2.99 (OSO_2Me), and 3.52 (OCH_3).

Anal. Calc. for $C_{41}H_{50}N_4O_{14}S$: C, 57.60; H, 5.90; N, 6.55. Found: C, 58.79; H, 6.28; N, 7.12.

*2',6'-Di-*N*-benzyloxycarbonyl-2-deoxy-1,2-epimino-2-*epi*-fortimicin B 4,5-carbamate* (**18**). — A stirred mixture of compound **15** (25.6 g), potassium carbonate (30.2 g), water (180 mL), and methanol (360 mL) was boiled for 65 min under reflux and allowed to cool. The crystalline product that separated was collected by filtration and dissolved in chloroform (1 L). The solution was washed with water, dried, and evaporated to give a quantitative yield of **18**. The analytical sample was prepared by recrystallization from ethanol (needles); m.p. 193–196°, $[\alpha]_D^{19}$ +39° (*c* 1, pyridine); $\tilde{\nu}_{max}^{CDCl_3}$ 3440, 1747, 1708, and 1500 cm^{-1}; $\delta(CDCl_3)$: 1.17 d ($J_{6',7'}$ 6.5 Hz, 6'-CH_3), 2.85 (NCH_3), 3.39 (OCH_3), 2.36 m (H-1, H-2), and 4.97 d ($J_{1',2'}$ 3.2 Hz, 1'-H).

Anal. Calc. for $C_{32}H_{40}N_4O_9$: C, 61.52; H, 6.45; N, 8.97. Found: C, 60.93; H, 6.33; N, 8.83.

*1,2',6'-Tri-*N*-benzyloxycarbonyl-2-*epi*-fortimicin B 4,5-carbamate* (**16**) *and 2',6'-di-*N*-benzyloxycarbonyl-2-*epi*-fortimicin B 1,2;4,5-bicarbamate* (**17**). — (*a*) A stirred solution of compound **15** (0.427 g), ammonium acetate (0.116 g), water (3 mL), and 1,2-dimethoxyethane (6 mL) was boiled for 21 h under reflux. The resulting solution was cooled and poured into 5% aqueous sodium hydrogencarbonate. Extraction with chloroform gave 0.386 g of a mixture of **16** and **17**.

A sample (3.01 g) of the mixture of **16** and **17**, prepared as just described, was chromatographed on a column of 250 g of silica gel with 9 : 1 ethyl acetate–1,2-dichloroethane. Initial fractions gave 1.24 g (41%) of **16**; $[\alpha]_D^{21}$ +8.5° (*c* 1.0, methanol); $\tilde{\nu}_{max}^{CDCl_3}$ 3442, 3328 (s), 1743, and 1698 cm^{-1}; $\delta(CDCl_3)$: 1.05 d ($J_{6',7'}$ 6.2 Hz, 6'-CH_3), 2.83 (NCH_3), and 3.43 (OCH_3).

Anal. Calc. for $C_{40}H_{48}N_4O_{12}$: C, 61.48; H, 6.23; N, 7.21. Found: C, 61.64; H, 6.37; N, 7.25.

Further elution of the column gave 0.965 g (37%) of **17**; $[\alpha]_D^{21}$ +7.3° (*c* 1.0,

methanol); $\tilde{\nu}_{max}^{CDCl_3}$ 3443, 3323, 1749, and 1699 cm^{-1}; $\delta(CDCl_3)$: 1.17 d ($J_{6',7'}$ 6.8 Hz, 6'-CH_3), 2.94 (NCH_3), and 3.52 (OCH_3).

Anal. Calc. for $C_{33}H_{41}N_4O_{11}$: C, 59.18; H, 6.17; N, 8.37. Found: C, 59.50; H, 6.06; N, 8.09.

(*b*) A solution of 2',6'-di-*N*-benzyloxycarbonyl-2-*epi*-fortimicin B 1,2-(2-benzyloxy)oxazoline 4,5-carbamate (**21**, 1.5 g), ammonium acetate (0.435 g), water (11.3 mL) and 1,2-dimethoxyethane (23 mL) was boiled under reflux for 3 h. A similar reaction was carried out with an additional 0.100 g of **21**, 0.029 g of ammonium acetate, 0.75 mL of water, and 1.5 mL of 1,2-dimethoxyethane. The two solutions were combined and shaken with a mixture of 5% aqueous sodium hydrogencarbonate and chloroform. The chloroform solution was separated and the aqueous solution washed with additional chloroform. The extracts were combined, dried, and evaporated to 1.46 g of a glass. The latter was chromatographed on a column of 150 g of silica gel with 1 : 1 ethyl acetate–1,2-dichloroethane to yield 0.583 g (38%) of **16** and 0.153 g (12%) of **17**. The products (**16** and **17**) were identical with those prepared directly from the methanesulfonate **15** with ammonium acetate in aqueous 1,2-dimethoxyethane as already described.

An additional 0.256 g of a mixture of **17** and a more-polar product (~1 : 1 mixture by t.l.c.) was isolated from later chromatography fractions.

2',6'-Di-N-*benzyloxycarbonyl-2*-epi-*fortimicin B 1,2;4,5-biscarbamate* (**17**). — A stirred solution of 13.0 g of a mixture of **16** and **17**, prepared from the methanesulfonate **15** (15.4 g) as already described, sodium hydrogencarbonate (8.0 g), and methanol (350 mL) was boiled for 1 h under reflux. The resulting solution was cooled, and shaken with a mixture of chloroform and 5% aqueous sodium hydrogencarbonate. The organic extract was dried and evaporated to give 13.0 g of product. Chromatography of the latter on a column of 450 g of silica gel packed and eluted with 9 : 1 ethyl acetate–1,2-dichloroethane gave 9.34 g (78%) of **17**, identical with that prepared as already described.

2',6'-Di-N-*benzyloxycarbonyl-2*-epi-*fortimicin B 1,2-(2-benzyloxy)oxazoline 4,5-carbamate* (**21**). — A stirred mixture of compound **15** (2.0 g), sodium hydrogencarbonate (1.22 g), water (7.4 mL), and oxolane (30 mL) was heated for 5 days at 67°. The resulting mixture was poured into 5% aqueous sodium hydrogencarbonate. The aqueous suspension was extracted with chloroform and the dried extract evaporated to give 1.77 g (100%) of **21** as a light-yellow glass; $[\alpha]_D^{23}$ +6° (*c* 1.0, methanol); $\tilde{\nu}_{max}^{CDCl_3}$ 3444, 3327, 1759, 1711, and 1665 cm^{-1}; $\delta(CDCl_3)$: 1.19 d ($J_{6',7'}$ 6.6 Hz, 6'-CH_3), 2.92 (NCH_3), and 3.47 (OCH_3).

2-epi-*Fortimicin B 1,2;4,5-biscarbamate* (**23**). — Compound **17** (1.0 g) in 0.4M hydrochloric acid in methanol (30 mL) was hydrogenated for 4 h under 3 atm of hydrogen in the presence of 1 g of 5% palladium-on-carbon. The catalyst was removed by filtration and the solvent was evaporated. Residual hydrochloric acid was removed by evaporation of methanol from the residue, leaving 0.717 g (100%) of the dihydrochloride salt of **23**; $[\alpha]_D^{22}$ +36° (*c* 1.0, methanol); $\tilde{\nu}_{max}^{KBr}$ 1738 and 1723 cm^{-1}; $\delta(D_2O)$: 1.79 d ($J_{6',7'}$ 6.8 Hz, 6'-CH_3), 3.42 (NCH_3), 4.04 (OCH_3), and

5.56 d ($J_{1',2'}$ 3.6 Hz, 1'-H); (M^{+}·) Calc. for $C_{17}H_{28}N_4O_7$: 400.1958, measured 400.1933.

2-epi-*Fortimicin B* (3) *and 2*-epi-*Fortimicin B 1,4-urea* (**24**). — (*a*) A solution of the dihydrochloride salt (0.680 g) of compound **23** in M aqueous sodium hydroxide (80 mL) was heated for 24 h at 75°. The resulting solution was brought to pH 7 by addition of M hydrochloric acid and then evaporated to dryness. Residual water was removed by evaporation of ethanol from the product. To the residue was added ethanol (100 mL), and the resulting suspension was briefly heated to boiling, cooled, and filtered. The insoluble residue was washed thoroughly with ethanol. The ethanol was evaporated, leaving 0.550 g of a light-yellow glass. This material was chromatographed on a column of 40 g of silica gel with the lower phase of a 2:2:1:1 mixture of chloroform–methanol–concentrated ammonium hydroxide–water. Early fractions gave 0.160 g (32%) of 3, identical with that prepared as described later.

Further elution gave 0.088 g (16%) of **24**; $[\alpha]_D^{22}$ +12° (*c* 1.0, methanol); $\delta(D_2O)$: 1.48 ($J_{6',7'}$ 6.6 Hz, 6'-CH_3), 3.50 (NCH_3), 3.96 (OCH_3), and 5.46 d ($J_{1',2'}$ 3.4 Hz, 1'-H); (M^{+}·) calc. for $C_{16}H_{30}N_4O_6$: 374.2165, measured 374.2193; (diamino sugar fragment), calc. for $C_7H_{15}N_2O$: 143.1184, measured 143.1173; (cyclitol fragment) calc. for $C_9H_{15}N_2O_4$: 215.1032, measured 215.1035.

(*b*) A solution of the dihydrochloride salt (4.94 g) of compound **23** in M aqueous sodium hydroxide (500 mL) was heated for 66 h at 75°. The resulting solution was cooled, brought to pH 7 with M hydrochloric acid, and evaporated to dryness. The residue was treated with several portions of boiling ethanol, and the resulting suspensions were filtered and combined. Evaporation of the ethanol left 4.12 g of a glass. The product was chromatographed on a column of 450 g of silica gel packed and eluted with 10:10:1:1 chloroform–methanol–concentrated ammonium hydroxide–water to yield 3.0 g (82%) of 3; $[\alpha]_D^{23}$ +78° (*c* 1.0, methanol); $\delta(D_2O)$: 1.50 d ($J_{6',7'}$ 6.8 Hz, 6'-CH_3); 2.83 (NCH_3), 3.99 (OCH_3), and 5.38 d ($J_{1',2'}$ 3.4 Hz, 1'-H); (M^{+}·) Calc. for $C_{15}H_{32}N_4O_5$: 348.2373, measured 348.2391; (diamino sugar fragment) calc. for $C_7H_{15}N_2O$: 143.1184, measured 143.1180; (cyclitol fragment) calc. for $C_8H_{19}N_2O_4$: 207.1345, measured 207.1346.

(*c*) A stirred solution of 1,2',6'-tri-*N*-acetyl-2-*epi*-fortimicin B 4,5-carbamate (**31**, 1.50 g) in 2M aqueous sodium hydroxide (50 mL) under an atmosphere of nitrogen was heated for 2 days at 95°. The resulting solution was cooled to room temperature and brought to pH 4 by addition of 2M hydrochloric acid. The solvent was evaporated and residual water was removed by evaporation of methanol. The residue was triturated several times with methanol, and the supernatant solution was separated from the insoluble salts. The extracts were combined and evaporated, leaving 2.68 g of brown solid. The latter was chromatographed on a column of 90 g of silica gel with 10:10:0.1 chloroform–methanol–concentrated ammonium hydroxide to yield 1.21 g of product. The latter was dissolved in an excess of 0.2M hydrochloric acid in methanol. The solvent was evaporated and residual water removed by evaporation of methanol, leaving 1.18 g (74%) of the tetrahydrochloride salt of 2-*epi*-fortimicin B (**3**).

An aqueous solution of the tetrahydrochloride salt of 2-*epi*-fortimicin B (**3**) was passed through a column containing an excess of AG2-X8 (OH^-) resin. Water was evaporated from the fractions containing the product and residual water was removed by evaporation of methanol to give 2-*epi*-fortimicin B (**3**), identical with that prepared from 2-*epi*-fortimicin B 1,2;4,5-biscarbamate (**23**) as already described.

1,2′,6′-Tri-N-*acetylfortimicin B* (**26**). — A stirred mixture of 1,4,2′,6′-tetra-*N*-acetylfortimicin B[3] (**25**, 33.4 g), sodium hydrogencarbonate (20 g), water (300 mL), and methanol (1 L) was boiled overnight under reflux. The major portion of the solvent was evaporated and residual water was removed by evaporation of several portions of ethanol. The residue was triturated with several portions of warm chloroform. The supernatant was filtered and evaporated, leaving 29.1 g of crude **26**.

A sample (5.13 g) of **26** thus prepared was chromatographed on a column of 400 g of silica gel with 18:6:0.5 chloroform–95% aqueous methanol–concentrated-ammonium hydroxide to yield 4.37 g (81% based on **25**) of **26**; $[\alpha]_D^{21}$ +28° (*c* 1.0, methanol); $\tilde{\nu}_{max}^{CDCl_3}$ 3553, 3439, 3333, and 1655 cm^{-1}: $\delta(CDCl_3)$: 1.16 d ($J_{6',7'}$ 6.2 Hz, 6′-CH_3), 1.94, 1.98, 1.99 ($COCH_3$), 2.41 (NCH_3), 3.45 (OCH_3), and 5.2 d ($J_{1',2'}$ 3 Hz, 1′-H); ($M^{+\cdot}$) calc. for $C_{21}H_{38}N_4O_8$: 474.2690, measured 474.2685; (diamino sugar fragment) calc. for $C_{11}H_{19}N_2O_3$: 227.1396, measured 227.1392; (cyclitol fragment) calc. for $C_{10}H_{21}N_2O_4$: 249.1451; measured 249.1439.

1,2′,6′-Tri-N-*acetyl-4*-N-*ethoxycarbonylfortimicin B* (**27**). — A stirred solution of 1,2′,6′-tri-*N*-acetylfortimicin B (**26**, 0.613 g), ethyl chloroformate (0.270 mL), and methanol (30 mL) was stirred for 4.5 h at room temperature. Solid sodium hydrogencarbonate (0.427 g) was added and stirring was continued for 1 h. The resulting suspension was filtered, and the filtrate was evaporated to dryness. The residue was washed with chloroform, and the supernatant solution was filtered and evaporated to dryness, leaving 0.629 g of white glass. The latter was chromatographed on a column of 60 g of silica gel with 9:1 chloroform–methanol to yield 0.379 g (54%) of **27**; $\tilde{\nu}_{max}^{CDCl_3}$ 3537, 3337, and 1657 cm^{-1}: $\delta(CDCl_3)$: 1.15 d ($J_{6',7'}$ 6.4 Hz, 6′-CH_3), 1.27 t (*J* 7.7 Hz, OCH_2CH_3), 1.97, 1.98, 1.99 ($COCH_3$), 3.02 (NCH_3), and 3.42 (OCH_3).

1,2′,6′-Tri-N-*acetylfortimicin B 4,5-carbamate* (**28**). — (*a*) A solution prepared from compound **27** (0.347 g), 1,5-diazabicyclo[5.4.0]-5-undecene (0.418 g), and benzene (20 mL) was heated for 5 days under reflux. The benzene was evaporated and the residue was chromatographed on a column of 60 g of silica gel with 87:13 chloroform–methanol to yield 0.279 g (88%) of **28**, identical with that prepared as described next.

(*b*) To a stirred suspension of crude 1,2′,6′-tri-*N*-acetylfortimicin B (**26**, 29.1 g), prepared as just described, sodium hydrogencarbonate, (21 g), and methanol (1.4 L) was added dropwise ethyl chloroformate (14 mL). The resulting suspension was stirred overnight at room temperature, and then boiled for 1.5 h under reflux. The methanol was evaporated off and the residue was triturated with chloroform. The supernatant was filtered and the chloroform was evaporated, leaving 30.9 g of glass. The latter was chromatographed on a column of 750 g of silica gel with 4:1 1,2-

dichloroethane–methanol to yield 26.9 g (100%) of **28**; $[\alpha]_D^{21}$ +4.8° (*c* 1.0, methanol), $\tilde{\nu}_{max}^{CDCl_3}$ 3552, 3440, 3402, 3315, 1753, and 1658 cm^{-1}; $\delta(CDCl_3)$: 1.18 d ($J_{6',7'}$ 7.0 Hz, 6'-CH_3), 2.00 (3 H), 2.04 (6 H) ($NHCOCH_3$), 2.91 (NCH_3), 3.48 (OCH_3), and 4.88 d ($J_{1',2'}$ 3 Hz, 1'-H); (M^+·) calc. for $C_{22}H_{36}N_4O_9$: 500.2482; measured 500.2473; (diamino sugar fragment) calc. for $C_{11}H_{19}N_2O_3$: 227.1396; measured 227.1390; (cyclitol fragment) calc. for $C_{11}H_{17}N_2O_5$: 257.1137; measured 257.1132.

*2',6',Di-*N*-acetyl-2-*epi*-fortimicin B 1,2-(2-methyl)oxazoline-4,5-carbamate* (**30**). — To a stirred solution of compound **28** (5.09 g) in pyridine (50 mL), cooled in an ice bath, was added methanesulfonic anhydride (3.50 g). Stirring was continued with cooling for 1 h, and then overnight at room temperature. The resulting mixture was shaken with a mixture of 5% aqueous sodium hydrogencarbonate and chloroform. The chloroform solution was separated, and the aqueous solution again extracted with chloroform. The combined extracts were dried and evaporated to give 4.47 g (97%) of **30**; $[\alpha]_D^{22}$ +38° (*c* 1.0, methanol); $\tilde{\nu}_{max}^{CDCl_3}$ 3442, 3321, 1746, and 1649 cm^{-1}; $\delta(CDCl_3)$: 1.19 d ($J_{6',7'}$ 6.2 Hz, 6'-CH_3), 1.99 ($NHCOCH_3$), 2.04 (N=C-CH_3), 2.97 (NCH_3), and 3.56 (OCH_3); (M^+·) calc. for $C_{22}H_{34}N_4O_8$: 482.2377, measured 482.2364; (cyclitol fragment) calc. for $C_{11}H_{17}N_2O_5$: 257.1137, measured 257.1161; (diamino sugar fragment) calc. for $C_{11}H_{19}N_2O_3$: 227.1396, measured 227.1415.

*1,2',6'-Tri-*N*-acetyl-2-*epi*-fortimicin B 4,5-carbamate* (**31**). — A stirred solution of compound **30** (4.40 g), 0.4M hydrochloric acid (45 mL), and oxolane (180 mL) was kept for 0.5 h at room temperature. Sodium hydrogencarbonate (150 mL, 5% aqueous) was added. The major portion of the solvent was evaporated and residual water was removed by evaporation of ethanol. The residue was triturated with 400 mL of boiling chloroform. The supernatant solution was separated by filtration and the chloroform-insoluble residue washed several times with fresh chloroform. The extracts were combined and evaporated to leave 4.66 g of a glass. The latter was chromatographed on a column of 250 g of silica gel with 9:1 dichloromethane–methanol to yield 3.64 g (80%) of **31**; $[\alpha]_D^{23}$ +31° (*c* 1.0, methanol); $\tilde{\nu}_{max}^{CDCl_3}$ 3439, 3320, 1752, and 1652 cm^{-1}; $\delta(CDCl_3)$; 1.21 d ($J_{6',7'}$ 6.7 Hz, 6'-CH_3), 1.98, 2.00, 2.03 ($COCH_3$), 2.89 (NCH_3), and 3.48 (OCH_3); (M^+·) calc. for $C_{22}H_{36}N_4O_9$: 500.2502, measured 500.2502; (diamino sugar fragment) calc. for $C_{11}H_{19}N_2O_3$: 227.1396, measured 227.1376; (cyclitol fragment) calc. for $C_{11}H_{19}N_2O_6$: 275.1243, measured 275.1242.

ACKNOWLEDGMENTS

The authors thank Ms. S. Mueller for mass spectra, Mr. W. H. Washburn for i.r.-spectra, Mr. J. E. Leonard for t.l.c. assays, and Ms. Julie Hood for microanalyses.

REFERENCES

1 T. NARA, M. YAMAMOTO, I. KAWAMOTO, T. TAKEYAMA, R. OKACHI, S. TAKASAWA, T. SATO, AND S. SATO, *J. Antibiot.*, 30 (1977) 533–540.

2 R. OKACHI, S. TAKASAWA, T. SATO, M. YAMAMOTO, I. KAWAMOTO, AND T. NARA, *J. Antibiot.*, 30 (1977) 541–551.
3 R. S. EGAN, R. S. STANASZEK, M. CIROVIC, S. L. MUELLER, J. TADANIER, J. R. MARTIN, P. COLLUM, A. W. GOLDSTEIN, R. L. DEVAULT, A. C. SINCLAIR, E. E. FAGER, AND L. A. MITSCHER, *J. Antibiot.*, 30 (1977) 552–563.
4 (a) J. TADANIER, J. R. MARTIN, P. KURATH, A. W. GOLDSTEIN, AND P. JOHNSON, *Carbohydr. Res.*, 79 (1980) 91–102; (b) J. TADANIER, J. R. MARTIN, P. JOHNSON, A. W. GOLDSTEIN, AND R. HALLAS, *ibid.*, 85 (1980) 61–71; (c) P. KURATH, D. GRAMPOVNIK, J. TADANIER, J. R. MARTIN, R. S. EGAN, R. S. STANASZEK, M. CIROVIC, W. H. WASHBURN, P. HILL, D. A. DUNNIGAN, J. E. LEONARD, P. JOHNSON, AND A. W. GOLDSTEIN, *J. Antibiot.*, 32 (1979) 884–890.
5 J. TADANIER, R. HALLAS, J. R. MARTIN, AND M. CIROVIC, *Carbohydr. Res.*, 92 (1981) 207–218.
6 (a) R. D. GUTHRIE, *Introduction to Carbohydrate Chemistry*, 4th edn., Clarendon Press, Oxford, 1974, pp. 58–59; (b) O. C. DERMER AND G. E. HAM, *Ethylenimine and Other Aziridines, Chemistry and Applications*, Academic Press, New York, 1974, pp. 48–52.
7 P. J. L. DANIELS, J. WEINSTEIN, R. W. TEACH, AND J. MORTON, *J. Antibiot.*, 27 (1974) 150–154.
8 (a) H. SANO, T. TSUCHIYA, S. KOBAYASHI, H. UMEZAWA, AND S. UMEZAWA, *Bull. Chem. Soc. Jpn.*, 50 (1977) 975–978; (b) R. E. CARNEY, J. B. MCALPINE, M. JACKSON, R. S. STANASZEK, W. H. WASHBURN, M. CIROVIC, AND S. L. MUELLER, *J. Antibiot.*, 31 (1978) 441–450.
9 J. B. STOTHERS, *Carbon-13 NMR Spectroscopy*, Academic Press, New York, 1972, pp. 269–271.
10 L. M. JACKMAN AND S. STERNHELL, *Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry, 2nd edn.*, Pergamon Press, London, 1969, pp. 100–102.