Carbohydrate Research, 92 (1981) 168–175
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

Note

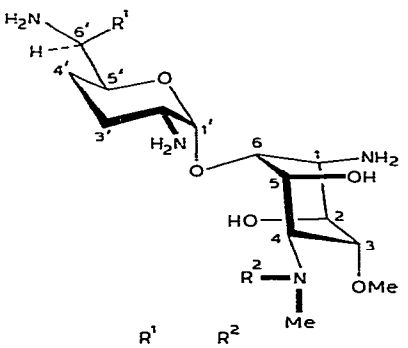
Formation of 2′-*N*-acetylfortimicins by acyl migration of 4-*N*-acylfortimicins

KUNIKATSU SHIRAHATA, TAKAO IIDA, MORIYUKI SATO, AND KENICHI MOCHIDA
Tokyo Research Laboratory of Kyowa Hakko Kogyo Co. Ltd., 3-6-6 Asahimachi, Machida, Tokyo 194 (Japan)

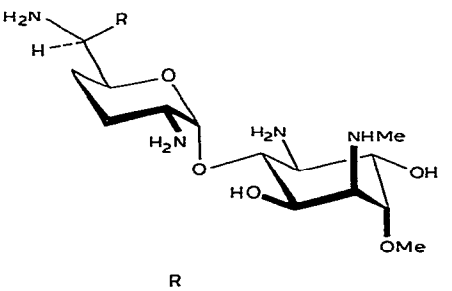
(Received October 24th, 1979; accepted for publication in revised form, June 25th, 1980)

During studies on the structures of fortimicins (Chart 1), it was found that hydrolysis of fortimicin D (FM-D, $C_{16}H_{33}N_5O_6$) with barium hydroxide gave a rearrangement product (**1**) and fortimicin KE (FM-KE), together with glycine[1]. The rearrangement product contains a glycyl group (i.r., 1650 cm^{-1}; ^{13}C n.m.r., 44.7 and 175.5 p.p.m., Table I) its molecular formula is the same as that of FM-D (M^+ = *m/z* 391.2431, $C_{16}H_{33}N_5O_6$ requires 391.2430), and it showed intense peaks at *m/z* 374, 361, 217, 207, 186, and 181 in its mass spectrum. The peak at *m/z* 186 strongly suggested that the glycyl group is present on the purpurosamine moiety instead of the cyclitol (fortamine B, Scheme 1). That the glycyl group in **1** is located on the 2′-amino group was shown by the lack of significant protonation (deuteration) shifts of the C-1′ and C-3′ signals in its ^{13}C-n.m.r. spectrum (Table II).

For compounds containing fortamine B moieties, the meaning of "deuteration

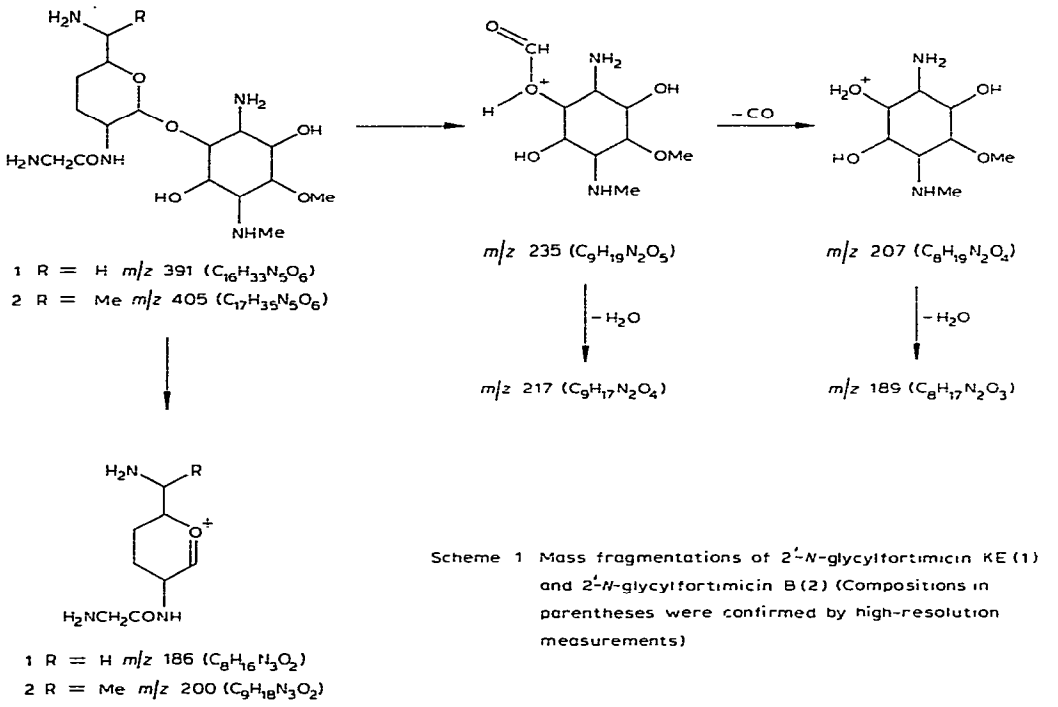


	R^1	R^2
fortimicin A	Me	$COCH_2NH_2$
fortimicin B	Me	H (in acidic media)
fortimicin C	Me	$COCH_2NHCONH_2$
fortimicin D	H	$COCH_2NH_2$
fortimicin KE	H	H (in acidic media)



	R
fortimicin B	Me (in basic media)
fortimicin KE	H (in basic media)

Chart 1. Structures of the fortimicins



Scheme 1 Mass fragmentations of 2′-*N*-glycylfortimicin KE (1) and 2′-*N*-glycylfortimicin B (2) (Compositions in parentheses were confirmed by high-resolution measurements)

TABLE I

^{13}C-N.M.R. CHEMICAL SHIFTS OF 2′-*N*-ACYLFORTIMICINS

Compounds	*2′-N-Glycyl-FM-KE* (1)	*2′-N-Glycyl-FM-B* (2)	*2′-N-Hydantoyl-FM-B* (3)
Carbon atoms	pD 11.3	pD 10.9	pD 10.8
1	54.2	54.3	54.2
2	71.0	71.0	71.0[a]
3	79.8	79.8	79.8
4	61.3	61.2	61.3
5	71.0	71.0	71.2[a]
6	83.1	83.6	82.1
3-OMe	59.3	59.2	59.3
4-NMe	35.5	35.5	35.3
1′	99.2	99.4	98.8
2′	50.0	50.1	50.0
3′	23.9	23.8	23.6
4′	27.8	26.8	26.9
5′	71.4	75.3	74.9
6′	45.8	50.4	50.6
6′-Me		18.8	18.3
CH_2NH	44.7	44.7	43.9
CO	175.5	175.7	173.0
$CONH_2$			162.1

[a]These assignments may be interchanged.

TABLE II

DEUTERATION SHIFTS IN THE ^{13}C-N.M.R. SPECTRA (p.p.m.)

Compounds	*C-1'*	*C-2'*	*C-3'*
FM-KE	5.8	1.2	5.2
2'-*N*-Gly-FM-KE (1)	1.0	1.0	1.1
FM-B	6.1	1.0	5.4
2'-*N*-Gly-FM-B (2)	1.4	1.1	1.2
2'-*N*-Hydantoyl-FM-B (3)	1.0	1.1	0.5

shifts" (or β-shifts) is different from that in the ordinary sense, because the conformations of their fortamine moieties in acids are different from those in basic media[2]. Nevertheless, the stereochemistry of the fortamine moieties are considered to exert little effect on the ^{13}C-n.m.r. chemical shifts of the purpurosamine moieties; for example, the carbon atoms of the purpurosamine moieties of FM-D and FM-KE in basic media resonate within 0.2 p.p.m. of the same frequencies, except for the anomeric carbon atoms. The anomeric carbon atom of the former resonates 2.2 p.p.m. to higher field than that of the latter[1]. The rearrangement product, therefore, was established to be 2'-N-glycylfortimicin KE. The same type of acyl migration from N-4 to N-2' was found in fortimicin A (FM-A)[2] and fortimicin C (FM-C)[1]. The rearrangement products are 2'-*N*-glycylfortimicin B (**2**) and 2'-*N*-hydantoylfortimicin B (**3**), respectively. Acyl migrations in FM-A and 4-*N*-acetylfortimicin B have also been investigated by the Abbott group[3].

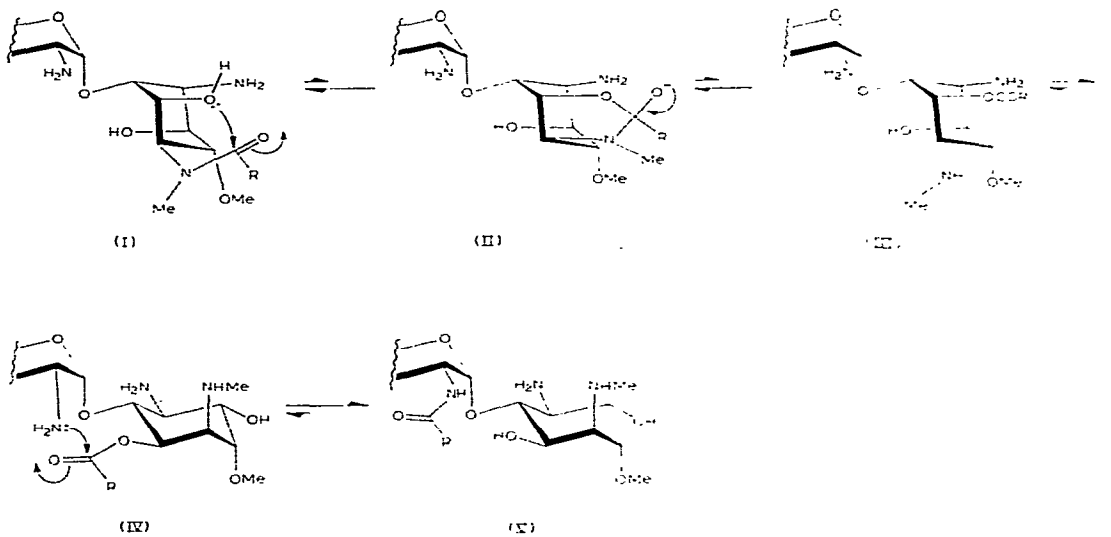
Treatment of FM-A with alkali in the presence of alanine did not give any 2'-*N*-alanylfortimicin B, suggesting that the rearrangement products are not formed by recombination of fortimicin B (FM-B)[2] with such acids as glycine or hydantoic acid, which are generated from the parent compounds by simple hydrolysis. On the other hand, 5-*O*-benzylfortimicin A (**4**) is very stable in alkaline solution. Thus, it seems that the presence of a hydroxyl group at C-5 is essential for the rearrangement. Scheme 2 shows a plausible mechanism. This postulated mechanism involves the following steps.

(*a*) Initial attack of the free 5-hydroxyl group on C=O of the 4-*N*-acyl group to form an oxazolidine intermediate (II).

(*b*) Breakdown of the oxazolidine intermediate, either by reconversion into the 4-*N*-acyl starting material or into the 5-*O*-acylfortimicin B derivative (III).

(*c*) Interconversion of 5-*O*-acylfortimicin B from the $^1C_4$ to the $^4C_1$ conformation (This is in accord with the known, predominant conformations of fortimicin A and fortimicin B in aqueous base; see Chart 1).

(*d*) Irreversible migration of the 5-*O*-acyl group to the 2'-amino group. (X-ray analysis[4] of fortimicin B base shows close proximity of the 2'-amino and the 5-hydroxyl groups. N.O.e. experiments in aqueous solution of fortimicin B show a 17% increase in the H-1' signal area on irradiation at H-1, supporting the postulate



Scheme 2. Postulated mechanism of the acyl migration from N-4 to N-2' of 4-*N*-acylfortimicins

that the conformation of fortimicin B base in solution approximates that in the crystal.

To confirm the mechanism, 5-*O*-benzoylfortimicin B (**5**) was prepared and its base stability was investigated. Interestingly, **5** was extremely unstable in alkaline media; even in phosphate buffer of pH 7.3 it was converted into 4-*N*-benzoylfortimicin B (**6**), which was identified by comparison with an authentic sample prepared by another route. If the intermediate (III) is converted into II much faster than into IV, these finding are not necessarily contrary to the postulated mechanism. In aqueous solution adjusted to pH 12 with sodium hydroxide, 4-*N*-benzoylfortimicin B was converted into FM-B and 2'-*N*-benzoylfortimicin B. After 2 days, the ratio of starting material (**6**) to FM-B and 2'-*N*-benzoylfortimicin B was approximately 1 : 2 : 1, as determined by t.l.c.

The 2'-*N*-acylfortimicins mentioned here have only weak antimicrobial activities. Compounds **2** and **3** were also isolated from the broth of *Micromonospora olivoasterospora*, the fortimicin-producing organism[5].

EXPERIMENTAL

General methods. — Low- and high-resolution mass spectra were obtained with a JEOL JMS-01SG spectrometer equipped with a field desorption–field ionization–electron-impact combination ion-source, model MS-FD-01. ^1H and ^{13}C n.m.r. spectra were recorded with JEOL PS/PFT 100, JEOL FX 100, or Varian T-60 (60 MHz) spectrometers, in the c.w. or f.t. mode, and chemical shifts for ^1H-n.m.r. are reported in p.p.m. downfield from internal DSS (in D_2O) or Me_4Si (in organic solvents), unless otherwise stated.

The chemical shifts for ^{13}C-n.m.r. were measured in D_2O from internal 1,4-dioxane (67.4 p.p.m.) and are reported in p.p.m. downfield from Me_4Si. I.r. spectra were taken on a Shimazu IR-27G spectrometer. Elemental analyses were obtained on a Yanagimoto CHN Corder MT-1. Optical rotations were measured with a Perkin–Elmer model 141 polarimeter. Reported pD values are uncorrected readings for solutions in deuterium oxide with an Okakura model AH 21 pH meter. Thin-layer chromatography (t.l.c.) was performed on pre-coated plates, Merck Art 5714. Abbreviations for the t.l.c. solvents are as follows: C, chloroform; M, methanol; I, isopropyl alcohol; and A, 28% aqueous ammonia.

2'-N-Glycylfortimicin KE (**1**). — Formation and isolation of this compound had been reported[1], $[\alpha]_D^{25}$ +59.6° (*c* 0.5, water); ν_{max}^{KBr} 3350, 2920, 1650, 1570, 1470, 1330, 1090, and 1030 cm^{-1}.

2'-N-Hydantoylfortimicin B (**3**). — An aqueous solution of 2.00 g of FM-C free base was heated for 24 h at 60° and applied to a column of Amberlite CG-50 (NH_4^+) resin (100 mL) after the pH had been adjusted to 7 with M hydrochloric acid. The column was washed with 300 mL of water and then eluted with 0.1M aqueous ammonia. Fractions containing the compound having R_F 0.44(C/I/A 1:2:1) were combined and evaporated to afford 638 mg (32.9%) of **3**; ^1H-n.m.r. (free base): δ 1.12 (3 H, d, *J* 6.6 Hz, 6'-Me), 1.3–2.0 (4 H, m, H-3' and 4'), 2.38 (3 H, s, NMe), 3.78 (2 H, s, CH_2 of hydantoyl), 3.47 (3 H, s, OMe), 4.01 (1 H, dd, *J* 9.3 and 4.6 Hz, H-5), and 5.24 (1 H, d, *J* 3.4 Hz, H-1'); m.s. (f.d.): MH^+ = *m/z* 449.2678. Calc. for $C_{18}H_{37}N_6O_7$: *m/z* 449.2723.

Anal. Calc. for $C_{18}H_{36}N_6O_7 \cdot H_2CO_3$: C, 44.69; H, 7.50; N, 16.46. Found: C, 44.76; H, 7.65; N, 16.57.

Further elution of the column with 0.3M aqueous ammonia gave 789 mg (50.7%) of FM-B, R_F 0.56(C/I/A 1:2:1).

2'-N-Glycylfortimicin B (**2**). — FM-A sulfate (100 g) dissolved in water (2 L) was heated under reflux for 4 h after the pH had been adjusted to 10 with 5M sodium hydroxide. The mixture was made neutral with hydrochloric acid and applied to a column of Amberlite IRC-50 (NH_4^+) (2 L). The column was washed with water (10 L) and eluted with 0.1M aqueous ammonia. Fractions containing the compound of R_F 0.45(C/I/A 1:2:1) were combined and the solvent was evaporated to give 37.6 g (61.8%) of **2**; ^1H-n.m.r. (pD 11.2): δ 1.04 (3 H, d, *J* 6.8 Hz, 6'-Me), 1.2–1.9 (4 H, m, H-3' and 4'), 2.36 (3 H, s, NMe), 3.26 (2 H, s, CH_2 of glycyl), 3.44 (3 H, s, OMe), ~3.9 (m, H-2'), 4.01 (1 H, dd, *J* 9 and 4.5 Hz, H-5), and 5.11 (1 H, d, *J* 3.8 Hz, H-1'); *m/z* 406 (MH^+, Calc. for $C_{17}H_{36}N_5O_6$: 406.2665. Found: 406.2637), 388 ($M^+ - NH_3$), 235, 207, 200, 190, and 189; ν_{max}^{KBr} (sulfate); 3400, 3100–2950, 1680, 1620, 1520, 1110, and 615 cm^{-1}.

Anal. Calc. for $C_{17}H_{35}N_5O_6 \cdot H_2CO_3 \cdot 2/3\ H_2O$: C, 45.08; H, 8.06; N, 14.61. Found: C, 44.95; H, 8.15; N, 14.52.

Further elution of the column with 0.2M aqueous ammonia gave 18.4 g of FM-B.

5-O-Benzylfortimicin A (**4**). — *Benzylation.* A mixture of tetra-*N*-benzyloxy-

carbonylfortimicin A[6] (940 mg), α-bromotoluene (260 mg), and silver oxide (170 mg) in *N*,*N*-dimethylformamide (10 mL) was stirred vigorously for 18 h at room temperature. Insoluble material was filtered off and the filtrate was poured onto ice–water and extracted with ethyl acetate. The combined extracts were successively washed, dried, and evaporated. The crude product was purified by column chromatography on silica gel with 49 : 1 chloroform–methanol to give 760 mg (73.6%) of pure sample; ^{1}H-n.m.r. (60 MHz, $CDCl_3$): δ 1.13 (3 H, d, *J* 6.0 Hz, 6′-Me), 2.92 and 2.98 (total 3 H, NMe)*, 3.34 (3 H, s, OMe), 5.04 (10 H, s, CH_2 of benzyl), and 7.20–7.33 (25 H, phenyl).

Debenzylation. Method A. 5-*O*-Benzyl-tetra-*N*-benzyloxycarbonylfortimicin A (7) (210 mg) and 10% palladium-on-carbon (40 mg) in a mixture of methanol (18 mL) and 2M hydrochloric acid (2 mL) was shaken under hydrogen for 1.5 h. The catalyst was filtered off and the filtrate evaporated. The residue was dissolved in water (5 mL), the pH adjusted to 6, and the solution applied to a column of CG-50 (NH_4^+) resin that was eluted with 0.15M ammonium hydroxide.

Evaporation of the solvent afforded 66 mg (65.4%) of **4**; t.l.c. R_F 0.57 (lower layer of C/M/A 2 : 1 : 1); *m/z* 495 (M^+, calc. for $C_{24}H_{41}O_6N_5$: 495.3056. Found: 495.3033), 382, 354, 336, 143, and 91; ^{1}H-n.m.r. (pD 11.0): δ 1.04 (3 H, d, *J* 6.6 Hz, 6′-Me), 2.94 (3 H, s, NMe), 3.40 (3 H, s, OMe), 3.90 (2 H, s, CH_2 of benzyl), 4.75 (1 H, d, *J* 3.4 Hz, H-1′), 4.82 (1 H, dd, *J* 11.7 and 2.7 Hz, H-4), and 7.43 (5 H, s, phenyl).

Debenzylation. Method B. A solution of **7** (100 mg) in 2M methanolic potassium hydroxide (20 mL) was heated overnight under reflux, and then made neutral and the solvent evaporated. The residue was applied to a column of CG-50 resin. Processing as for method A gave **4**.

5-O-Benzoylfortimicin B (**5**). — A solution of tetra-*N*-benzyloxycarbonylfortimicin A (940 mg, 1 mmol), *tert*-butylchlorodimethylsilane, (190 mg, 1.2 mmol) and imidazole (180 mg, 2.4 mmol) in *N*,*N*-dimethylformamide (4 mL) was kept for 3 days, poured into ice–water, and extracted with ethyl acetate. The organic layer was washed, dried, and evaporated. The residue was applied to a column of silica gel that was eluted with 1% methanol in chloroform to give 810 mg (76.6%) of tetra-*N*-benzyloxycarbonyl-2-*O*-*tert*-butyldimethylsilyl fortimicin A; t.l.c. R_F 0.45 (C/M 39 : 1); ^{1}H-n.m.r. (60 MHz, $CDCl_3$): δ 0.12 (6 H, s, Me_2Si), 0.90 (9 H, s, *tert*-butyl). 1.08 (3 H, d, *J* 6.1 Hz, 6′-Me), 2.94 and 3.05 (3 H, a pair of s, NMe)*, 3.34 (3 H, s, OMe), 5.03 (8 H, s, CH_2 of benzyl), and 7.34 (20 H, s, phenyl).

A solution of 530 mg of the foregoing product in a mixture of 0.5 mL of 2M sodium hydroxide and 20 mL of methanol was kept for 3 h at room temperature. T.l.c. was showed a clear spot of a new product, 1,2′,6′-tri-*N*-benzyloxycarbonyl-2-*O*-*tert*-butyldimethylsilylfortimicin B (**8**), at R_F 0.15 (C/M 19 : 1).

To the solution was added benzyl chloroformate (150 mg) and M aqueous

*The ^{1}H-n.m.r. spectrum of some FM-A derivatives exhibited two signals because of the 4-*N*-Me group.

potassium carbonate (0.5 mL). After stirring for 1 h, **8** had almost disappeared and a new spot, the 4-*N*-benzyloxycarbonyl derivative of **8**, was observed at R_F 0.73 (C/M 39:1) in t.l.c. The mixture was evaporated, and the residue dissolved in ethyl acetate. The solution was washed, dried, and evaporated.

A solution of the residue thus obtained and benzoyl chloride (180 mg) in dry pyridine (10 mL) was kept for 20 h at 60°, poured onto ice–water, and extracted with ethyl acetate. The extract was washed successively with diluted hydrochloric acid, water, and then aqueous sodium hydrogencarbonate. The crude product (5-benzoate) obtained by evaporation of the solvent was treated with a mixture of 2M hydrochloric acid (2 mL) and methanol (18 mL) for 18 h at room temperature. The mixture was evaporated and the residue extracted with ethyl acetate. The extract was washed with aqueous sodium hydrogencarbonate, dried, evaporated, and the residue applied to a column of silica gel (25 g), that was eluted with dichloromethane–methanol (99:1–49:1). Evaporation of the solvent gave 250 mg of pale-yellow powder; t.l.c. R_F 0.39 (C/M 24:1); ^{1}H-n.m.r. (60 MHz, CD_3OD): δ 1.11 (3 H, d, J 6.0 Hz, 6′-Me), 3.06 (3 H, s, NMe), 3.36 (3 H, s, OMe), 5.16 (6 H, s, CH_2 of benzyl), 7.31 (20 H, s, phenyl of benzyl), 7.3–8.2 (5 H, m, benzoyl).

A mixture of 5-*O*-benzoyl-tetra-*N*-benzyloxycarbonylfortimicin B just obtained 10% palladium-on-carbon (20 mg), and 0.5M sulfuric acid (0.81 mL) in methanol (10 mL) was shaken under hydrogen for 2.5 h. The catalyst was filtered off and the filtrate concentrated to dryness to afford 90 mg of **5** as solid; ^{1}H-n.m.r. (pD 1.5): δ 1.34 (3 H, d, J 6.8 Hz, 6′-Me), 2.89 (3 H, s, NMe), 3.62 (3 H, s, OMe), 4.11 (1 H, dd, J 3.8 and 2.9 Hz, H-4), 4.30 (1 H, dd, J 8.3 and 2.9 Hz, H-3), 5.58 (1 H, d, J 3.4 Hz, H-1′), 5.9 (1 H, m, H-5), and 7.5–7.1 (5 H, m, benzoate); ^{13}C-n.m.r. (pD 1.5) δ 15.0 (6′-Me), 21.3 (C-3′), 25.4 (C-4′), 33.6 (NMe), 49.1 (C-2′), 51.4 (C-6′), 53.5 (C-1), 56.0 (C-4), 58.7 (OMe), 65.2 (C-2), 70.7 (C-5′), 71.4 (C-5 and C-6), 74.7 (C-3), 94.9 (C-1′), 128.2, 129.8 (2 × C), 130.7 (2 × C), 135.7 (phenyl), and 167.0 (CO).

Anal. Calc. for $C_{22}H_{36}N_4O_6 \cdot 2\ H_2SO_4 \cdot 2\ H_2O$: C, 38.59; H, 6.48; N, 8.18. Found: C, 38.63; H, 6.69; N, 7.88.

4-N-*Benzoylfortimicin B* (**6**). — A mixture of 1,2′,6-tri-*N*-benzyloxycarbonylfortimicin B (ref. 6, 300 mg), benzoyl chloride (100 mg), and M potassium carbonate (0.2 mL) in methanol (12 mL) was stirred for 1 h at room temperature, and then the mixture was shaken under hydrogen following addition of 2M hydrochloric acid (1.5 mL) and 10% palladium-on-carbon (30 mg). Isolation as for **4** (method *A*) gave 80 mg of solid; t.l.c. R_F 0.32 (lower layer of C/M/14% A, 2:1:1); *m/z* 452 (M^+, Calc. for $C_{22}H_{36}O_6N_4$: 452.2635. Found: 452.2634), 311, 207, and 143; ^{1}H-n.m.r. (pD 1.1, 80°): δ 1.34 (3 H, d, J 6.6 Hz, 6′-Me), 3.15 (3 H, s, NMe), 5.34 (1 H, d, J 3.4 Hz, H-1′), and 7.5 (5 H, s, phenyl); at room temperature, some doubling of signals occurred; δ 1.25 (d, J 5.1 Hz) and 1.36 (d, J 6.8 Hz) (total 3 H, 6′-Me), 3.13 and 3.25 (total 3 H, each s, NMe), 5.00 (dd, J 11.5 and 2.2 Hz) and 5.17 m (total 1 H, H-4), and 5.38 (1 H, d, J 3.4 Hz, H-1′).

REFERENCES

1 T. IIDA, M. SATO, I. MATSUBARA, Y. MORI, AND K. SHIRAHATA, *J. Antibiot.*, 32 (1979) 1273–1279.
2 R. S. EGAN, R. S. STANASZEK, M. CIROVIC, S. L. MUELLER, J. TADANIER, J. R. MARTIN, P. COLLUM, A. W. GOLDSTEIN, R. L. DE VAULT, A. C. SINCLAIR, E. E. FAGER, AND L. A. MITSCHER, *J. Antibiot.*, 30 (1977) 552–563.
3 J. TADANIER, J. R. MARTIN, P. JOHNSON, A. W. GOLDSTEIN, AND R. HALLAS, *Carbohydr. Res.*, 85 (1981) 61–71.
4 N. HIRAYAMA, K. SHIRAHATA, Y. OHASHI, Y. SASADA, AND J. R. MARTIN, *Acta Crystallogr., Sect. B*, 34 (1978) 2648–2650.
5 T. NARA, M. YAMAMOTO, I. KAWAMOTO, K. TAKAYAMA, R. OKACHI, S. TAKASAWA, T. SATO, AND S. SATO, *J. Antibiot.*, 30 (1977) 533–540.
6 J. TADANIER, J. R. MARTIN, P. KURATH, A. W. GOLDSTEIN, AND P. JOHNSON, *Carbohydr. Res.*, 79 (1980) 91–102.