NOTE

THE BASE CATALYZED REARRANGEMENT OF 3-*O*-DEMETHYLFORTIMICIN A

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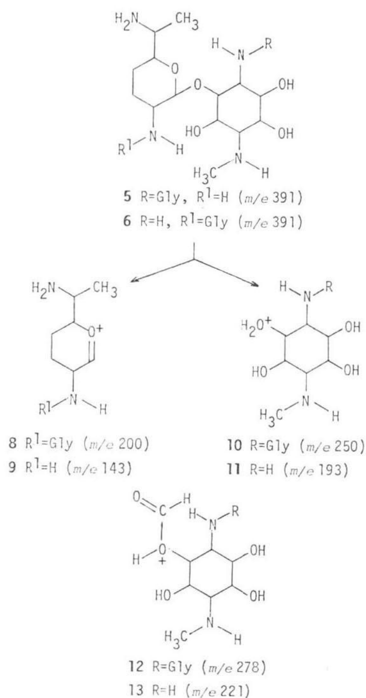
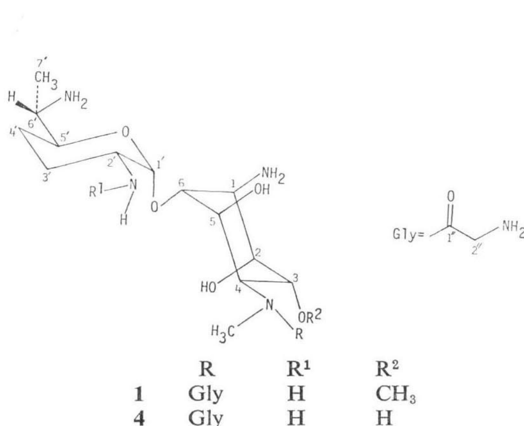
(Received for publication June 21, 1980)

Fortimicins A (**1**) and B (**2**) are aminoglycoside antibiotics. Both compounds consist of a 6-*epi*-purpurosamine B moiety and the aminocyclitol fortamine. Fortimicin A differs from B in having a glycyl substituent at its 4-*N*-position. Base catalyzed loss of glycine from the 4-amino group of **1** to give **2** and a rearrangement product, 2′-*N*-glycylfortimicin B or isofortimicin (**3**), has been reported by TADANIER, *et al.*[1)] In that study, a number of 4-*N*-acylfortimicin analogs

Scheme 1. Some mass spectral fragments of **5** and **6**.





	R	R^1	R^2
1	Gly	H	CH_3
4	Gly	H	H

	R	R^1	R^2
2	H	H	CH_3
3	H	Gly	CH_3
5	Gly	H	H
6	H	Gly	H
7	H	H	H

Scheme 2. Proposed mechanism for the rearrangement of **4**.

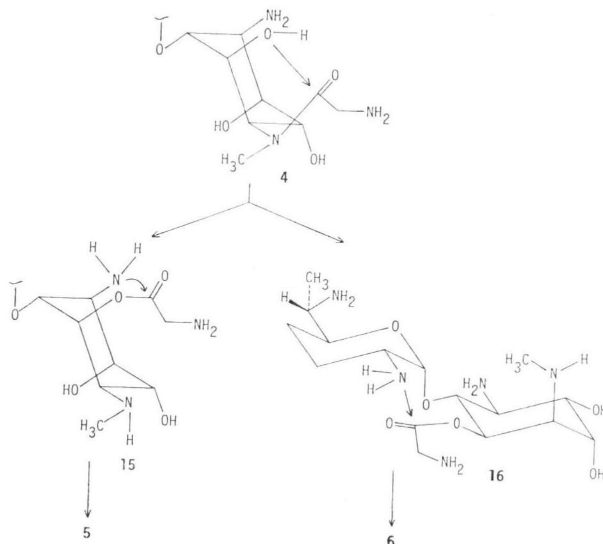


Table 1. 25 MHz CMR parameters of compounds **5** and **6**.

	5			**6**		
	pD 1.16	pD 10.04	β-Shift	pD 2.64	pD 10.89	β-Shift
C-1	53.4	53.1	—	53.7	53.9	0.2
C-2	71.4	72.9	0.5	70.7	74.8	4.1
C-3	65.8	69.2	3.4	65.0	69.6	4.6
C-4	58.4	64.6	6.2	60.1	65.1	5.0
C-5	65.8	69.7	3.9	67.1	70.9	3.8
C-6	75.3	76.5	0.7	73.7	83.1	9.4
C-1′	95.5	98.8	3.3	97.1	99.1	2.0
C-2′	51.8	51.2	—	52.1	50.7	—
C-3′	21.6	26.8	5.2	22.4	23.7	1.3
C-4′	26.3	26.9	0.6	27.1	26.8	—
C-5′	70.8	72.0	1.2	68.5	71.6	3.1
C-6′	49.3	50.3	1.0	49.0	50.1	1.1
C-7′	15.2	17.1	1.9	15.1	18.4	3.3
C-2″	41.4	45.2	3.8	41.3	44.8	3.5
N-CH_3	31.6	35.3	3.7	32.6	35.5	2.9

were shown to undergo similar acyl migrations. SHIRAHATA, *et al.*[2] also reported similar rearrangements for fortimicin D (6′-demethylfortimicin A) and some further 4-*N*-acylfortimicin analogs. In both studies, however, the only rearrangement products observed were of acyl migration from the 4-amino to the 2′-amino group. Thus the mechanism for the rearrangement has been postulated to involve intramolecular migration of the 4-*N*-acyl moiety *via* the 5-hydroxy group to the 2′-amino group.

We wish to report that 3-*O*-demethylfortimicin A (**4**), a synthetic analog[3] of **1**, undergoes base catalyzed rearrangement to afford 1-*N*-glycyl-3-*O*-demethylfortimicin B (**5**) and 3-*O*-demethylisofortimicin (**6**). Compound **5** has been reported by MCALPINE, *et al.*[4] as a minor fermentation product (fortimicin AN) of *Micromonospora olivoasterospora*, the producing organism of **1**. Compound **6** has not hitherto been reported.

The compounds were obtained by treatment of **4** with 10 M NH_4OH for 18 hours, the resulting mixture was neutralized and chromatographed over IRC-50 (NH_4^+) using an aqueous-IM NH_4OH gradient. The fractions collected were neutralized and combined after thin-layer chromatography (t.l.c.) using silica gel and the solvent system; CH_2Cl_2–MeOH–NH_4OH (1: 1: 1, lower phase). The combined fractions were desalted over Sephadex G-10 and lyophilized to afford **5**, **6** and 3-*O*-demethylfortimicin B (**7**) in 51, 28 and 19% yields, respectively. Identification of **5** as fortimicin AN was by direct comparison of spectra and tlc characteristics. The product of simple glycine cleavage, compound **7**, was similarly shown to be identical with an authentic sample[3] of 3-*O*-demethylfortimicin B. The spectral characteristics that led to the identification of the rearrangement products are the following:

Compound **5**; MS (Scheme 1) *m/e*: 391 (M^+), 392 ($M+H^+$), 374 (M^+-NH_3), 278 (**12**), 250 (**10**), 143 (**9**); PMR (D_2O) δ ppm from external TMS: 1.5 (3H, d, J=7 Hz, CH_3-7′), 1.7~2.4 (4H, CH_2-3′, 4′), 2.9 (3H, S, $HNCH_3$-4), 3.1~3.4 (2H, CH-2′, 6′), 3.4 (1H, CH-4), 3.85 (2H, S, CH_2-2″), 4.0 (1H, CH-5′), 4.2~4.5 (2H, CH-2, 6), 4.6~4.7 (2H, CH-3, 5), 4.8 (1H, HC-NCO-1), 5.8 (1H, d, J=3.5, CH-1′); IR (KBr) cm^{-1}: 1570, 1650 (HN–C=O).

Compound **6**; MS *m/e*: 391 (M^+), 392 ($M+H^+$), 374 (M^+-NH_3), 221 (**13**), 200 (**8**), 193 (**11**); PMR (D_2O) δ ppm from external TMS: 1.7 (3H, d, J=7 Hz, CH_3-7′), 2.1~2.5 (4H, CH_2-3′, 4′), 3.0 (3H, S, $HNCH_3$-4), 3.4~3.7 (2H, CH-1, 6′), 3.8 (1H, CH-4), 4.0 (1H, CH-5′), 4.2 (2H, S, CH_2-2″), 4.3~4.4 (2H, CH-2, 6), 4.5 (1H, CH-2′), 4.6~4.7 (2H, CH-3, 5); IR (KBr) cm^{-1}: 1570, 1650 (HN–C=O).

The 4-N–CH_3 singlets at δ 2.9 and 3.0 in the PMR spectra of **5** and **6**, respectively, indicated that the 4-amino group in each case was unsubstituted by an acyl group, since **4**, which has a glycyl substituent on the 4-amino group, shows a chemical shift of δ 3.62 for the 4-N–CH_3 group.[3] Hence the point of attachment of glycine was not the 4-N–CH_3 position in either **5** or **6**. The position of glycine substitution was determined for each compound by spin decoupling experiments. For compound **5**, the C-1 proton was determined to be at δ 4.8 by decoupling the resonances for the C-2 and C-6 protons. Similarly, the C-2′ proton was located at δ 4.5 for compound **6** by decoupling the C-1′ proton. The chemical shifts for the C-1 and C-2′ protons of **5** and **6** have been reported[5] as 4.0 and 3.3, respectively. The downfield shifts observed for the C-1 proton in **5** and the C-2′ proton in **6** indicated the position of glycine substitution to be the 1-NH_2 group in **5** and the 2′-NH_2 group in **6**.

The *m/e* 250 (**10**) and 278 (**12**) fragments (Scheme 1) in the mass spectrum of **5** was consistent with glycine substitution on the 3-*O*-demethylfortamine cyclitol. On the other hand, the spectrum for **6** showed peaks at *m/e* 193 and 221, consistent with a fortamine cyclitol lacking a glycyl substituent, and a peak at *m/e* 200, confirming that glycine was attached to the 6-*epi*-purpurosamine B moiety. In addition, **5** showed a peak at *m/e* 143, consistent with an *epi*-purpurosamine B moiety.

The CMR spectra of **5** and **6** are summarized in Table 1. A comparison of the β-shifts observed for the various carbon assignments in the spectra of the two compounds showed differences in magnitude that were diagnostic. Thus **5**, which is acylated at the 1-NH_2 group showed only small shifts for C-2 and C-6, upon titration, as compared to the same carbons in **6**. Similarly, the shifts for C-1′ and C-3′ in **6**, which is acylated at the 2′-NH_2 group were smaller than those observed for the same carbons in **5**. The spectral evidence is thus consistent with the assigned structures of the rearrangement products of **4**.

It is of interest that 4-*N*-acyl fortimicins undergo base catalyzed rearrangements to 2′-*N*-acyl derivatives exclusively,[1,2] whereas **4**, which differs from the above compounds only in lacking a 3-*O*-methyl group, rearranges to give 1-*N*- and 2′-*N*-acyl products. We suggest an intramolecular mechanism involving the formation of a 5-*O*-acyl derivative **14** (Scheme 2) of the type described by SHIRAHATA, *et al.*[2] In this case however, the two conformers, **15** and **16**, may both be stable and undergoing rearrangement. Hence direct migration of glycine from the 5-position of **15** to the 1-*N* affords **5**, whereas migration from 5-*O* to 2′-*N* in **16** affords **6**.

Acknowledgements

We are indebted to the following members of the spectroscopy group of Abbott Laboratories, North Chicago; Mr. MOMIR CIROVIC for PMR data, Mrs. RUTH STANASZEK for CMR, Mr. WILLIAM WASHBURN for IR, Mrs. SANDRA MUELLER and Mr. PRESTON HILL for mass spectra.

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