THE STRUCTURES OF COMPONENT A_1 (=LL-AB664) AND COMPONENT A_2 (=LL-AC541), STREPTOTHRICIN-LIKE ANTIBIOTICS

Sir:

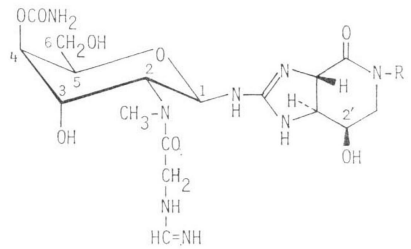
Two streptothricin-like antibiotics effective against *Serratia marcescens,* tentatively designated as components A_1 and A_2, were isolated from broth filtrate of *Streptoverticillium olivoreticuli*[1,2]. Component A_1 was identified to be LL-AB664 (=BD-12)[3~6] by Avicel and silica gel TLC[2] and component A_2 as the LL-AC541 (=BY-81, citromycin, E-749-C)[5~9], respectively. Streptothricin group antibiotics give streptolidine, D-gulosamine, L-β-lysine, ammonia and carbon dioxide by acid hydrolysis, while streptolidine or N-methylstreptolidine, N-methyl-D-gulosamine, glycine with or without formic acid, ammonia and carbon dioxide were found in the acid hydrolysate of streptothricin-like antibiotics. Though, structures have been proposed for several streptothricin-like antibiotics, the location of the carbamoyl group has not yet been fully determined, it is either on the C_3– or C_4–OH group of the N-methyl-D-gulosamine moiety. Thus, ^{1}H-NMR and ^{13}C-NMR spectra of component A_2 (I-b) and its partial hydrolysis product, N-*guan*-streptolidyl N′-methyl-β-D-gulosaminide (II), were studied to determine the location of the carbamoyl group on the aminosugar. The partial hydrolysis product II was obtained from I-b according to the method reported by BORDERS *et al.*[4].

^{1}H-NMR spectra of I-b and II were taken in D_2O. The complete assignment of the proton resonances of II has already been achieved by BORDERS *et al.*[4]. They have also partly assigned the proton resonances of I-b, however the protons of the aminosugar moiety has been left unidentified except C_1–H and C_6–H.

The ^{13}C-NMR spectra of both compounds in D_2O were assigned by the help of proton selective decoupling experiments. For the spectrum of II every proton has been properly assigned. For the spectrum of I-b, carbon signals of the streptolidine moiety were assigned by irradiation of known proton resonance position*. By the same method, C_1 and C_6 signals of the aminosugar moiety of I-b were assigned to δ 77.8 and 61.1, respectively. ^{13}C-Chemical shifts for the N-methylgulosamine moiety of I-b (see below) and II are shown in Table 1.

NAGANAWA *et al.* reported the ^{13}C-NMR of bleomycins, in which the effects of substitution of a carbamoyl group on sugar hydroxyl groups were precisely studied[10]. They found that the α-carbon signal was shifted downfield by 1.9~5.0 ppm on substitution, while the β-carbon

Fig. 1.



Component A_1 (I-a): R=CH_3
Component A_2 (I-b): R=H

Fig. 2. N-*guan*-Streptolidyl N′-methyl-β-D-gulosaminide (II).

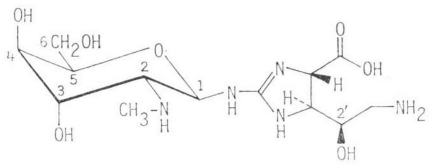
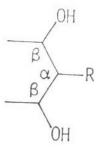


Fig. 3.



R=OH⟶$OCONH_2$
$\Delta\delta=\delta_{OCONH_2}-\delta_{OH}$
α: $\Delta\delta=+1.9\sim5.0$ ppm
β: $\Delta\delta=-0.3\sim-2.7$ ppm

Table 1. ^{13}C-Chemical shifts for N-methyl-D-gulosamine moiety of component A_2 (I-b) and N-*guan*-streptolidyl N′-methyl-β-D-gulosaminide (II).

	C_1	C_2	C_3	C_4	C_5	C_6
I-b	77.8	61.7	61.7	71.5	74.7	61.1
II	78.4	57.5	65.7	68.2	75.4	61.6
$\Delta\delta$	−0.6	+4.2	−4.0	+3.3	−0.7	−0.5

Measured at 25.15 MHz using D_2O as a solvent. Chemical shifts were calculated from internal dioxane (δ 67.4).

* The chemical shift of C_2′-H (δ 5.2) reported by BORDERS *et al.* should be corrected to δ 4.8 according to our decoupling experiment.

resonance was shifted upfield by 0.3~2.7 ppm (Fig. 3).

The resonance of C_5 of the aminosugar moiety of I-b was assigned to δ 74.7 by consideration of shift trend, which would be shifted at most 3 ppm on substitution of the carbamoyl group either on the C_3– or C_4–OH group. Among remaining three doublet signals (actually two, due to overlapping), the C_4, C_3 and C_2 signals were tentatively assigned to δ 71.5, 61.7 and 61.7 respectively, based on the assumed C_4 substitution. By this assignment, C_4 (α-carbon) was shifted downfield by 3.3 ppm, while C_5 and C_3 (β-carbons) were shifted upfield by 0.7 and 0.4 ppm, respectively. The large shift of C_3 of I-b was explained by the additive β-effect of the amido formation at C_2–N. An additional β-effect of C_2–N substitution was observed at C_1 (−0.6 ppm). C_2 Carbon (α-position) was probably shifted downfield by 4.2 ppm. Alternative assignments of C_2, C_3 and C_4 could not reasonably explain the shift induced by substitution. This corraborated our tentative assignment. The carbon resonances of the aminosugar moiety of I-a in D_2O were also identical to those of I-b.

In conclusion, the position of the carbamoyl group was determined to be on the C_4–OH group. Thus, the structures of components A_1 and A_2 were determined to be I-a and I-b as shown in Fig. 1.

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