LL-AM31 ANTIBIOTIC COMPLEX: AMINOALDITOL ANTIBIOTICS FROM A *STREPTOVERTICILLIUM*

Sir:

In the search for novel, clinically useful antibiotics, we encountered a complex of basic antibiotics designated LL-AM31α, β, and γ. These antibiotics are perhaps most similar to the aminocyclitols[1]; however, they contain an aminoalditol instead of deoxystreptamine or streptamine. To our knowledge, natural products known to contain aminoalditol moieties are relatively rare.[2] The LL-AM31 antibiotics are produced by a *Streptoverticillium* species when it is fermented in a complex medium,[3] and in the following discussion are shown to be identical to the recently described sorbistin antibiotics which are produced by a bacterium.[4] These studies also provide useful characterization data previously not described for these compounds.

The LL-AM31 complex, $[\alpha]_D^{25}+87.0°$ (*c* 0.56, H_2O), was isolated from the fermentation filtrate by sequential chromatography on ion-exchange resins such as Amberlite IRC-50 (NH_4^+) eluted with ammonium hydroxide and Dowex 1-X2 (OH^-) eluted with water. The complex was readily resolved into its components by GC (0.7% OV-1 on Gas Chrom Q with N_2 carrier at 230°C) of the N-acetyl-O-trimethylsilyl derivative, or by partition chromatography of the underivatized material on silica gel eluted with MeOH - $CHCl_3$ - NH_4OH (7: 5: 5).

The molecular formulas of the α, β, and γ

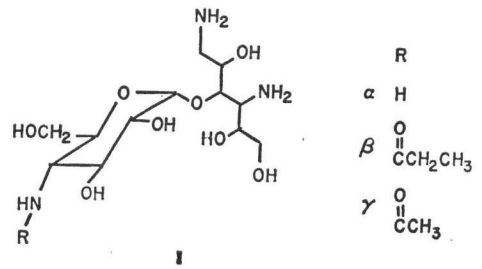

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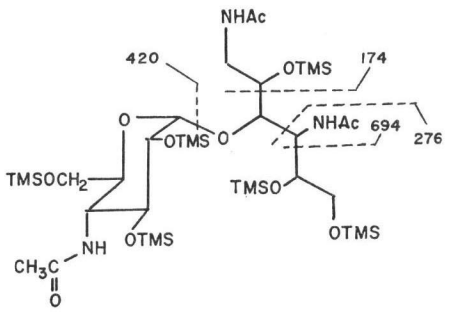
Table 1. Fragmentation patterns of LL-AM31γ N-Ac-O-TMS derivative



Relative abundance (%) for Ac	Observed mass[a]		calcd. mass	Assignment for Ac
	Ac-d_3	Ac	Ac	
6	890	884.4238	884.4252	$C_{33}H_{78}N_3O_{11}Si_6$ (M^+-15)
9	700	694.3380	694.3406	$C_{28}H_{60}N_3O_9Si_4$
33	638	635.2995	635.3035	$C_{26}H_{55}N_2O_8Si_4$
3	587	581.2909	581.2929	$C_{23}H_{53}N_2O_7Si_4$[b]
100	423	420.2056	420.2057	$C_{17}H_{38}NO_5Si_3$
4	279	276	—	$C_{11}H_{26}NO_3Si_2$
50	189	186	—	$C_8H_{16}NO_2Si$[c]
25	177	174	—	$C_7H_{16}NO_2Si$

[a] Data for N-Ac-OTMS and N-Ac-d_3-OTMS derivatives.

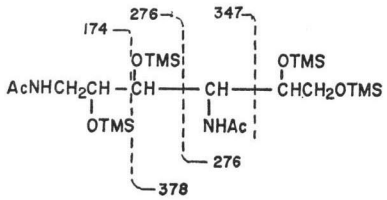
[b] Rearrangement ion of alditol: $C_6H_8(NHAc)_2(OTMS)_3(\overset{+}{O}CHOTMS)$

[c] *m/e* 276 $\xrightarrow{-HOTMS}$ *m/e* 186

components were established as $C_{12}H_{27}N_3O_8$, $C_{14}H_{29}N_3O_8$, and $C_{15}H_{31}N_3O_8$ by mass spectra of the N-acetyl-O-trimethylsilyl derivatives and were consistent with ^{13}C nmr data. In addition, the fragmentation patterns were consistent with the proposed structures (I$\alpha\sim\gamma$) and were supported by peak matching studies and a comparison of N-acetyl-O-trimethylsilyl and N-acetyl-d_3-O-trimethylsilyl derivatives (Table 1). Peak shifts in the latter mass comparisons indicated the number of acetylated nitrogens on each fragment ion.

When the LL-AM31 complex was refluxed for four hours in 2 N NaOH, the product was essentially LL-AM31α and could be purified by chromatography on an ion-exchange resin (Dowex 50-X2 eluted with ammonium hydroxide) to obtain a homogeneous amorphous solid: $[\alpha]_D^{25}+88°$ (c 0.537, H_2O); calcd. for $C_{12}H_{27}N_3O_8$: C, 42.2; H, 7.98; N, 12.3. Found: C, 42.2; H, 7.77; N, 11.7. Treatment of LL-AM31α with acetic anhydride-pyridine at room temperature for several days followed by evaporation afforded the peracetyl derivative as an oil. Crystallization from chloroform, hexane gave colorless needles: mp 183~185°; $[\alpha]_D^{25}+65$ (c 0.21, CH_3OH); calcd. for $C_{30}H_{45}N_3O_{17}$: C, 50.0; H, 6.30; N, 5.83; MW 720. Found: C, 49.7; H, 6.53; N, 5.67; m/e (M^+) 720.

Table 2. Alditol fragmentation pattern as N-acetyl-O-trimethylsilyl derivative



Exact mass		Composition
Observed	Calculated	
537.2668	537.2659 (M^+-15)	$C_{21}H_{49}N_2O_6Si_4$
378.1952	378.1942	$C_{15}H_{36}NO_4Si_3$
347.1822	347.1818	$C_{14}H_{31}N_2O_4Si_2$
276.1421	276.1450	$C_{11}H_{26}NO_3Si_2$
174.0929	174.0950	$C_7H_{16}NO_2Si$

The LL-AM31 complex was heated with 6 N HCl in a sealed vial at 110~140°C for 16 hours to yield the diaminoalditol $[\alpha]_D^{25}-8°$ (c 0.135, H_2O) which was purified by chromatography on Dowex 1-X2 (OH^-). The mass spectrum of the N-acetyl-O-trimethylsilyl derivative of diaminoalditol had M^+-15 at m/e 537 with expected fragmentations (Table 2) for the indicated substitution sequence but was ambiguous for a terminal NH_2CH_2CHOH– or $HOCH_2CHNH$– group. However, the primary amino group pattern was defined for the LL-AM31 antibiotics from ^{13}C nmr which had a NH_2CH_2– triplet at 43.00 ppm.

Methanolysis of the complex in 1.5 N methanolic HCl at 100°C for 30 minutes in a sealed vial yielded both the sugar, as a methylglycoside, and the alditol. The positional and tentative stereo isomeric form of the hexosamine was deduced from ^{13}C nmr and comparative studies of the N-acetyl-O-trimethylsilyl derivative of the LL-AM31 sugar methylglycoside with the corresponding derivatives of authentic 2-, 3-, 4- and 6-gluco-

Table 3. GC-mass data for glucosamine derivatives[a]

Glucosamine[b] isomer	Relative abundance[c] m/e							Retention Time[d] (min)
	73	146	173	186	204	316	436 ($M-15$)	
2	100	3	83	29	40	3	6	14.5
3	100	21	95	2	6	2	9	13.3, 13.6[e]
4	100	45	40	49	79	16	20	14.4, 14.6[e]
6	82	54	43	10	100	10	5	16.1, 16.8[e]
X	100	40	38	43	71	14	18	14.4, 14.6[e]

[a] N-Acetyl-O-trimethylsilyl derivatives of methylglucosides.
[b] 2-, 3-, 4-, and 6-glucosamine and the LL-AM31 sugar (X) derivatives.
[c] Abundances shown for selected ions.
[d] 1.84 m×2 mm glass column, SP2250 (Supelco); 140°C, $\Delta t=6°$/min. to 280°; He, 35 ml/min.
[e] Two peaks corresponding to α and β methylglucosides.

samines. No significant pH shifts[5] were observed in the ^{13}C nmr for the anomeric carbon of the α-component; therefore, a 2-aminosugar was excluded. The GC retention time[6] and mass fragmentation patterns[7] (Table 3) of the LL-AM31 sugar derivative were essentially identical to those of 4-glucosamine and significantly different from those of the other glucosamines when all samples were run under the same conditions.

The nature of the acyl side chain for the β and γ components was readily established as propionyl (δ 2.34q and 1.15t, J=7.5 Hz) and acetyl (δ 2.08s), respectively, by nmr spectra. An anomeric proton at δ 5.21 (J=3.0 Hz) indicated an α-glycoside linkage for the glucosamine. Attachment of the glycosidic bond to the 3-position of the alditol was deduced from the mass fragmentation of N-acetyl-O-trimethylsilyl derivatives of the antibiotics. For example, the γ component derivative had expected fragmentations at *m/e* 174, 276 and 694 (Table 1).

The absolute configuration of the alditol was established as the 1,4-diamino-1,4-dideoxy-D-glucitol through X-ray analysis of the dihydrochloride salt, $C_6H_{16}N_2O_4 \cdot 2HCl \cdot H_2O$, by the anomalous dispersion effect of chlorine.[8] Details of this work will be published separately.

A consideration of the data allowed assignment of structures (Iα, β, and γ) for LL-AM31α, β, and γ, and indicated identity to the sorbistins. Subsequent to the recent publications on the isolation and structure determination of the sorbistin antibiotics,[9~11] authentic samples of the sorbistins D, A, and B were obtained and found indistinguishable from LL-AM31α, β, and γ, respectively, by a number of the same chromatographic and spectral methods discussed above.

Sorbistin A_2, having a butyryl side chain, was not detected in the fermentations of the organism producing the LL-AM31 antibiotics. The production of identical antibiotics by organisms as different as those for the sorbistins and the LL-AM31 antibiotics is a relatively rare phenomenon.

Acknowledgements

The authors wish to thank Mr. K. ANGYL for technical assistance in high resolution mass measurements, Mr. L. BRANCONE and staff for micro-analytical analyses, Dr. H. TRESNER and staff for isolation and taxonomic studies of the organism, Dr. J. N. PORTER and Mr. A. J. SHAY and staffs for fermentation material, Messrs. M. DANN and M. HERTZ for antibiotic purification, and Mr. W. MULLER for initial GC studies. The authors also wish to thank Dr. H. KAWAGUCHI of Bristol-Banyu Research Institute, Ltd., for samples of the sorbistin antibiotics.

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(Received February 3, 1977)

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