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THE PARTIAL STRUCTURE OF LL-AV290† - A NEW ANTIBIOTIC

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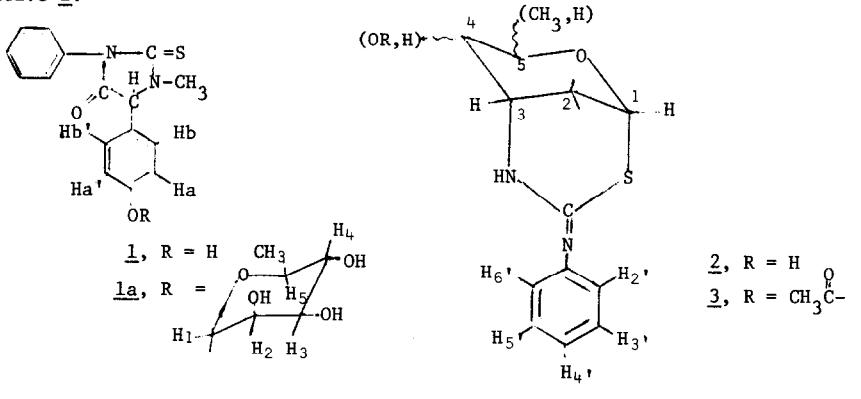
Previous communications[1,2] from these Laboratories have described the isolation and characterization of a new antibiotic, designated as LL AV290, that exhibited both in vivo and in vitro activity against gram positive bacteria. We now wish to report some results on the partial structure of this antibiotic.†† Initial degradative studies[1] indicated this material to be a glycopeptide with some structural similarities to both vancomycin[3] and the ristocetins.[4] Elemental analysis[1] on LL AV290 indicated the presence of carbon, hydrogen, nitrogen, chlorine and N-alkyl.

An initial Edman reaction[6] on LL AV290 yielded p-hydroxyphenylsarcosine as the thiohydantoin derivative 1 (λ_{max}^{MeOH} 270, log ε 3.2) and a 2,3,6-trideoxy-3-aminosugar as the phenyl isothiocyanate derivative 2.



† The generic name, avoparcin, has been adopted by the USAN Council.

†† We have not yet assigned a molecular weight to this antibiotic since molecular weight determinations by a variety of methods have been inconclusive. A potentiometric titration[5] on vancomycin gave a value of 1600.

Since the second step of the Edman reaction is a cyclization in HCl/CH_3COOH, some of the acetate 3 was isolated along with 2. The exact point of attachment of the amino sugar in the molecule is unknown but the isolation of 2 represents first a normal thiourea formation between a primary amine (C_3) and phenyl isothiocyanate with subsequent nucleophilic attack of the sulfur atom on C_1 of the sugar and concomitant displacement of the C-1 oxygen to yield 2.

The high resolution mass spectrum of 1 showed the highest mass ion at 298 m/e ($C_{16}H_{14}N_2O_2S$) and of 3 at 306 m/e ($C_{15}H_{18}N_2O_3S$). In both cases the fragmentation patterns were consistant with the assigned structures.

The ^1H n.m.r. spectrum of 1 in deuterated dimethylsulfoxide contained the following peaks: 3.14 δ (s, N-CH_3), 5.40 δ (s, hydantoin ring proton), 6.89 δ (d, J=8.5 Hz, Ha & Ha'), 7.15 δ (d, J=8.5 Hz, Hb & Hb'), and 7.43 δ (m, N-phenyl protons).

The ^1H n.m.r. spectrum of 3 in deuterated dimethylsulfoxide contained the following signals: 1.20 δ (d, J=6.5 Hz, 5-CH_3), 2.03 δ (s, 4-acetyl), 2.18 δ (broadened d, J=12 Hz, H_{2e}), 2.26 δ (m, J=4, 3.5, 12 Hz, H_{2a}), 4.12 δ (broadened d, J=3.5 Hz, H_3), 4.24 δ (d, J=5.5 Hz, H_4), 4.75 δ (d q, J=5.5, 6.5 Hz, H_5), 5.70 δ (d, J=4 Hz, H_1); aromatic protons: 6.89 δ (m, $H_{4'}$), 7.18 δ (m, $H_{3'}$ & $_{5'}$), 7.36 δ (d, J=8 Hz, $H_{2'}$ & $_{6'}$). These assignments were confirmed by decoupling experiments.

The ^1H n.m.r. spectrum of 2 in deuterated dimethylsulfoxide contained the following signals: 1.07 δ (d, J=6.5 Hz, 5-CH_3), 2.08 δ (broadened d, J=12 Hz, H_{2e}), 2.26 δ (m, J=12, 4, 4.5 Hz, H_{2a}), 3.38 δ (d q, J=6, 6.5 Hz, H_5), 3.93 δ (d, J=6 Hz, H_4), 4.18 δ (broadened d, J-4 Hz, H_3), 4.82 δ (broad s, OH), 5.69 δ (d, J-4 Hz, H_1); aromatic protons: 6.87 δ (m, $H_{4'}$), 7.17 δ (m, $H_{3'}$ & $_{5'}$), 7.33 δ (d, J=8.5 Hz, $H_{2'}$ & $_{6'}$).

In all the above cases the n.m.r. spectra were in agreement with the assigned structures.

Recently,[7,8] an amino sugar was isolated from vancomycin. This material has an additional methyl group at C_3.

The reaction sequence normally used in the Edman procedure[6] is (a) coupling of the unknown peptide with phenyl isothiocyanate in pyridine-water (50/50) to yield a phenylthiocarbamyl derivative (b) followed by cyclization in acid solution to yield the hydantoin of the terminal amino acid.

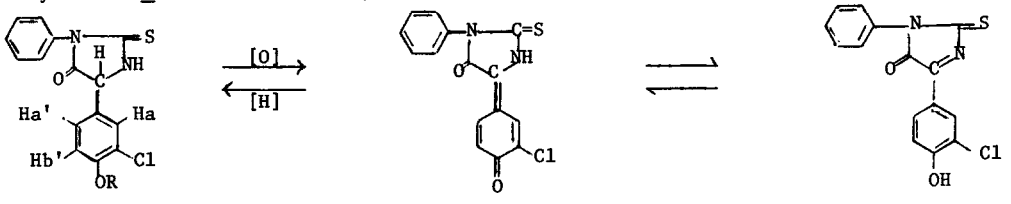
We have found that purification of the reaction mixture by tlc (silica gel using the system $CHCl_3$/MeOH: 90/10) after the first step yields directly the hydantoin derivative 1a. The isolation of this material, 1a, clearly shows that in this case step (b) is unnecessary for

cyclization. In addition, the elimination of the acid step permitted not only the isolation of a new deoxysugar fragment, (tentatively assigned as rhamnose based on an n.m.r. and tlc comparison with rhamnose) but also disclosed its point of attachment in the molecule.

Apparently, in our first experiment when acid was used, the deoxysugar readily hydrolyzed to yield only the thiohydantoin 1.

The high resolution mass spectrum of 1a showed the highest mass ion at 444 m/e ($C_{22}H_{24}N_2O_6S$). The ^1H n.m.r. spectrum of 1a in deuterated dimethylsulfoxide exhibited signals at 1.13 δ (d, J=6 Hz, 5-CH_3), 3.14 δ (s, N-CH_3), 3.34 δ (m, J=9, 10, 5 Hz, H_4), 3.52 δ (dq, J=10, 6 Hz, H_5), 3.69 δ (m, J=9, 3, 4 Hz, H_3), 3.87 δ (m, J=1, 3, 6 Hz, H_2), 4.68 δ (d, J=6 Hz, C_2-OH), 4.83 δ (d, J=5 Hz, C_4-OH), 5.02 δ (d, J=4 Hz, C_3-OH), 5.42 δ (d, J=1 Hz, H_1), 5.53 δ (s, hydantoin ring proton), 7.15 δ (d, J=8.5 Hz, Ha & Ha'), 7.31 δ (d, J=8.5 Hz, Hb & Hb'), 7.42 δ (m, N-phenyl protons). The sugar ring proton peaks were assigned after exchange of hydroxyl protons with deuterated methanol.

A second Edman reaction, on the residue that remains after the first reaction, afforded the thiohydantoin 4 of the amino acid, 3-chloro-4-hydroxyphenylglycine. This material 4 proved to be



4, R = H

5, R = - $\overset{O}{\overset{\|}{C}}$-$CH_3$

6 6a

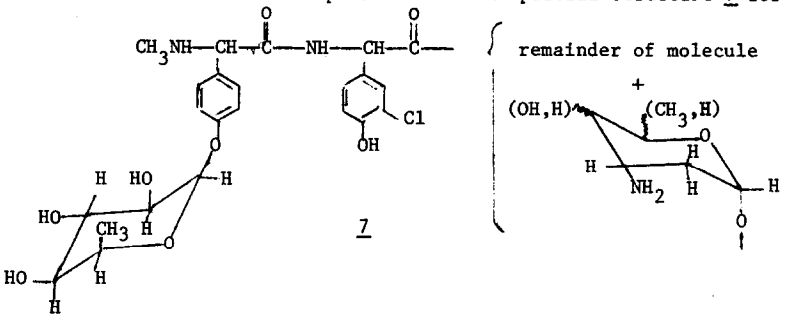
an extremely elusive molecule. We found it readily oxidized to the quinone methide 6 (λ_{max}^{MeOH} 274, 552, 557, log ε 3.1, 1.5, 1.5) during purification (tlc, silica gel). The high resolution mass spectrum of the chromatographed material showed both a mass ion of 4 at 318 m/e ($C_{15}H_{11}N_2SO_2Cl$) and a mass ion of 6 at 316 m/e ($C_{15}H_9N_2SO_2Cl$).

The ^1H n.m.r. spectrum (in d_6 DMSO) of 6 is rather complex, probably due to this mixture of tautomeric forms (i.e. 6a), however, in the presence of trifluoroacetic acid a fairly sharp aromatic pattern can be obtained as two doublets at 6.82 δ and 7.04 δ and of course no hydantoin ring proton is observed; however, after an in situ reduction (to 4) with sodium hydrosulfite the hydantoin ring proton appears at 5.48 δ.

Comparison of both 1 and 4 to synthetic samples[9] confirmed these assignments.

Acetylation of 4 affords the monoacetate 5 (λ_{max}^{INHCl} 284 log ε 3.25). A similar reaction on 6 yields a mixture of di and triacetates.

These results are incorporated into the partial structure 7 for this antibiotic.



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