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STRUCTURE DETERMINATION OF LAVENDAMYCIN- A NEW ANTITUMOR ANTIBIOTIC FROM STREPTOMYCES LAVENDULAE

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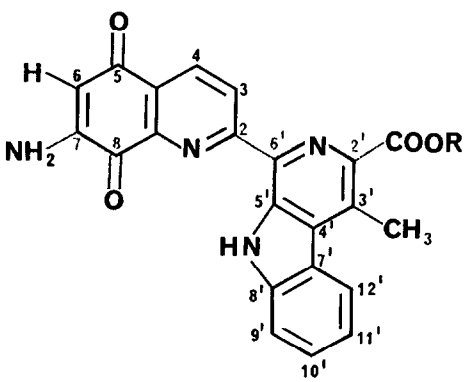
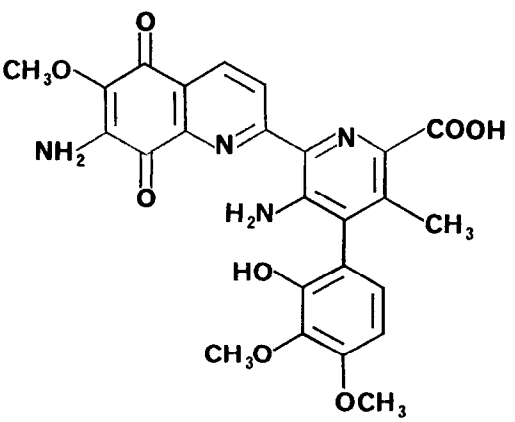
Summary The structure elucidation of lavendamycin **(Ia)**, a new antitumor antibiotic related to streptonigrin **(2)** is described. The NMR spectra of **Ia** and several model compounds **3**, **4** and **5a-b** are also discussed.

Recently we have isolated a novel antitumor antibiotic lavendamycin **(Ia)** from Streptomyces lavendulae, strain C22030.[2] Lavendamycin was obtained as a dark red solid, mp >300° dec. It had only limited solubility in organic solvents which precluded our efforts to grow crystals for x-ray analysis. The IR spectrum of **Ia** had bands characteristic of a carboxylic acid and a quinone (IR bands at 2800-3800, 1740, 1692, 1610 (strong), 1590). The UV spectrum of **Ia** in methanol showed three maxima at 234 (a=49.2), 246 (a=49.8) and 391 (a=21.1) nm. On addition of dilute acid the maxima shifted to 252 (a=47.4), 277 (a=36.0) and 385 (a=19.0) nm. Addition of dilute base gave bands at 245 (a=94.1), 309 (a=42.3) and 390 (a=39.6) nm. These results indicated the amphoteric nature of **Ia** and the uv spectrum was similar to that of streptonigrin **(2)** (uv maxima at 245 and 382). The elemental formula, $C_{22}H_{14}N_4O_4$, was determined by elemental analysis.[4] The mass spectrum of **Ia** showed a molecular ion M/e 398 and a parent ion at M/e 354 ($M-CO_2$). The ^1H NMR spectrum of **Ia** in DMSO-d_6 exhibited resonances at δ 3.08 (s, 3H, C-3'-CH_3) 5.91 (s, 1H, C6-



Ia R=H
b R=CH3



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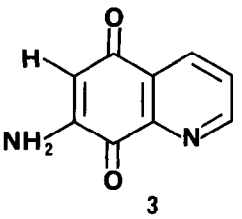

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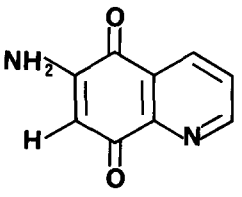

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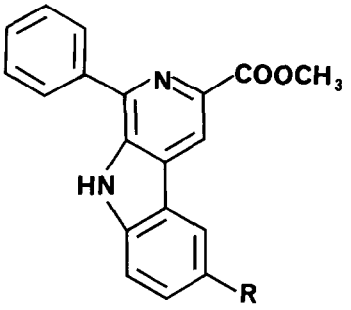
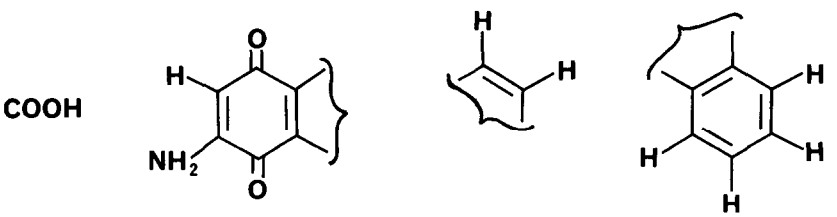


5a H
b NO_2

H) 7.37 (dd, 1H, J=8.6, 6.0, C-11'H), 7.40 (bs, 2H, NH_2), 7.61 (m, 2H, C-10' and C-9' H's), 8.28 (d, 1H, J=8.6, C-12'H), 8.37 (d, 1H, J=8.0, C-3H),8.93(d,1H,J=8.0,C-4H),11.86 (s, 1H, NH). When the ^1H NMR spectrum was recorded in CF_3CO_2D, an exchange of the signal at δ 5.91 was observed. Treatment of **la** with methanolic hydrogen chloride gave the ester **lb.** The IR and NMR spectra of **lb** were consistent with this interpretation. The MS of **lb** had a molecular ion at M/e 412 (also the parent ion). The CMR spectrum of **la** (Table I) showed resonances at δ 180.7 and 180.0 (suggestive of a quinone), 167.3 (carboxylic acid) and a signal at δ 102.2 for a protonated carbon which was absent in the spectrum when it was recorded in CF_3CO_2D. The foregoing evidence was suggestive of the following structral fragments in **la;**



In addition to the similar uv spectrum lavendamycin **(la)** also behaved similarly to streptonigrin in various bioassays.[3] This led us to suggest **la** as a possible structure for lavendamycin. All of the physical data was consistent with this assignment.[5] In order to confirm the assignment, we decided to do a ^{13}C nmr study on **la** in comparison with several model compounds, i.e. **3, 4** and **5a,b.**[7]

We were especially interested in establishing the position of substitution of the amino group on the quinone since it was not possible to establish this point directly and our original assignment had been made solely based on the structural analogy with streptonigrin. Comparison of the signals for C5, C6, C7, C8 in lavendamycin with the corresponding signals in the model compounds **3** and **4** indicated that the amino group of lavendamycin **(la)** resides at C7 as in **3**[10], inasmuch as the chemical shifts for these carbons were virtually identical in **3** and **la.** The carbon at 180.0 δ in **la** and **3** showed long range coupling to the C6 proton. The signal at 180.7 was uncoupled. In compound **4** the carbonyl at 181.8 was coupled to the C7 proton while that at 180.4 was uncoupled. Overall the model compound **3** more closely resembled **la** than did **4** supporting our assignment to the quinoloquinone portion of the molecule.

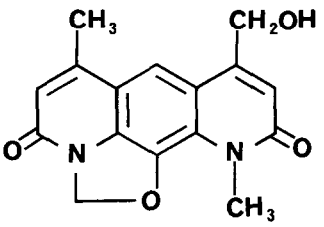
Table I. ^{13}C NMR Spectral Assignments to **1a**, **3**, **4**, **5a**, and **5b**[a]

Carbon	Compound			Carbon	Compound		
	1a	**3**	**4**		**1a**	**5a**[b]	**5b**[b]
C2	157.9	152.4	154.2	C2'	132.7	134.6	137.0
C3	134.4	133.2	133.6	C3'	128.8	116.6	117.5
C4	125.1	128.2	126.3	C4'	(131.9)[c]	129.2	129.7
C4a	(129.8)[c]	130.2	127.4	C5'	134.6	142.1	143.2
C5	180.7	180.8	181.8	C6'	136.9	136.7	138.1
C6	102.2	101.7	149.8	C7'	121.3	121.1	121.0
C7	150.7	150.9	103.6	C8'	140.3	137.6	144.7
C8	180.0	180.0	180.4	C9'	112.2	112.8	113.2
C8a	145.5	146.6	148.7	C10'	128.7	128.6	123.7
				C11'	121.0	120.3	136.0
				C12'	124.0	121.9	119.6
				$\overset{*}{C}O_2R$	167.3	166.0	165.7
				OCH_3	-	51.9	52.0
				C3'-CH_3	16.4		

a. Recorded at 90.5 MHz in DMSO-d6 on a Bruker WH-360 spectrometer.
b. The carbons for the phenyl group resonated at δ 141.5 (C1), 128.8 (C2), 128.6 (C3) and 128.9 (C4).
c. Bracketed assignments may be reversed.

As a model for the β-carboline portion of **1a**, we have used **5a**. The spectrum of **5b** was used to aid in the assignments to **5a**. Compound **5a** differs in two important respects from **1a**; it lacks the C3' methyl substituent, and the C6' phenyl is replaced in lavendamycin (**1a**) by the amino quinoloquinone chromophore. Despite these differences, the ^{13}C nmr spectrum of **5a** closely resembles that of **1a**. The signal for C12' in **1a** is shifted ~2ppm downfield from the corresponding carbon in **5a**. We ascribe this to a steric compression. The signal at 128.8 in **1a** shows coupling to the C3'-methyl group thus aiding in its assignment. The carbon atoms most perturbed by the substitution of the methyl group at C3' (other than C3' itself) were C8' and C5'. This may be due to some skewing about the C4'-C7' axis as well as direct electronic effects.

It is interesting to speculate on the possible biogenetic role of **1a** with respect to the biosynthesis of streptonigrin. One of us (S.G.) has earlier proposed that a substituted β-carboline, such as **1a**, may be an intermediate.[11] If this were the case, it would indicate that oxygenation of the benzene rings occurs at a late stage in the biosynthesis. This would be consistent with the finding that O-methylation of streptonigrin precursors occurs late in the biosynthesis.[12] In view of the structures of **1a** and **2** it is likely that 4-amino anthranilic acid is a biogenetic intermediate in the formation of both compounds, each containing a m-phenylenediamine (C_6N_2) unit. Since the carbocyclic ring of nybomycin **(6)** has been shown to be derived via a shikimate pathway,[13] it, too, may now be viewed as biogenetically part of the same family. The ionophore A-16239 **(7)** also has a m-phenylenediamine grouping, but in this case it is part of an isomeric 6-aminoanthranilic acid.[14]



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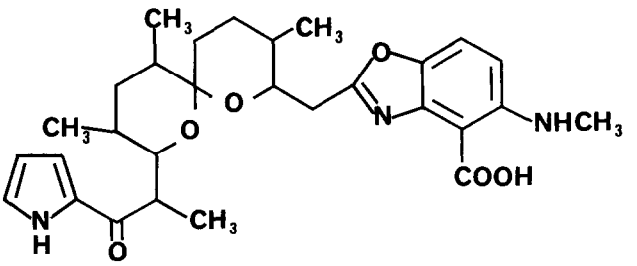

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References and Notes

1. S.G. is a Career Development Awardee of the National Cancer Institute (CA-00627) 1979-1984.
2. The isolation and biological activities of **(Ia)** will be reported upon separately.[3].
3. D.M. Balitz, J.A. Bush, W.T. Bradner, F.A. O'Herron, D.E. Nettleton and T.W. Doyle, to be submitted to J. Antibiotics.
4. Compound **(Ia)** analyzed correctly for the monohydrate. Taken together with the molecular ion in the MS at 398, this indicated a molecular formula of $C_{22}H_{14}N_4O_4$.
5. Recently Shibata et al[6] have reported isolation of antibiotic K82-A which has very similar UV and IR spectra to **Ia** as well as possessing the same molecular formula.
6. M. Shibata, M. Uyeda, Y. Kido, N. Toya, R. Nakashima, and R. Terzumi, J. Antibiotics 33, 1231-1235 (1980).
7. We thank Dr. J. W. Lown of the University of Alberta for a generous gift of **4**. Compounds **3**, **5a** and **5b** were synthesized in connection with related studies on streptonigrin.[8] A full account of their synthesis will be published elsewhere.[9]
8. S.J. Gould and D.E. Cane, Abstract 190, Division of Organic Chemistry, 181st ACS National Meeting, Atlanta, Georgia, 1981.
9. S.J. Gould, unpublished results.
10. The ^{13}C nmr assignments were made unequivocally by comparison of model compounds, off resonance and gated decoupling techniques.
11. S.J. Gould, C.C. Chang, D.S. Darling, J.D. Roberts, M. Squillacote, J. Am. Chem. Soc., 102, 1707-1712 (1980).
12. S.J. Gould and C.C. Chang, J. Am. Chem. Soc., 102, 1702-1707 (1980).
13. A.M. Nazdan and K.L. Rinehart, Jr., J. Am. Chem. Soc., 98, 5012 (1976).
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