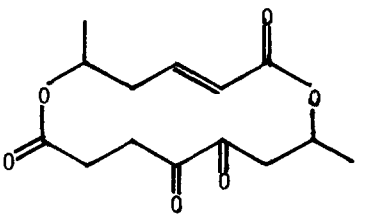
Tetrahedron Letters Vol. 21, pp 681-684 0040-4039/80/0215-0681$02.00/0

GRAHAMIMYCIN A_1: A NOVEL DILACTONE ANTIBIOTIC FROM CYTOSPORA

Robert C. Ronald* and S. Gurusiddaiah
Department of Chemistry and Bioanalytical Laboratory
Washington State University
Pullman, Washington 99164

By means of spectral and chemical studies the structure of Grahamimycin A_1 1, a novel macrocyclic dilactone antibiotic from Cytospora, is established.

As part of a program investigating fungi of the genus Cytospora as a source of novel biologically active substances, we observed that aerobically grown cultures of one isolate of Cytospora, a saprophytic fungus from lodge pole pine [Pinus contorta var. Latifolia Engelm][1] designated as Cytospora sp. Ehrenb W.F.P.L. (Western Forest Products Laboratory, Vancouver, B.C., Canada) 13A (ATCC 20502), exhibited antibacterial activity in a modified disc-zone assay[2] against a variety of pathogenic microorganisms including species of Bacillus, Pseudomonas, Salmonella, Staphylococcus and Strepococcus. Preliminary investigations demonstrated that the antimicrobial activity of these cultures could be completely extracted into organic solvents. From these extracts we have obtained an antimicrobially active, bright yellow, dilactone-dione which we have designated Grahamimycin A_1 1.



1

Cultures of the fungus were grown aerobically at 23-34°C in a potato extract-dextrose medium for 9 days, at which time antimicrobial activity reached a maximum level. Essentially all of the antimicrobial activity could be extracted from the culture broth and mycelial material with chloroform. The chloroform extracts of both the culture broth and mycelial material were combined and concentrated to a small volume, then precipitated by pouring into a large volume of petroleum ether which removed inactive oily material. The precipitate was taken up in ether which separated an inactive solid residue, concentrated to a small volume

and then reprecipitated with petroleum ether. The precipitated material was applied to a silica gel column and eluted with a mixture of 2% ethyl ether in dichloromethane. Antimicrobial activity eluted as an intensely yellow band with this solvent mixture. From 4.6g of crude, chloroform soluble extract 115mg of oily material was collected. Repeated recrystallization from CH_2Cl_2-hexane and finally from diisopropyl ether afforded bright yellow crystalline Grahamimycin A_1, 1, mp 91-92°, $[\alpha]_D^{22}$ -14.7° (0.76, $CHCl_3$). Purified Grahamimycin A_1 exhibits antibacterial activity at the microgram level in disc-zone inhibition assays against the test organisms Bacillus subtilis and Pseudomonas nigrifaciens.

The structure of Grahamimycin A_1 was assigned on the basis of a combination of spectral and chemical evidence. Combustion analysis (59.81% C and 6.44% H) in conjunction with the mass spectrum which had a molecular ion of 282 amu were consistent with a molecular formula of $C_{14}H_{18}O_6$ (calculated for 59.57% C and 6.38% H).

The ultraviolet spectrum of 1 in ethanol had λ_{max} 426nm ε 16 and was otherwise featureless except for strong end absorption. The band at 426nm coupled with the lack of any significant absorption around 260nm was suggestive of an α-diketone chromophore with trans, anti-planar carbonyl groups which were not significantly enolized in alcoholic solution. A conjugated ester would account for the strong end absorption observed.

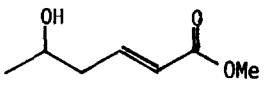
The infrared spectra of 1 taken in KBr and also in $CHCl_3$ showed strong carbonyl absorptions between 1750 and 1700 cm^{-1} and a band at 1650 cm^{-1} indicated the presence of at least one unsaturated carbonyl system which was also supported by the UV data. In KBr there was a weak hydroxyl absorption which is nearly absent in the spectrum taken in chloroform. Treatment of a small amount of 1 with acetic anhydride afforded a single slightly more polar product by thin layer chromatography which had new unsaturated carbonyl absorptions at 1760 cm^{-1} and 1680 cm^{-1}. This behavior would be more consistent with the formation of an enol acetate rather than the acetylation of a hydroxyl group.[3] Hydrogenation of 1 over platinum at atmospheric pressure afforded a much more polar product by thin layer chromatography which showed a single broad carbonyl absorption at 1720 cm^{-1}, a strong hydroxyl at 3490 cm^{-1} suggesting reduction of a carbonyl group or enol, and loss of the absorption for the unsaturated carbonyl at 1650 cm^{-1}. Both acetylation and hydrogenation occurred with loss of the yellow color of 1. These reactions are consistent with an enolizable α-dicarbonyl system. Borohydride reduction of 1 afforded a complex mixture of products.

The proton magnetic resonance spectrum in $CDCl_3$ if 1 showed four groups of resonances. An AB quartet integrating for 2 protons between 5.4-7.2 δ J = 16 Hz in which the lower field doublet centered at 6.8 δ was split into triplets J = 8 Hz. This pattern is consistent with a $-CH_2-CH=CH-COO-$ moiety in which the double bond has the E geometry. A complex multiplet integrating for 2 protons 5.6-4.8 δ which was shown to be coupled to a pair of methyl doublets at 1.6-1.2 δ J = 7 Hz. These bands are consistent with a $CH_3-CH-O-$ subgroup. A very complex band 3.8-2.0 δ integrated for 8 protons and there was no observable exchange upon treatment with D_2O.

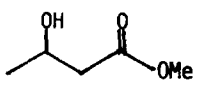
Due to the small amounts of 1 available only ten of the possible fourteen carbon-13 resonances were observed. A very weak proton coupled spectrum allowed assignment of the methyl groups at 19.95 and 20.67 ppm (relative to TMS), and four methylene groups at 28.46, 31.46, 38.28 and 39.71 ppm. The methine carbons bearing the oxygens and the olefinic carbons were at 68.89 and 70.64 and 123.68 and 144.92 ppm respectively.[4]

The mass spectrum obtained at 15 ev had a molecular ion at 282 amu. Prominent fragment ions occurred at: 254 amu (m-28), loss of CO; 154 amu (M-128), loss of succinic anhydride from (M-28) with a corresponding metastable peak at 93.4 amu; and 113 amu (M-169), an ionized hexenoic acid. Other fragment ions at 95 amu and 96 amu were also assigned to the hexenoate moiety.

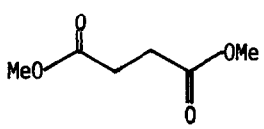
Since from spectral evidence 1 appeared to be an ester (or lactone) a small amount (1 mg) was treated with dilute aqueous sodium hydroxide which resulted in immediate reaction to form an intense red-brown color. After all of the starting material had reacted (5 min), the mixture was acidified with dilute sulfuric acid and extracted into chloroform. Esterification of this extract with diazomethane afforded a single volatile product 2 as determined by gas chromatography at 200° on a 3mm x 2.6m glass column packed with 3% OV-17 on 80/100 Chromosorb W HP. No other fragments were observed either by GC or by thin layer chromatography. On the



2



3



4

basis of the infrared spectrum of 2 and also from the proton nmr spectrum of 1, the volatile fragment was assigned a hydroxy hexenoate structure, and thus accounts for six of the fourteen carbon atoms of 1. This assignment was confirmed by the conversion of 2 with refluxing methanol-sulfuric acid to a new ester identical by GC and IR to methyl sorbate.

The final verification of the structure as shown in 1 was achieved by cleavage of the ring with aqueous, methanolic periodic acid followed by hydrolysis and finally esterification. The periodic cleavage afforded further chemical evidence for the α-diketone structure implicated by the UV and IR data, and the fragments produced by these reactions fixed its position in the structure. Treatment of 10.8mg of 1 with 25mg (2 equivalents) periodic acid in 1:1 water-methanol resulted in slow fading of the yellow color. After 1 hr the TLC indicated that all of the starting material had reacted. The crude diacid obtained was hydrolyzed by addition of aqueous 2M NaOH which also precipitated excess sodium periodate and sodium iodate. After standing overnight the mixture was filtered to remove suspended solids and evaporated to dryness. The residue was acidified with dilute aqueous HCl and

esterified with diazomethane to afford three volatile products 2, 3, and 4 in approximately equal amounts as determined by GC. These were separated by preparative gas chromatography on a 4mm x 2.4m glass column packed with 3% OV-17 on Chromosorb W HP to afford: methyl 3-hydroxybutyrate 3 (R.T. at 125°, 1.4 min), identical by coinjection on GC and IR with an authentic sample prepared from the hydroxy acid; methyl succinate 4 (R.T. at 125°, 2.9 min), identical by coinjection on GC and by IR with an authentic sample; and the aforementioned methyl 5-hydroxy-trans-2-hexenoate 2 (R.T. at 125°, 6.4 min), identical to the sample prepared by base hydrolysis of 1. The formation of succinate 4 and butyrate 3 accounts for the remaining eight carbon atoms of 1; and isolation of these fragments concomitantly with the previously indicated hydroxyhexenoate residue uniequivocally establishes the structure of Grahamimycin A_1 as that shown in 1. Hydroxyhexenoic acids have also been isolated in the alkaline degradation of a related series of macrocyclic dilactone metabolites from Colletotrichum capsici, a pathogen of the pepper plant.[5] However, dione 1 is distinct from any known Colletotrichum metabolites, and furthermore, no antibiotic activity has been reported for these compounds. We are currently engaged in studies to determine the absolute configuration and the mode of antibacterial action of this novel antibiotic.

The generous support of this work by the National Institute of Allergy and Infectious Disease and the Washington State University Research Foundation is gratefully acknowledged.

References and Notes

1. H. W. Eades and J. W. Roff, Canada Department of Northern Affairs and National Resources Bulletin, 1025, 1 (1959).

2. Y. H. Loo, et al., J. Bact., 50, 701 (1945).

3. K. Nakanishi, Infrared Absorption Spectroscopy, Holden-Day, San Francisco, 1962; R. M. Silverstein and G. C. Bassler, Spectrometric Identification of Organic Compounds, J. Wiley, New York, 1963.

4. The carbon-13 resonance spectra were obtained on a Bruker WH-90 spectrophotometer provided by a grant from the National Science Foundation to Washington State University and the University of Idaho.

5. J. MacMillan and T. Simpson, JCS Perkin 1, 1487 (1973); for other dilactones see also: W. Keller-Schlerlein, Fortschr. Chem. Org. Naturstoffe, 30, 313 (1970); M. Binder and C. Tamm, Angew. Chem. Internat. Ed., 12, 370 (1972); S. Nozoe, et al., Tet. Lett., 4675 (1975).

(Received in USA 24 October 1979)