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REVIEW ARTICLE

Ziracin, a Novel Oligosaccharide Antibiotic†

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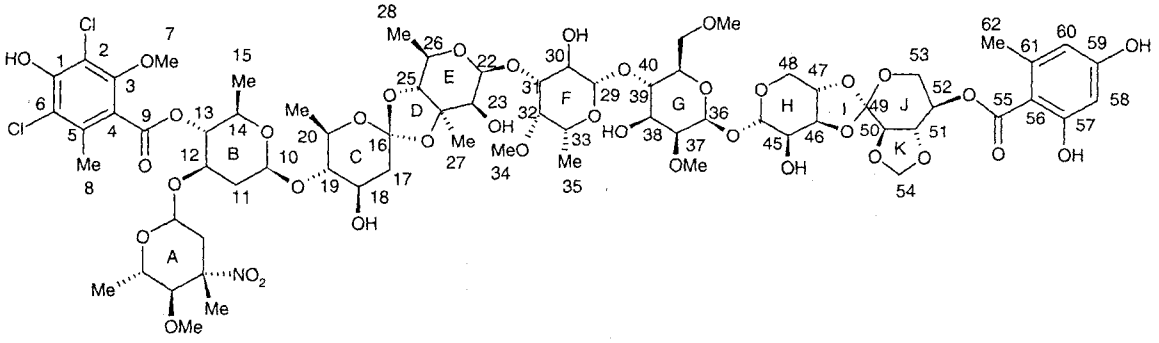
Ziracin is produced by *Micromonospora carbonacea*[1] and is highly active against Gram-positive bacteria. In particular it is highly active against methicillin resistant staphylococci and vancomycin resistant enterococci. Ziracin, $C_{70}H_{97}NO_{38}Cl_2$, contains two orthoester linkages, a nitro sugar, a methylene dioxy group, two aromatic ester residues and thirty five centres of assymmetries. In this paper a brief description of the structural elucidation of ziracin is presented along with the chemical modification of the antibiotic which has led to the identification of several potent antibacterials.

Since the discovery of penicillin in 1928 by ALEXANDER FLEMMING and large scale production of this class of antibiotics during the second world war enormous progress has been made towords the discovery of newer penicillins, cephalosporins, aminoglycosides, quinolones, rifamycins and macrolide antibiotics. Availability of the above antibiotics has made it possible to conquer many of the infectious diseases which were the main causes of mortality around the world in the early part of the last century. However, with the overuse of antibiotics and with increasing number of immunocompromised patients *etc.* microorganisms have grown resistant to some of these antibiotics which is of great concern. In particular methicillin and vancomycin resistant *Staphylococcus* and *Enterococcus* are becoming more common and there are not too many alternative antibiotics available to cure infections caused by them.

In this paper we would like to review the chemistry and microbiological activities of ziracin and its analogs. Ziracin (**1**) is a novel oligosaccharide antibiotic which is active against methicillin and vancomycin resistant organisms. When tested against a large number of methicillin sensitive and resistant strains of *S. pneumoniae* ziracin shows MIC's in the range of 0.032~0.125 μg/ml. In the same experiment Pen-G shows MIC's in the range of 0.125~8 μg/ml. Against vancomycin sensitive and resistant strains, ziracin shows MIC's in the range of 0.45~2 μg/ml whereas against the same organisms vancomycin shows MIC's in the range of 1~421.4 μg/ml. In *in vivo* experiments in mice infected with *S. pneumoniae* ziracin shows good activity with an ED_{50} at 2.5 mg/kg. In human volunteers ziracin when administered by i.v. it gave significant blood level *i.e.* 10 μg/ml at 1 mg/kg and it is well tolerated.

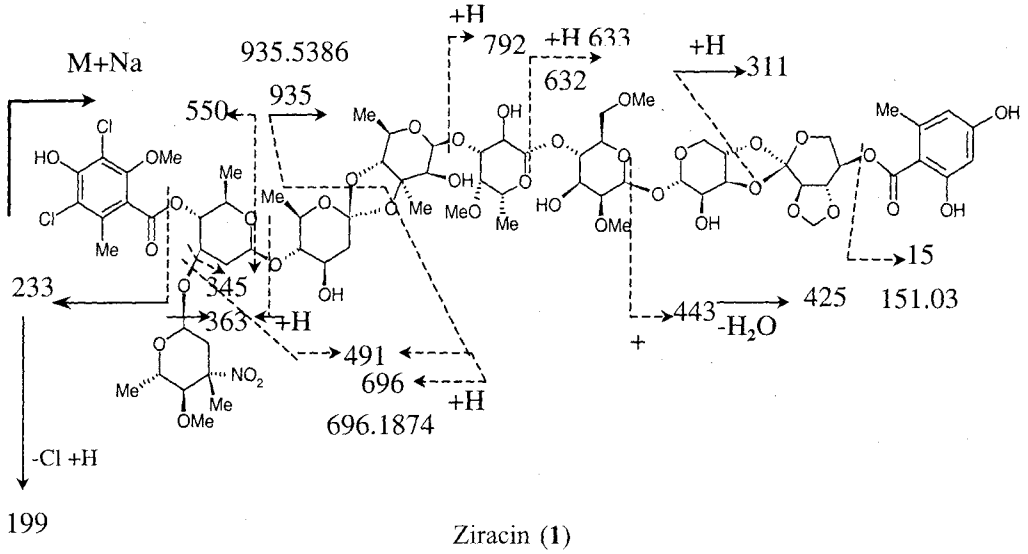
Ziracin belongs to the orthosomycin family of antibiotics. Other members of this class of antibiotics include everninomicins, curamycin, olivamycin and avilamycin. The structures of everninomicins[2] were the first to be deduced amongst the orthosomycins by the present author and his colleagues at Schering-Plough Research Institute. We have extensively reviewed the chemistry of everninomicins and in this article we shall only refer to it when it becomes necessary to compare the chemistry of ziracin and everninomicins.

† Dedicated to the memory of Professor E. ABRAHAM for his many brilliant contributions in the area of infectious diseases.

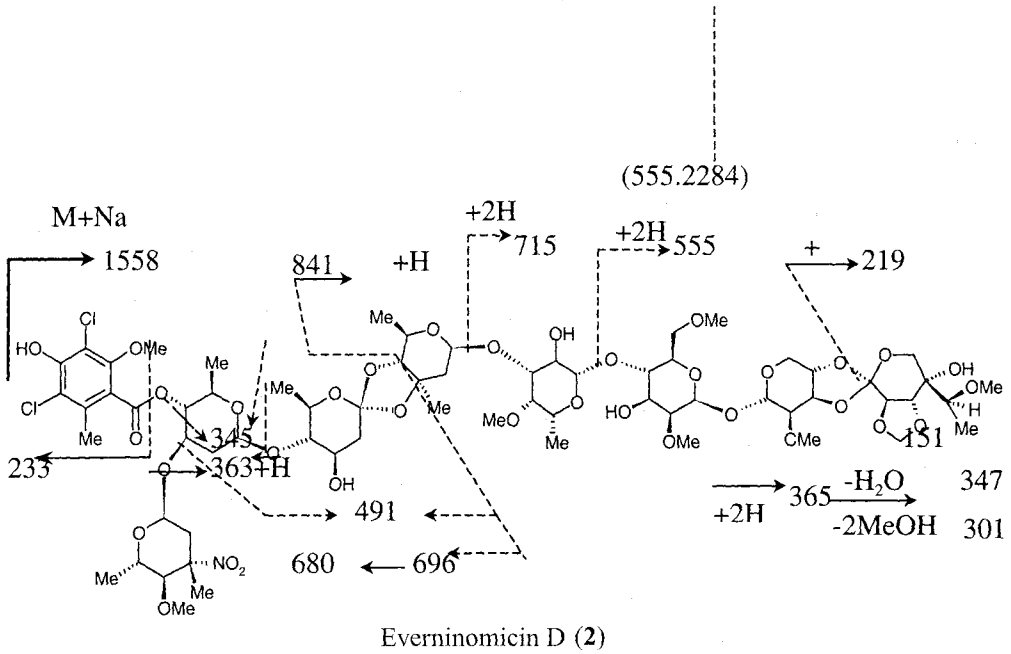


Ziracin (**1**)

Scheme 1.



Ziracin (**1**)



Everninomicin D (**2**)

Physico-chemical Properties and Structure Elucidation

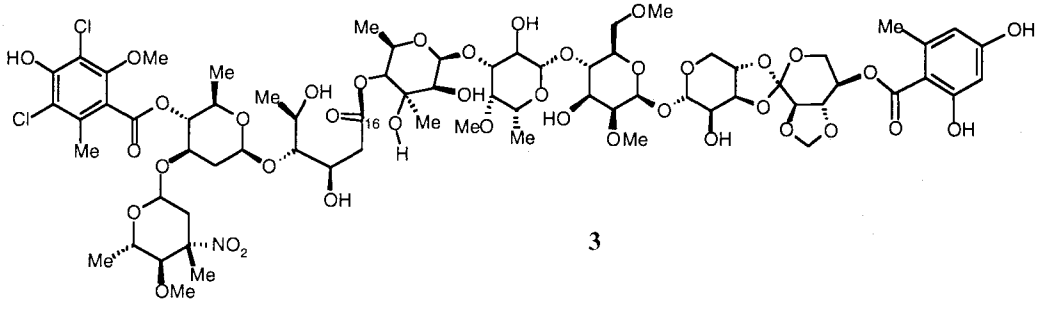
Ziracin[3] (**1**) is an amorphous solid, $C_{70}H_{97}NO_{38}Cl_2$ (mol. wt. 1629) $[\alpha]_D$ −47.2° and ν_{max} 1540 cm^{-1} (nitro group). Comparison of the high resolution mass spectra of ziracin **1** and everninomicin-D **2** (see Scheme 1) reveals similarities and differences between the two molecules. For example, a) the two antibiotics have identical compositions of A, B and C rings, b) the E-ring in ziracin has an extra hydroxyl group, c) in the H-ring ziracin has a hydroxyl group compared to the presence of a methoxyl group at the same position in everninomicin-D and d) the substitution patterns at C52 are different in the two antibiotics.

^{1}H-NMR spectrum of ziracin **1** indicates the presence of five methyl doublets, two tertiary methyl groups, two aromatic methyls and five methoxyl groups. ^{13}C-NMR spectrum of ziracin shows the presence of two orthoester carbons at δ 119.2 and 120.4, which are characteristics of this class of antibiotics and hence they are classified as orthosomycins. In an earlier publication[4] we have completely assigned all the protons and carbon atoms of ziracin. When the ^{13}C-NMR spectra of ziracin **1** and everninomicin-D **2** are compared it reveals the presence of an extra aromatic residue in ziracin **1** at C_{52} *e.g.* δ 170.77 (ester carbonyl), six extra aromatic carbons at δ 103.83, 165.5, 101.09, 162.77, 112.31 and 143.93.

High resolution mass spectrum indicates that the group attached at C_{52} in ziracin to be $C_8H_7O_4$ (*m/e* 167) and based on NMR spectrum it is assigned to be a 2 : 4-dihydroxy-6-methyl benzoyloxy residue.

Chemical degradations of ziracin and everninomicin-D yield either identical products or units which have similarities and also recognisable differences. Thus, when a solution of ziracin **1** in methylene chloride is stirred with 0.1 N hydrochloric acid it yields **3** in which the central orthoester linkage is cleaved. On treatment with diazomethane compound **3** yields lactone **4** and compound **5**. The lactone **4** is found to be identical (mp, NMR, MS, $[\alpha]_D$) with the lactone obtained by similar degradation of everninomicin-D[2].

Compound **5**, however, is quite different than olgose-D **6**, a degradation product of Everninomicin-D **2**. Compound **5**, $C_{43}H_{64}O_{25}$ (*m/e* 980.3832), $[\alpha]_D$ −46.9° is an amorphous solid which shows UV absorption in trifluoroethanol λ 242 nm (22231), 261 nm (11099), 278 nm (5233), 294 nm



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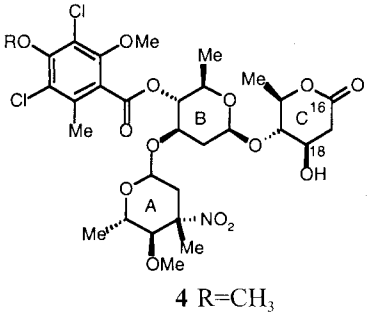
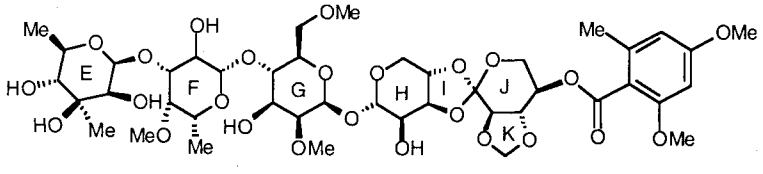


4 R=CH_3



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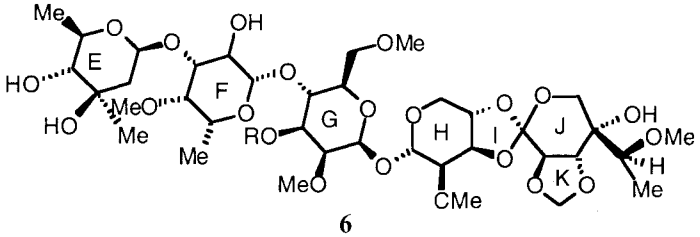

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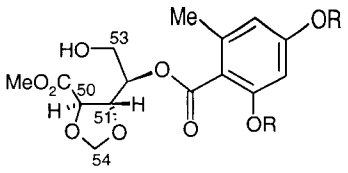
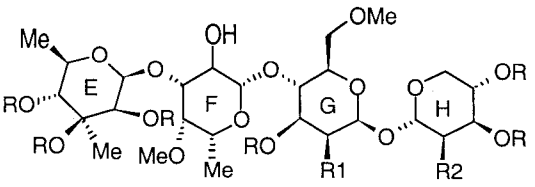
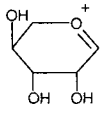


7 R=CH_3

12 R=H



8 R=H; R1= OMe; R2=OH
11 R=Me; R1=R2=OMe



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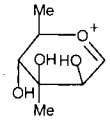

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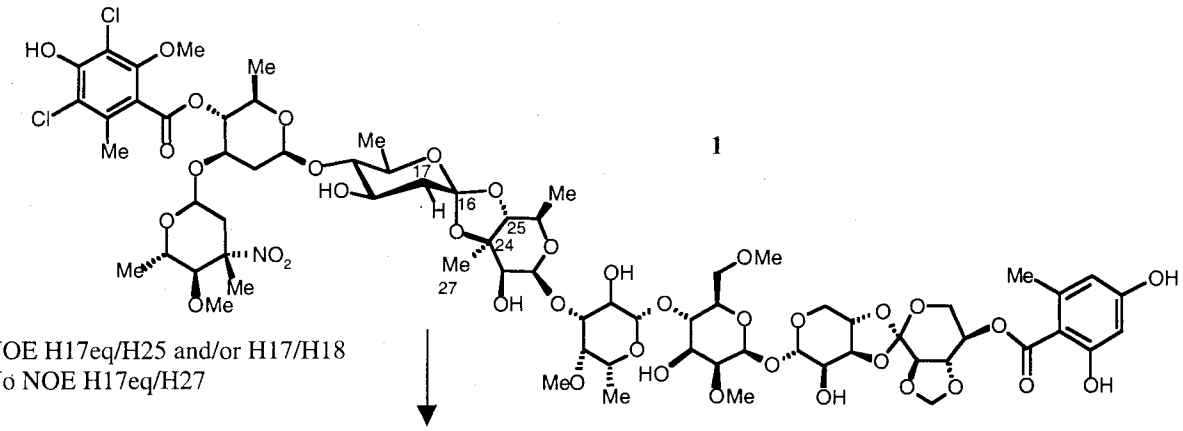
(2970) and IR absorption at ν_{max} 1735 cm^{-1} (ester). Olgose-D **6**, of course, does not show the above UV and IR absorptions. ^{1}H-NMR spectrum of **5** shows the presence of two methyl doublets (δ 1.31 and 1.33), a tertiary methyl (δ 1.2), an aromatic methyl (δ 2.5), five methoxyl groups, four anomeric hydrogens (δ 5.31, 4.27, 4.96 and 4.78) , two aromatic hydrogens (δ 6.33) and H_{52} appears at δ 5.43 ($J_{51,52}$=$J_{52,53a}$=9.7; $J_{52,53e}$=5.8 Hz) which suggests the presence of an axial hydrogen at C_{52}. Comparison of the ^{13}C-NMR spectra of **5** and **6** shows the presence of C_{45} in **5** and **6** at 72.5 and 80.5 respectively indicating that C_{45} in **5** bears a hydroxyl group compared to **6** wherein the hydroxyl group at the same position is methylated. Compounds **5** and **6** show characteristic singlets for H_{54} at δ 5.14 and δ 5.22 and H_{50} and H_{51} are axially oriented in both these compounds.

Hydrolysis of **5** with methanol and *para*-toluenesulphonic acid yields the UV absorbing methyl ester **7** and the non UV absorbing compound **8**. In the mass spectrum compound **8** shows ions corresponding to **9** and **10**. Based on all the above observations it is clear that **8** is 45-des-*O*-methyl evertetrose B. Permethylation of **8** yields permethylated evertetrose B **11** the structure and absolute stereochemistry of which we have determined in an earlier publication[2].

The structure of the methyl ester **7** follows from NMR and mass spectral studies. Compound **7**, oil, $C_{17}H_{22}O_9$ (*m/e* 370.1255) is obtained by methylation of **12** with diazomethane and **12** in turn is obtained from ziracin **1** by methanolysis with methanol and *para*-toluenesulphonic acid. Compound **12** $C_{15}H_{18}O_9$ (*m/e* 342.0942) shows an aromatic methyl at δ 2.50 ($J_{60,62}$=1 Hz) , two *meta* coupled aromatic protons at δ 6.23 (H_{58}; $J_{58,60}$=2 Hz) and 6.19 (H_{60}; $J_{60,62}$=1 Hz), a carbomethoxyl group at δ 3.80, two methylene dioxy protons assigned for H_{54} appear at δ 5.03 and 5.24 (J=0 Hz); H_{50} at δ 4.51 ($J_{50,51}$=5 Hz) and H_{51} appeared at δ 4.47 ($J_{51,52}$=5 Hz). As in Olgose-D **6** we concluded that compound **5** is derived from **7** and **8** with the loss of methanol and water involving the carbomethoxyl group and the hydroxyl groups at C_{53}, C_{46} and C_{47} thus forming the orthoester linkage in **5**. This would explain the presence of a carbomethoxy group in **7** and the orthoester linkage in **5**.

During the hydrolysis with mild acid ziracin **1** is converted to **3** in which the orthoester in the centre gets hydrolysed creating a new ester linkage which is absent in ziracin (**1**). On treatment with diazomethane **3** yields **4** and **5**. The above sequence of reaction is similar to the one we observed in the case of everninomicin-D **2**. As in the case of **2** the orthoester linkage in the center of ziracin **1** is derived by the elimination of a molecule of water between the ester carbonyl at C_{16} and the hydroxyl groups at C_{24} and C_{20}.

The stereochemistry of the orthoester linkage at C_{49} has been assigned using X-ray crystallography and the stereochemistry at C_{16} is deduced as follows[5]. When a solution of ziracin **1** in tetrahydrofuran is refluxed with *para*-nitrobenzoic acid it is partly converted into two isomeric products **13** and **14**. The structure of **13** was solved by comparing NOE studies with ziracin **1** and compound **13**. In ziracin **1** NOE is observed between H17e/H_{25} and no NOE is observed between H17e/H_{27} whereas in isomer **13** NOE is observed between H17e/H_{27} and no NOE is observed between H17e/H_{25}. As **1** and **13** have identical compositions and they yield the same ester **3** on mild acidic hydrolysis the difference between them must therefore be in the stereochemistry at the C_{16} orthoester carbon. Based on the above degradation experiments and NOE studies the structure and absolute stereochemistry of ziracin is established to be **1**. It should be noted that although ziracin **1** could be rearranged to the isomer **13** the reverse is not possible thus indicating that **13** is



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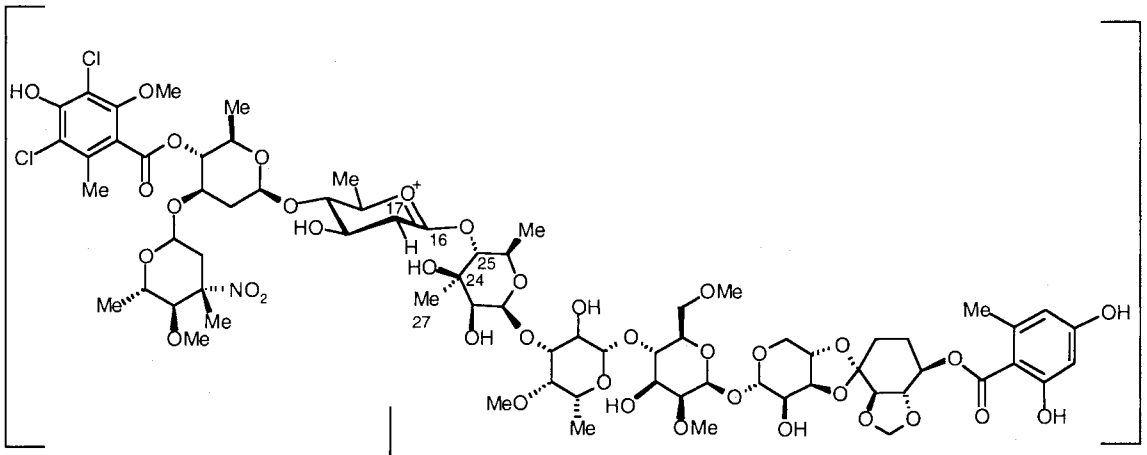
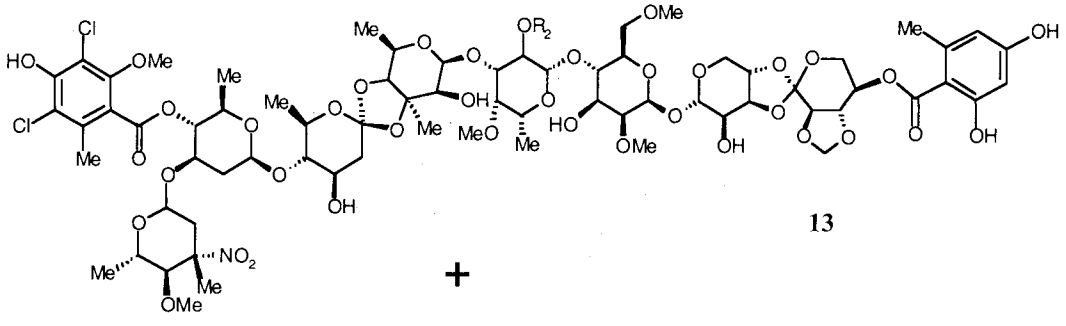
NOE H17eq/H25 and/or H17/H18
No NOE H17eq/H27





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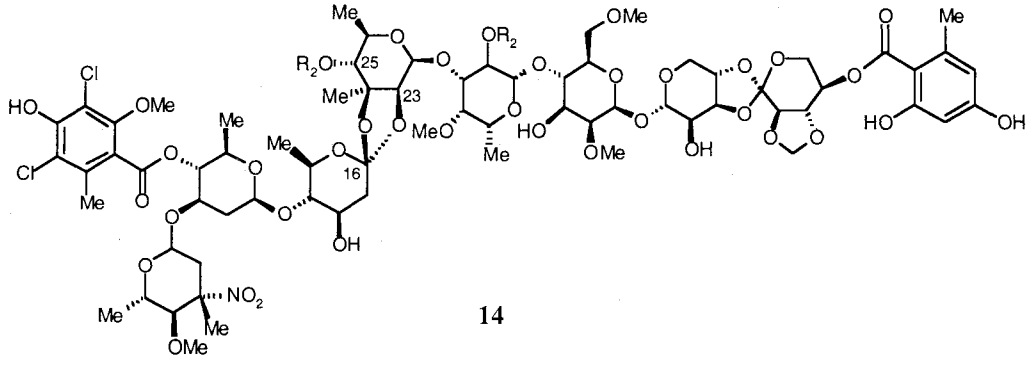

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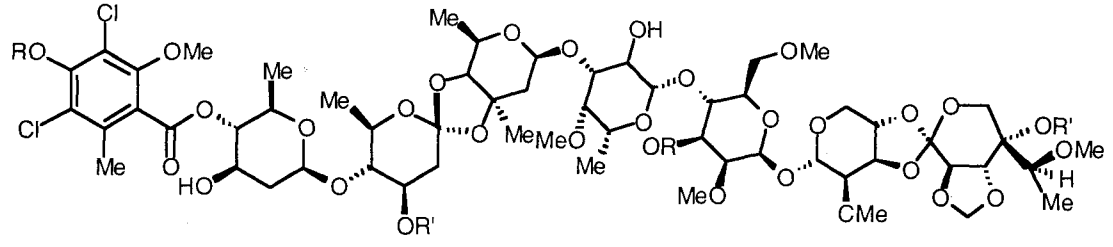
thermodynamically more stable.

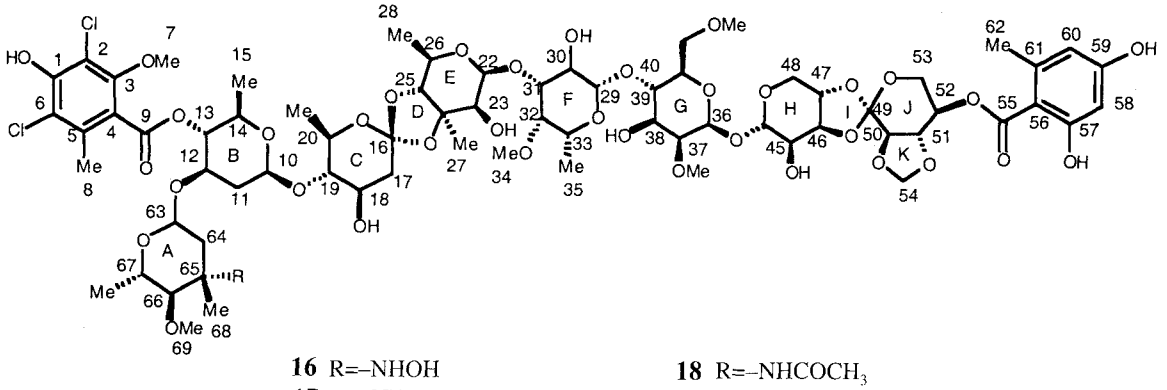
Structure Activity Relationship in Ziracin (1)

To retain the microbiological activity of ziracin the analogs must retain some of its structural features for example, the phenolic hydroxyl at C_1 must be free. Once it is derivatised as a methyl or allyl ether the activity is lost. Similarly the orthoester linkages are necessary for activity, for example compound **3** obtained by the hydrolysis of one of the orthoester linkages is found to be inactive. The stereochemistry at C_{16} is also very important because when it is inverted as in isomer **13** the compound is found to be inactive. The nitro sugar (ring A) is not essential for antimicrobial activity as we have shown during our work on everninomicin-2[6] **15**, which is equally active when compared to everninomicin-D **2**.

Although the presence of the nitro sugar is not necessary for activity, however the presence of the nitro function in ziracin **1** provides a very convenient handle for further modification. Reduction of the nitro group in ziracin **1** using zinc and ammonium chloride yields the corresponding hydroxylamino derivative **16** which possesses very potent antibacterial activity and when reduced with Raney-Nickel ziracin **1** produces the amino derivative **17** which also possesses antibacterial activity. It should be noted that both compounds **16** and **17** are produced during the fermentation of ziracin and are presumed to be biosynthetic precursors of **1**. Acylation of **17** yields antibacterials amongst which the acetamide derivative **18** possesses the highest activity.

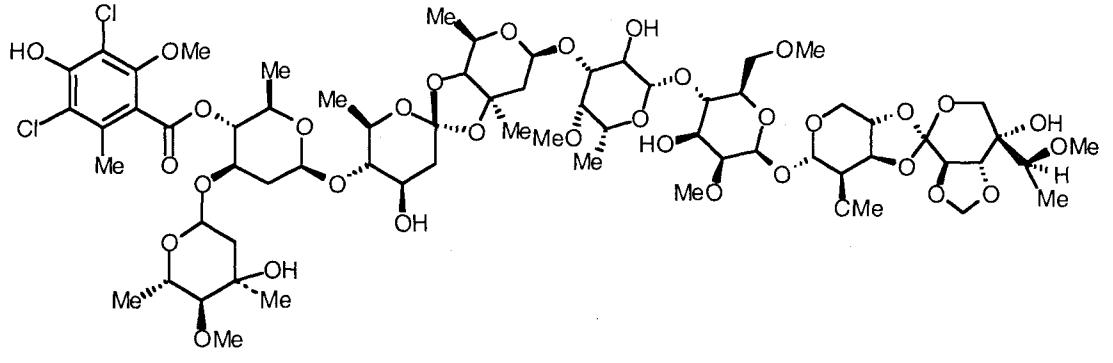


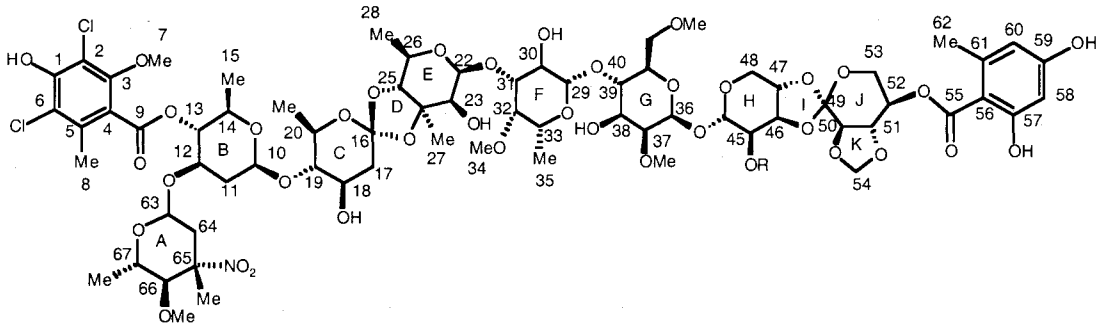
15 R′=R=H



16 R=–NHOH
17 R=–NH_2

18 R=–$NHCOCH_3$





20 R=–CH_2CH_2OH

Although not tried in ziracin, we have, however, demonstrated[6] that when everninomicin-D **2** is electrolysed in the presence of oxygen it produces the antibacterial everninomicin-7 **19** in which the nitro function is replaced with hydroxyl group with inversion of configuration.

The alcoholic hydroxyl groups in ziracin **1** when permethylated[8] results in loss of activity whereas partial derivatisation at C_{45} **20** maintains antibacterial activity.

Finally, the author wishes to note that a brilliant synthesis[7] of ziracin has been achieved very recently by Prof. K. C. NICOLAOU and his colleagues which verifies the structure and absolute stereochemistry of the antibiotics proposed by us.

Acknowledgement

The author wishes to sincerely thank Mrs. SARRE, Mr. MORTON, Mr. SZMULEWICZ and Drs. CHAN, GIRIJAVALLABHAN, GREEVES, MCCORMICK, PRAMANIK, and SAKSENA for all their contrbutions without which this exciting journey towards solving the structure of ziracin could not have been completed.

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