Structure of Everninomicin-2

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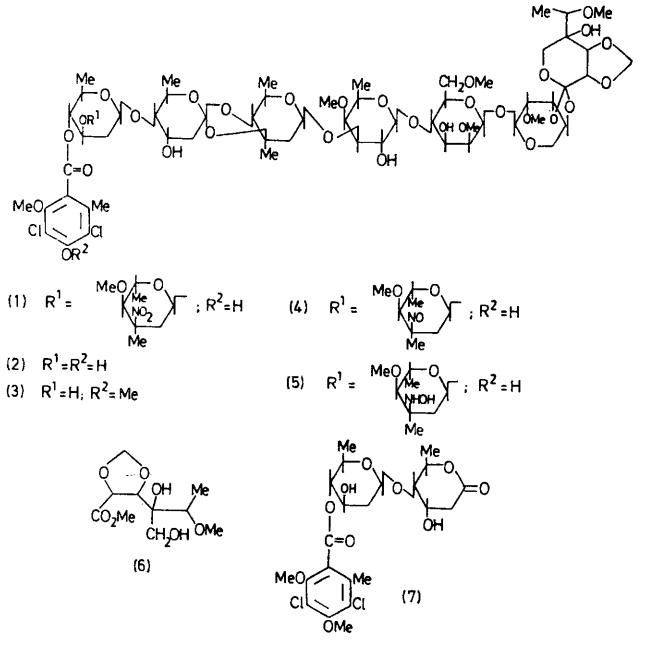
Summary The structure of everninomicin-2 (**2**) has been elucidated and a method of conversion of everninomicin D (**1**) into (**2**) is discussed.

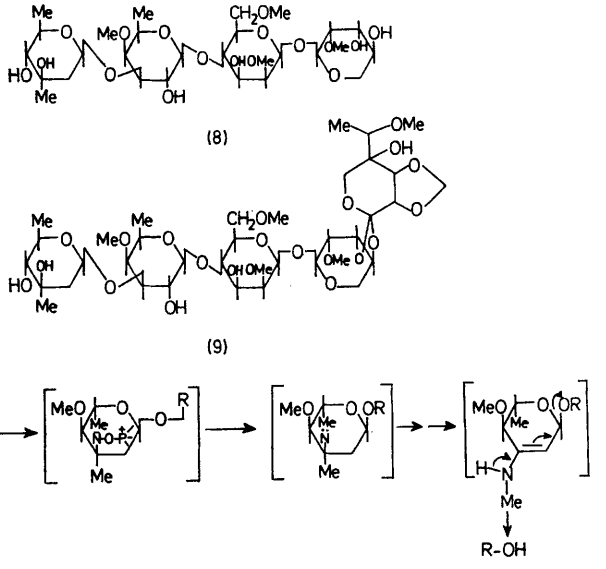
EVERNINOMICINS are produced by *Micromonospora Carbonaceae*.[1] They are highly active against gram-positive bacteria and are also active against strains resistant to penicillins, tetracyclines, lincomycins, rifampicin, macrolides, and chloramphenicol. In earlier publications we have disclosed the structures of everninomicin B,[2] C,[3] and D.[4] We report here the structure of everninomicin-2 and also describe a method of conversion of everninomicin D (**1**) into everninomicin-2 (**2**).



Everninomicin-2 (**2**) is a colourless crystalline solid, $C_{58}H_{86}Cl_2O_{31}$, m.p. 212—216 °C, $[\alpha]_D$ − 0·5°, ν_{max} 2·9 (OH) and 5·7 μm (ester). The molecular weight of (**2**) was determined by the application of the radioactive method[4] to be 1335 (calc. for $C_{58}H_{86}Cl_2O_{31}$ 1348). The ^{13}C n.m.r. spectrum of everninomicin-2 showed signals at δ 120·5 and 119·7 p.p.m. indicating the presence of two orthoester carbon atoms[4] in the molecule as in everninomicin B, C, and D. Everninomicin-2 (**2**) on methylation using diazomethane yielded a monomethyl ether (**3**), $C_{59}H_{88}Cl_2O_{31}$, m.p. 210—212 °C, $[\alpha]_D$ + 1·2°.

Compound (**3**) on solvolysis† yielded a mixture of products which were separated by chromatography on a silica gel column. Elution of the column with chloroform containing increasing proportions of methanol yielded the hydroxymethyl ester (**6**),[4] a lactone (**7**), and evertetrose (**8**).[5] Compound (**7**), $C_{22}H_{28}Cl_2O_{10}$ (M^+ 522) is a colourless crystalline solid, m.p. 200 °C, $[\alpha]_D$ + 10·8° ν_{max} 2·9 (OH) and 5·72 μm (ester, lactone). Its n.m.r. spectrum was consistent with the assigned structure and compared well with the n.m.r. spectrum of *O*-methyl flambolactone.[6] The mass spectrum of (**7**) was also consistent with the assigned structure. We have observed earlier that olgose (**9**)[4] on solvolysis yields (**6**) and evertetrose (**8**). Based on the fact that the monomethyl ether of everninomicin-2 on solvolysis yields (**6**), (**7**), and (**8**) and that the ^{13}C n.m.r. spectrum of everninomicin-2 (**2**) shows the presence of two orthoester carbon atoms, we propose structure (**2**) for everninomicin-2 and structure (**3**) for its monomethyl ether.

At this point the conversion of everninomicin D (**1**) into everninomicin-2 (**2**) was considered. This involved hydrolysis of only one of the several glycosidic bonds present in the molecule and more importantly the labile orthoester linkages had to be kept intact. It was conceived that nitrosoeverninomicin D (**4**) on treatment with triethyl phosphite or triphenylphosphine could be converted into a nitrene (see formula) which would rearrange with bond migration (one of the three possibilities shown) to an enamine which should on principle hydrolyse the required glycoside bond yielding everninomicin-2 (**2**).



Everninomicin D (**1**) on reduction[7] with aluminium amalgam in aqueous ethanol yielded hydroxylaminoeverninomicin D (**5**) as a colourless crystalline solid, $C_{66}H_{101}Cl_2NO_{34}$, m.p. 185—186 °C, $[\theta]_{255}$ (−17,400), ν_{max} 2·9 and 5·72 μm, no nitro-group absorption. It gave a positive colour reaction[8] with triphenyltetrazolium chloride for a hydroxylamino-group. Compound (**5**) was unstable to acid and was oxidised readily in air to nitrosoeverninomicin D.[4] For preparative purposes hydroxylaminoeverninomicin D (**5**) was oxidized in tetrahydrofuran solution using sodium hypobromite to nitrosoeverninocicin D (**4**), a blue amorphous solid, $[\theta]_{255}$ (−15,000). Nitrosoeverninomicin D (**4**) was refluxed in benzene solution with 1·1 mol. equiv. of triphenylphosphine until the blue colour disappeared (*ca.* 15 min). The reaction mixture was evaporated to dryness and the residue chromatographed on silica gel to yield everninomicin-2 (**2**) (identical on comparison with a

† Solvolysis in this communication refers to treatment of the compound with methanolic toluene-*p* sulphonic acid.

sample obtained from a natural source). The overall yield of (**2**) from (**1**) was *ca.* 30%.

Compounds (**1**), (**2**), (**4**), and (**5**) possessed equal *in vitro* activity against gram-positive bacteria. However, hydroxylaminoeverninomicin D (**5**) gave the highest blood level when administered intramuscularly to dogs.[9]

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[6] W. D. Ollis and C. Smith, *J.C.S. Chem. Comm.*, 1974, 882. After we completed our work, the paper on the structural elucidation of flambolactone appeared. As the structure of flambolactone was elucidated following similar procedures outlined by us (refs. **4** and A. K. Ganguly, O. Z. Sarre, D. Greeves, and J. Morton, *J. Amer. Chem. Soc.*, 1973, **95**, 942), for a related compound and the constants for compound (**5**) and the mono-*O*-methyl flambolactone were so similar, a direct comparison of the two samples for establishing their identity was felt unnecessary.
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