Electrochemical Modification of Everninomicin D

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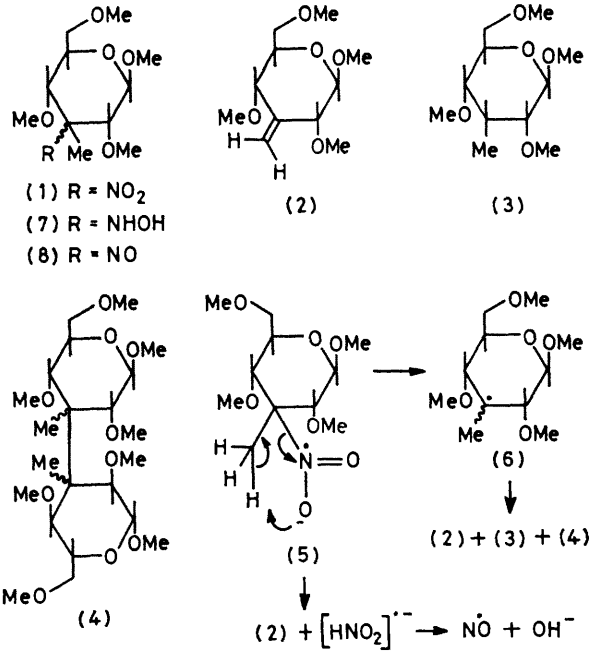
Summary Electrochemical reduction of everninomicin D yields everninomicin-2, -3, and -7 whose structures have been elucidated.

EVERNINOMICIN B,[1] C,[2] and D[3] are produced[4] by *Micromonospora carbonaceae*. The structure and stereochemistry of these antibiotics have been established in our laboratories. One of the many novel features in the structure of this class of antibiotics is the presence of a nitro sugar. In our attempts to improve the pharmacokinetics of these antibiotics while retaining their parent *in vitro* activity, we have studied electrochemical modifications of everninomicin D, the major constituent of the antibiotic complex produced in this fermentation. We report here our results.

Although electrochemical reduction of aliphatic tertiary nitro compounds has been documented,[5] similar reaction in a more complex situation, such as in the present case, has not been reported. In a model experiment, compound (**1**), $C_{11}H_{21}NO_7$, m.p. 104—105 °C, $[\alpha]_D$ −12·3°, ν_{max} 1543 cm^{-1} (nitro), was reduced in anhydrous acetonitrile using tetraethylammonium fluoroborate as the supporting electrolyte. Approximately one electron equivalent was consumed during the electrolysis. Work-up in the usual way yielded (**2**) (36%), (**3**) (14%), and (**4**) (2%). Compound (**2**), oil, $C_{11}H_{20}O_5$, $[\alpha]_D$ −17°, δ 4·06 (1H, d, *J* 8 Hz, 1-H) and 5·2 (q, 2 vinyl-H); (**3**), oil, $C_{11}H_{22}O_5$, $[\alpha]_D$ −30°, δ 0·96 (sec.

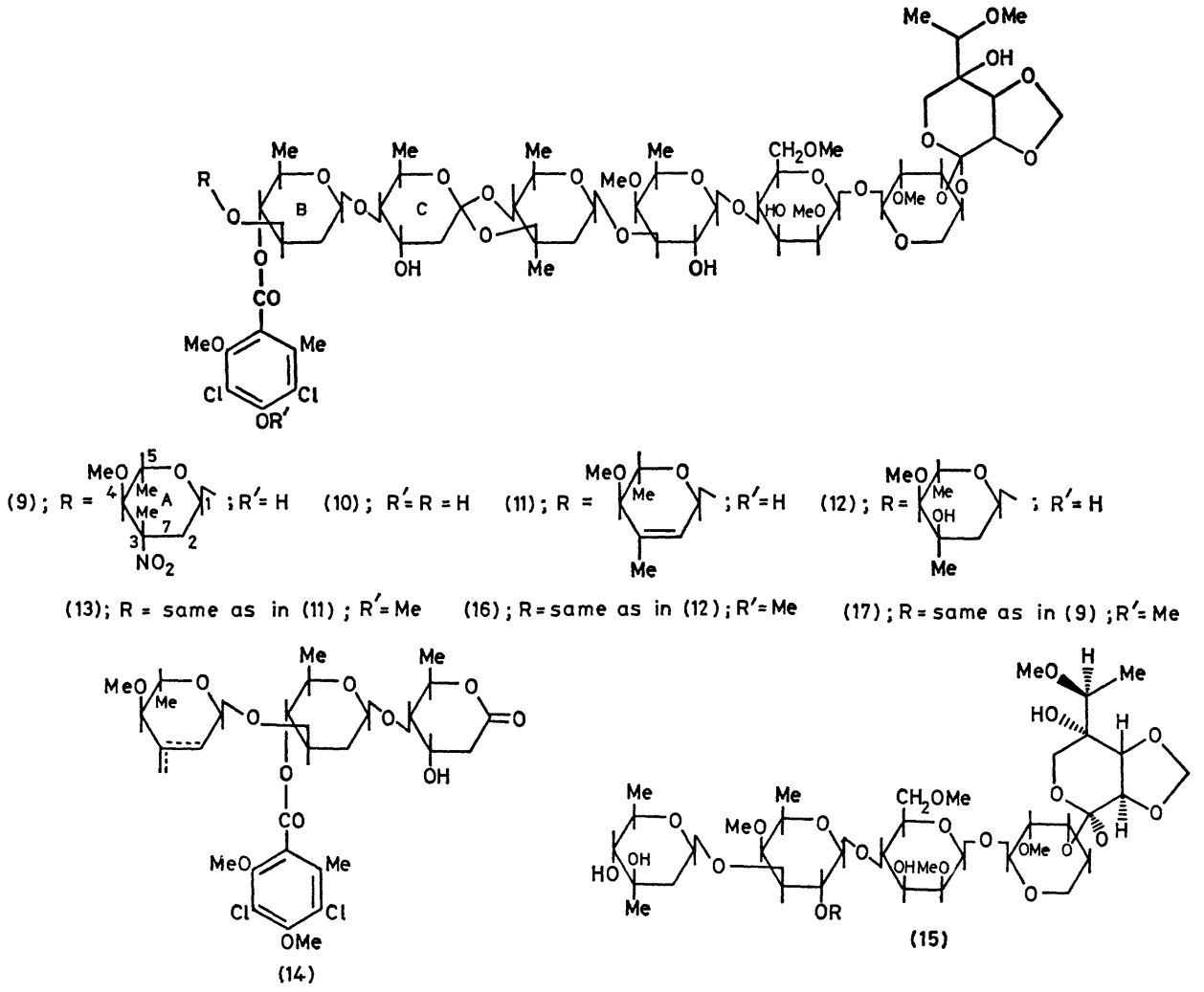


SCHEME 1

Me, J 7 Hz) and 4·49 (1H, J 8 Hz, 1-H); (**4**), oil, $C_{22}H_{42}O_{10}$, M^+ 466, $[\alpha]_D$ −36·8°, δ 1·26 (tert. Me, s) and 4·25 (d, J 8 Hz, 1-H). None of the above compounds showed the presence of OH, NO_2, or carbonyl group in the i.r. spectra. The stereochemistry at the point of attachment in (**4**) has not been determined.

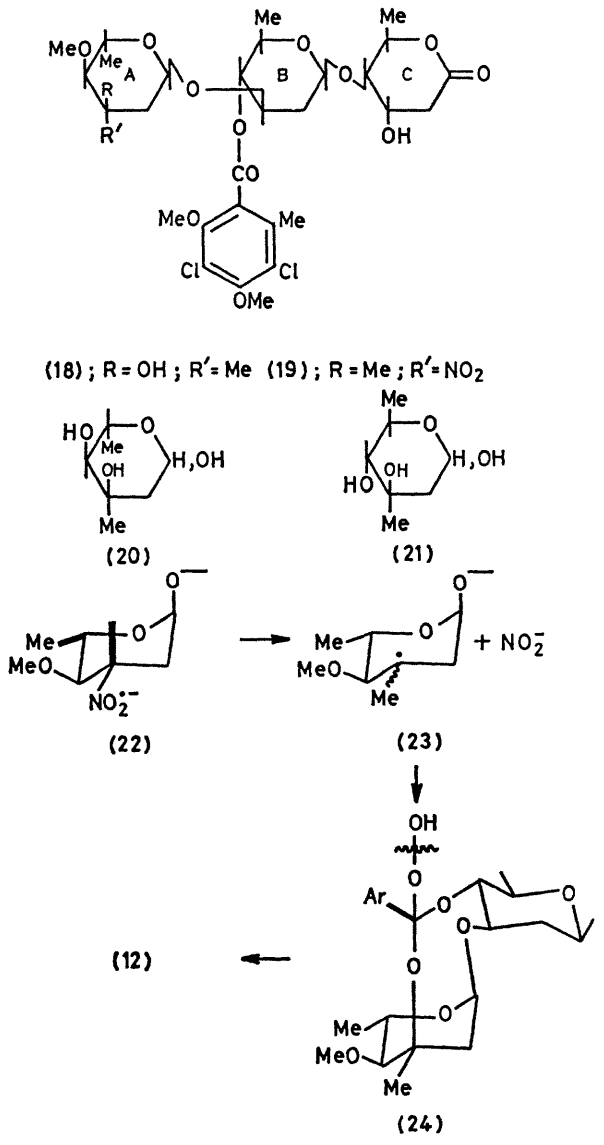
A proposed mechanism for the above conversion is summarized in Scheme 1. In principle, the anion radical (**5**) could dissociate into NO_2^- and a free radical (**6**) which could yield products (**2**), (**3**), and (**4**). If this were the only mechanism available, then one would expect approximately equal amounts of (**2**) and (**3**) in the electrolysis of (**1**). In view of the above product ratio and the fact that compound (**2**) is expected to be less stable than (**3**), an alternative pathway involving the rearrangement of (**5**) to (**2**) and HNO_2^- (the latter could dissociate to [NO]· and $[OH]^-$) is suggested. If the above electrolysis is carried out in aqueous acetonitrile, compound (**1**) consumes four electrons and is converted into the colourless crystalline hydroxylamine derivative (**7**), $C_{11}H_{23}O_6N$, m.p. 82—84 °C, $[\alpha]_D$ −25°. Compound (**7**) is easily oxidized with sodium hypobromite to a blue crystalline nitroso compound (**8**), $C_{11}H_{21}O_6N$, m.p. 49—50 °C.

Everninomicin D, (**9**) when reduced in anhydrous acetonitrile solution using tetraethylammonium fluoroborate as the supporting electrolyte, yielded after usual work-up and purification everninomicin-2 (**10**), -3 (**11**), and -7 (**12**). In an earlier publication[6] we have reported the structure of everninomicin-2 (**10**).



Everninomicin-3 (**11**), crystalline solid, m.p. 157—158 °C, $C_{66}H_{98}O_{33}Cl_2$, $[\alpha]_D$ −29·6°, shows the absence of a nitro group in the i.r. spectrum. On treatment with diazomethane it forms a colourless crystalline monomethyl ether (**13**), $C_{67}H_{100}O_{33}Cl_2$, m.p. 170 °C, $[\alpha]_D$ −29·2°. The ^{13}C n.m.r. spectrum of (**13**) shows the presence of *ortho* ester carbons at δ 120·2 and 120·8 p.p.m. and signals attributable to olefinic carbons at δ 140·1 and 135·3 p.p.m. Solvolysis† of (**13**) yielded a lactone (**14**) and olgose (**15**)[3,7] (identical

† Solvolysis refers to treatment of the compound in tetrahydrofuran solution with toluene-*p*-sulphonic acid.



SCHEME 2

when compared with an authentic sample). The lactone (**14**) is a colourless crystalline solid, $C_{30}H_{40}O_{12}Cl_2$ (M^+ 662), m.p. 165—166 °C. ^1H and ^{13}C n.m.r. spectra of (**14**) were consistent with the assigned structure (mixture of position isomers of the double bond). With the above observations and our earlier work on the structural elucidation of everninomicin D (**9**) the structure of (**11**) is firmly established.

Everninomicin-7 (**12**) is a colourless crystalline solid, $C_{66}H_{100}O_{34}Cl_2$, m.p. 173—175 °C, $[\alpha]_D$ −35·6°. On methylation with diazomethane, (**12**) yields (**16**), colourless crystalline solid, $C_{67}H_{102}O_{32}Cl_2$, m.p. 186—188 °C, $[\alpha]_D$ −34·8°. The ^{13}C n.m.r. spectrum of (**16**) shows the presence of two *ortho* ester carbons at δ 119·4 and 120·2 p.p.m. and a *C*-methyl group at δ 23·6 p.p.m. which is absent in the ^{13}C n.m.r. spectrum of the methyl ether of everninomicin D. Solvolysis of (**16**) yielded olgose (**15**) and a lactone (**18**). Compound (**18**) is a colourless crystalline solid, $C_{30}H_{42}O_{13}Cl_2$, M^+ 610. The mass spectrum of (**18**) not only confirms the composition but its fragmentation pattern demonstrates that the only difference between (**18**) and the lactone (**19**), obtained by solvolysis of the methyl ether of everninomicin D (**17**), is the replacement of the $-NO_2$ group in (**19**) by the –OH group in (**18**). This conclusion was verified by direct comparison of the ^1H and ^{13}C n.m.r. spectra of the two compounds. The ^{13}C n.m.r. spectrum of (**18**) shows no significant changes in B and C ring carbons compared with (**19**). However, there were significant changes in (**18**) at A_1 (δ 93·7), A_3 (δ 71·7), and A_7 (δ 23·6 p.p.m.). The downfield shift of A_1 along with the appearance of the A_7 methyl group at δ 23·6 p.p.m. in (**18**) is consistent only with the axial hydroxy-group at A_3. It is known that the tertiary methyl group in L-mycarose (**20**) appears at δ 23·6 and in D-evermicose (**21**) at 20·7 p.p.m.

As in the model experiment, the formation of everninomicin-3 (**11**) from everninomicin D (**9**) can be readily explained by the rearrangement of the anion radical (**22**). Everninomicin-2 (**10**) is probably derived from (**11**). To explain the formation of everninomicin-7 (**12**) we propose that (**22**) dissociates to form a carbon radical (**23**) and NO_2^- (Scheme 2). Owing to the proximity‡ of the aromatic ester carbonyl, (**23**) may rearrange to the more stable benzyl carbon radical which in turn could capture oxygen resulting in a structure such as (**24**). It is expected that (**24**) would cleave at the peroxide bond yielding (**12**).

Compound (**10**), (**11**), and (**12**) showed high activity against gram positive bacteria (*e.g.* mean MIC against *Staph. aureus* is 0·06 mcg/ml). Everninomicin-7 (**12**) showed improved pharmacokinetics compared to everninomicin D. The details of microbiological activities will be published elsewhere.

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‡ Deduced using ^{13}C n.m.r. analysis of everninomicin-D and its derivatives.

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