Hydrolysis Products of Everninomicin B

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Summary Hydrolysis of everninomicin B yields a mixture from which a heptasaccharide, everheptose B, has been isolated; everheptose B contains a new sugar, D-evalose, the structure and absolute stereochemistry of which has been established.

EVERNINOMICINS are oligosaccharide antibiotics produced by *Micromonospora carbonaceae*.[1] We report here the results of some degradation experiments on everninomicin B.

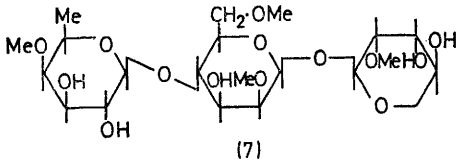
Everninomicin B on aqueous hydrolysis gave a crystalline heptasaccharide named everheptose B (**1**),† m.p. 135—137°; $[\alpha]_D$ — 68° ($CHCl_3$); $\nu_{max.}$ 1538 (NO_2) and 1730 (CO) cm^{-1}. As in the case of everheptose (**2**)[2] the carbonyl absorption was stronger than the nitro-absorption. Treatment of everheptose B with ethereal diazomethane gave the fragments (**3**) and (**4**) and evertetrose B (**5**). Compounds (**3**) and (**4**) were identified on the basis of their interconversion and comparison with authentic samples.[2]

Evertetrose B (**5**) is a non-reducing crystalline solid, m.p. 276—277°; $[\alpha]_D$ — 46·3° (H_2O). It has no selective u.v. absorption, and its i.r. spectrum does not show carbonyl absorption. Its n.m.r. spectrum shows the presence of four methoxy-groups, and one tertiary and two secondary methyl groups. Of the four anomeric proton signals, which appear at δ (pyridine) 4·7 (d, J 7 Hz), 5·19 (s, $W_{\frac{1}{2}}$ 2·5 Hz), 5·68 (d, J 2 Hz), and 5·45 (s, $W_{\frac{1}{2}}$ 2·5 Hz), all but the last have been assigned to the evertriose[3] portion of the molecule. Aqueous hydrolysis of (**5**) under controlled conditions gave evertriose (**7**)[3] and a new sugar, named D-evalose (**8**).

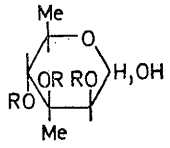
Exhaustive methylation[4] of evertetrose-B (**5**) and prolonged aqueous hydrolysis of the product (**6**) gave a mixture

† Satisfactory elemental analyses were obtained for all new compounds.

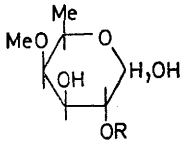
from which 2,3,4-tri-*O*-methyl-D-evalose (**9**) and 2-*O*-methyl-D-curacose (**10**)[4] were isolated by preparative t.l.c. This established that in (**5**), D-evalose (**8**) is linked through its anomeric oxygen atom to C-3 of D-curacose (**11**).



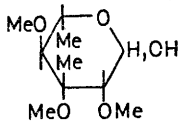
(7)



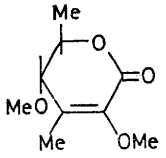
(8) R = H
(9) R = Me



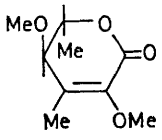
(10) R = Me
(11) R = H



(12)



(13)



(14)

D-Evalose (**8**) was obtained as a colourless glass, $[\alpha]_D$ — 4·7 → — 5·2° (24 h; H_2O). Its n.m.r. spectrum showed a secondary methyl doublet, a tertiary methyl singlet, and no methoxy-signals. On methylation and partial hydrolysis it formed the tri-*O*-methyl-ether (**9**), m.p. 115—120°; $[\alpha]_D$ + 18·3 → + 6·3° (24 h; MeOH). The n.m.r. spectrum of (**9**) (anomeric mixture) shows the presence of three methoxy-groups [δ ($CDCl_3$) 3·29, 3·51, and 3·54], a secondary methyl group [δ 1·29 (d, *J* 6·5 Hz)], and a tertiary methyl group (δ 1·37). A one-proton doublet at δ 3·08 (*J* 9 Hz, H-4) corresponds to the major anomer. The stereochemistry at C-2 (axial OMe) follows from the coupling constants of the anomeric protons [δ 4·78br (s, $W_{\frac{1}{2}}$ 3 Hz) and 5·26 (d, *J* 2·5 Hz)]. The above evidence establishes that (**9**) and L-nogalose (**12**)[6]‡ have the same constitution and relative stereochemistry at C-2, C-4, and C-5. A direct comparison with an authentic sample of L-nogalose (i.r., n.m.r., t.l.c.) established the identity of the two compounds.

The absolute stereochemistry of L-nogalose (**12**) has been unequivocally established by *X*-ray crystallography.[6] The trio-*O*-methyl-ether (**9**) must therefore be D-nogalose. This was confirmed as follows. Jones oxidation of compound (**9**) followed by β-elimination yielded (**13**), $[\theta]_{255}$ + 3639°; similarly L-nogalose gave (**14**),[6] $[\theta]_{255}$ — 4292°. Although (**13**) and (**14**) showed equal but opposite Cotton effects, the sign in each case was exactly the opposite of the expected value[7] for simpler αβ-unsaturated δ-lactones. This difference may be attributed to the presence of the enol ether chromophore. Some steroidal α-diketones[8] have also been reported to have abnormal c.d. The stereochemistry of the anomeric linkage of D-evalose in evertetrose B (**5**) was deduced as β-equatorial by application of Klyne's rule[5] and from the n.m.r. spectrum [δ 5·45 (s, $W_{\frac{1}{2}}$ 2·5 Hz; anomeric proton of D-evalose)]. The mass spectrum of (**6**) was also consistent with the assigned structure.

The linkage between the units (**4**) and (**5**) in everheptose B has been established from the results of experiments similar to those described for everheptose (**2**),[2] and will be described in the full paper.

We thank Dr. P. F. Wiley, Upjohn Company, U.S.A., for a sample of L-nogalose.

(*Received*, 25*th April* 1973; *Com.* 593.)

‡ L-Nogalose,[6] m.p. 115—121°; $[\alpha]_D$ — 17·1 → — 5·1° (24 h; MeOH).

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