Structure and Absolute Stereochemistry of Everninose, a Non-reducing Sugar obtained on Hydrolysis of Everninomicin D[1]

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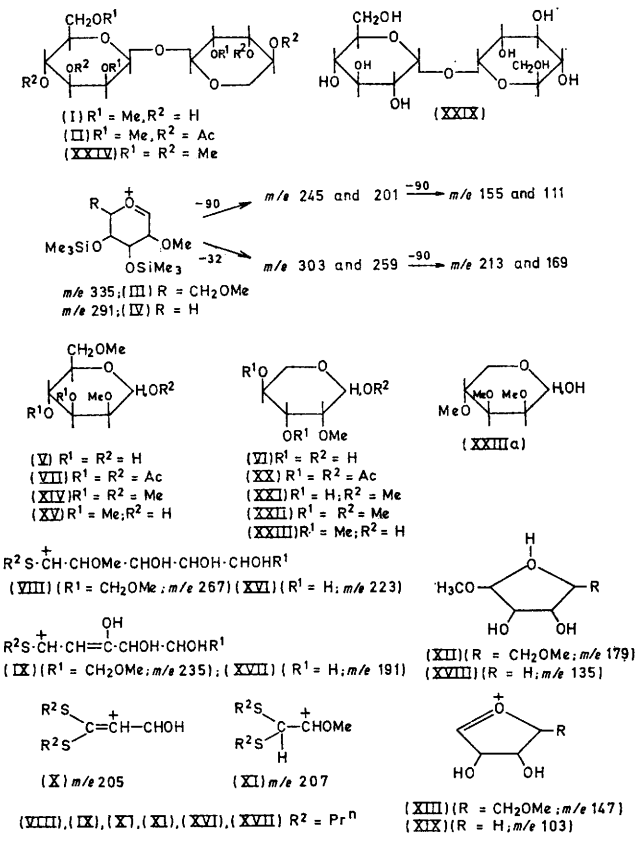
Summary Everninose, a hydrolysis product of everninomicin B and D, has been shown to possess structure (I).

EVERNINOMICIN D on hydrolysis gave a mixture of products from which everninose was isolated[2] by Herzog and his collaborators. Everninose (I), $C_{14}H_{26}O_{10}$,† m.p. 200—201°, $[\alpha]_D$ — 74·1° (water) is a non-reducing sugar, consumes two moles of periodic acid, and does not form a trityl derivative. The n.m.r. spectrum (pyridine) of everninose showed the presence of three methoxy-groups at δ 3·35, 3·5, and 3·65 and two anomeric protons at δ 5·25 (1H; $J_{W/2}$ *ca.* 1·5 Hz) and δ 5·7 (1H; J 2·5 Hz). Everninose forms a tetra-acetate (II),‡ $C_{22}H_{34}O_{14}$, m.p. 150—151°, $[\alpha]_D$ —77·1° which does not show the presence of any hydroxy-group in the i.r. spectrum; the n.m.r. spectrum shows the presence of three methoxy-groups, four acetate methyls, and two anomeric protons. The mass spectrum of the tetra-*O*-trimethylsilyl ether of everninose showed a strong M — 15 peak at *m/e* 627 besides a small molecular-ion peak at *m/e* 642. Other prominent peaks were at *m/e* 335 and 291 [ions (III) and (IV)]. Further fragmentation of ions (III) and (IV) followed the expected pattern as outlined by DeJongh *et al.*[3]



The above mass spectral fragmentation, together with the fact that everninose is a non-reducing sugar, clearly indicated that everninose was made up of a dimethoxy-hexose and a monomethoxy-pentose and that they were linked through their anomeric hydroxy-groups.

On prolonged heating with aqueous acid, everninose was hydrolysed into a mixture of two monosaccharides which were separated using preparative t.l.c., and their structures have been shown to be 2,6-di-*O*-methyl-D-mannose[4] (V) and 2-*O*-methyl-L-lyxose (VI) in the following way.

Compound (V), syrup, $[\alpha]_D$ + 7·9° (water; 72 hr.) is a reducing sugar (anomeric proton at δ 5·3; J 2 Hz) and contains two methoxy-groups. It forms a triacetate (VII) which sublimed at 85°/0·2 mm. Hg, as syrup, $C_{14}H_{22}O_9$, $[\alpha]_D$ + 55·7°. The n.m.r. spectrum of (VII) agreed with that published recently for triacetoxycuramicose.[4] The mass spectrum of the di-n-propyl mercaptal of (V), $C_{14}H_{30}O_5S_2$, m.p. 50°, $[\alpha]_D$ — 24·01° showed, in addition to a molecular-ion peak at *m/e* 342, prominent peaks at *m/e* 267 (VIII), 235 (IX), 205 (X), 207 (XI), 179 (XII), and 147 (XIII). The methyl glycoside of compound (V) on methylation[5] yielded (XIV), which on hydrolysis gave (XV), syrup, $C_{10}H_{20}O_6$, $[\alpha]_D$ + 2·3° (water; 24 hr.) identical with an authentic sample of 2,3,4,6-tetra-*O*-methyl-D-mannose,§ $[\alpha]_D$ + 2·3° (water; 24 hr.).

Compound (VI) crystallized from acetone, $C_8H_{16}O_5$, m.p. 122°, $[\alpha]_D$ + 6·2° (water; 72 hr.). It formed a di-n-propyl mercaptal, $C_{12}H_{26}O_4S_2$, m.p. 49°, $[\alpha]_D$ + 19·5°, the mass spectrum of which showed the molecular-ion peak at *m/e* 298 and also peaks at *m/e* 223 (XVI), 191 (XVII), 207 (XI), 205 (X), 135 (XVIII), and 103 (XIX), confirming[6] that compound (VI) was a 2-methoxypentose.

Compound (VI) formed a triacetate which sublimed at 80°/0·1 mm. Hg as a syrup, $C_{12}H_{18}O_8$, $[\alpha]_D$ — 10·5°. Besides three acetyl methyl groups, the n.m.r. spectrum (100 MHz) of the triacetate (XX) in benzene solution showed signals at δ 3·88 (1H; q; J 11·5 and 4·5 Hz; 5e-H), δ 3·69 (1H; q; J 11·5 and 7·0 Hz; 5a-H), δ 5·40 (1H; octet; J 4·5, 7·0, and 8·0 Hz; 4-H), 5·52 (1H; q; J 8·0 and 2·9 Hz; 3-H) 3·60 (1H; q; J 2·9 and 4·2 Hz; 2-H), and 6·29 (1H; d; J 4·2 Hz; 1-H). The above chemical shifts and coupling-constant values were obtained using spin–spin-decoupling experiments. From the above data one would conclude that compound (VI) was 2-methoxy-lyxose. To prove the gross structure and particularly its absolute stereochemistry, the methyl glycoside (XXI) was methylated[5] to (XXII) and then hydrolysed to (XXIII), sublimed at 60°/0·2 mm. Hg as syrup, $C_8H_{16}O_5$, $[\alpha]_D$ + 12·4° which was identical with an authentic sample§ of 2,3,4-trimethoxy-D-lyxose (XXIIIa) except for the opposite sign of rotation $[\alpha]_D$ — 21·6°. In

† Satisfactory elementary analyses were obtained for all new compounds; unless otherwise noted, i.r. spectra were recorded in chloroform solution and n.m.r. spectra were taken at 60 MHz in $CDCl_3$ with internal $SiMe_4$ standard; optical rotations were measured in chloroform solution at 25°.

‡ We thank Dr. H. Reimann for making these observations.

§ Authentic samples of (XV) and (XXIIIa) were prepared from D-mannose and D-lyxose, respectively, using conventional methods.

the n.m.r. spectrum of (XX) the higher coupling constants (J 4·2 Hz) of 1-H and 2-H and comparatively lower coupling constants of 4-H and 5-H (J 7·0 Hz) and 3-H and 4-H (J 8·0 Hz) suggests[7] that 2-*O*-methyl-1,3,4-triacetoxy-L-lyxose (XX) exists in a conformational equilibrium between 1*C* and *C*1 conformations approximately in the ratio of 3 : 2.

The absolute configurations of (V) and (VI) were further confirmed by c.d. measurements[8] of the cuprammonium complex of their methyl glycosides. Thus having had established the structure and absolute stereochemistry of (V) and (VI), it remained to prove the stereochemistry of the anomeric linkages in everninose (I). Everninose on methylation[5] yielded (XXIV), syrup, $C_{18}H_{34}O_{10}$, $[M]_D$ −356°. To apply Klyne's rule[9] we have prepared compounds (XXV)—(XXVIII) in the conventional way and determined their molecular rotation values (Table).

Assuming that Klyne's rule is valid in a structure like everninose, it follows from these results that the structure and absolute stereochemistry of everninose should be represented as (I). In trehalose (XXIX), the only other example of a naturally occurring disaccharide of this group, Klyne's rule has been successfully applied[10] to determine its stereochemistry.

TABLE

	Compound	$[M]_D$
(XXV)	Methyl 2,3,4,6-tetra-*O*-methyl-β-D-mannoside	−218°
(XXVI)	Methyl 2,3,4,6-tetra-*O*-methyl-α-D-mannoside	+132°
(XXVII)	Methyl 2,3,4-tri-*O*-methyl-β-D-lyxoside	−176°
(XXVIII)	Methyl 2,3,4-tri-*O*-methyl-α-D-lyxoside	+ 76°
(XXIV)	Tetra-*O*-methyl ether of everninose	−356°

We thank Dr. R. D. Guthrie and Miss S. T. K. Bhukhari of the University of Sussex, Brighton, for c.d. measurements on the cuprammonium complexes.

(*Received, October 23rd*, 1969; *Com.* 1607.)

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