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Note

Yersiniose, a new branched-chain sugar

RAISA P. GORSHKOVA, VLADIMIR A. ZUBKOV, VLADIMIR V. ISAKOV, AND YURY S. OVODOV

Pacific Institute of Bioorganic Chemistry, Far East Science Centre, Academy of Sciences of the U.S.S.R., Vladivostok 690022 (U.S.S.R.)

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In 1976, we reported[1] an unidentified sugar (yersiniose) as a component of the O-specific side-chain polysaccharide from the lipopolysaccharide (LPS) of *Yersinia pseudotuberculosis* VI serovar, which was not detected in the LPS of *Y. pseudotuberculosis* I–V serovars[2]. The sugar was shown to be related to a branched-chain trideoxyoctose isolated from *Streptomyces aureofaciens*[3] antibiotics and to methyl 2,6-dideoxy-4-*C*-(D-*glycero*-1-hydroxyethyl)-α-D-*ribo*-hexopyranoside synthesised by Paulsen and Sinwell[4]. We now report evidence which indicates yersiniose to be 3,6-dideoxy-4-*C*-(1-hydroxyethyl)-D-*xylo*-hexose.

P.c. of a hydrolysate of the O-specific side-chain polysaccharide from *Y. pseudotuberculosis* VI serovar LPS revealed yersiniose, which had a mobility higher than that of colitose and gave a characteristic brown colour with aniline hydrogenphthalate but did not react with 2-thiobarbituric acid[5]. Yersiniose was purified by repeated p.c., and its homogeneity was proved by g.l.c. and g.l.c.–m.s. of acetylated methyl yersinioside and yersinitol, respectively. Electrophoresis data demonstrated yersiniose to be a neutral sugar, and the i.r. bands at 2800–3000 cm^{-1} indicated the presence of methyl groups. Reduction of yersiniose with sodium borohydride gave yersinitol which, after acetylation, showed i.r. absorption at ~3600 cm^{-1} for hydroxyl consistent with the presence of a tertiary hydroxyl group resistant to acetylation.

The ^{1}H-n.m.r. spectrum (D_2O) indicated yersiniose to be 3,6-dideoxy-4-*C*-(1-hydroxyethyl)-D-*xylo*-hexose. The preponderant β-form gave signals at δ 4.71 ($J_{1,2}$ 8.25 Hz, H-1), 3.80 ($J_{2,3}$ 5.22, $J_{2,3}$ 11.82 Hz, H-2), 1.85 ($J_{3,3}$ 13.47 Hz, H-3*e*), 4.18 ($J_{5,6}$ 6.63 Hz, H-5), 1.30 (H-6), 3.93 ($J_{7,8}$ 6.6 Hz, H-7), and 1.35 (3 H-8). The values of coupling constants ($J_{2,1}$ 8.25, $J_{2,3}$ 11.85 Hz) indicated[6] H-2 to be axial. The chemical shift of the signal for H-2 indicated HO-4 to be axial (*cf.* δ 3.56 for HO-4 equatorial).

The ^{13}C-n.m.r. spectrum of β-yersiniose (Fig. 1) showed eight signals. The signal at 98.7 p.p.m. corresponded to C-1, and those at 13.4 and 16.4 p.p.m. indicated the presence of a *C*-methyl group. The ring deoxy group was indicated[7] by

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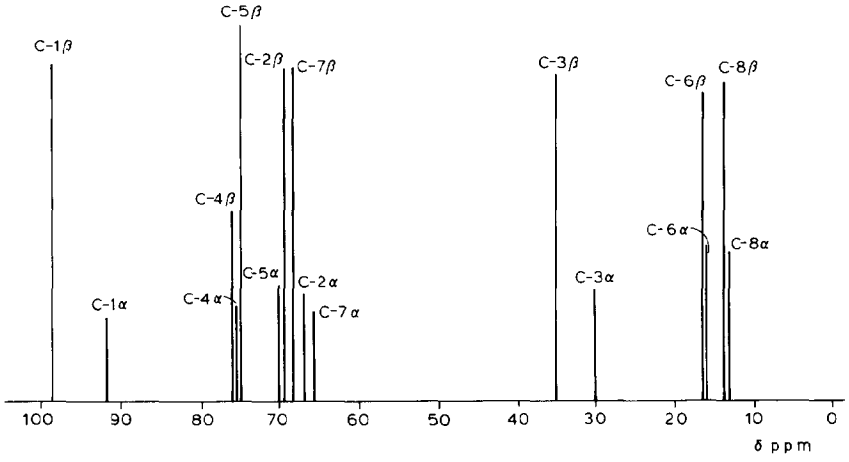
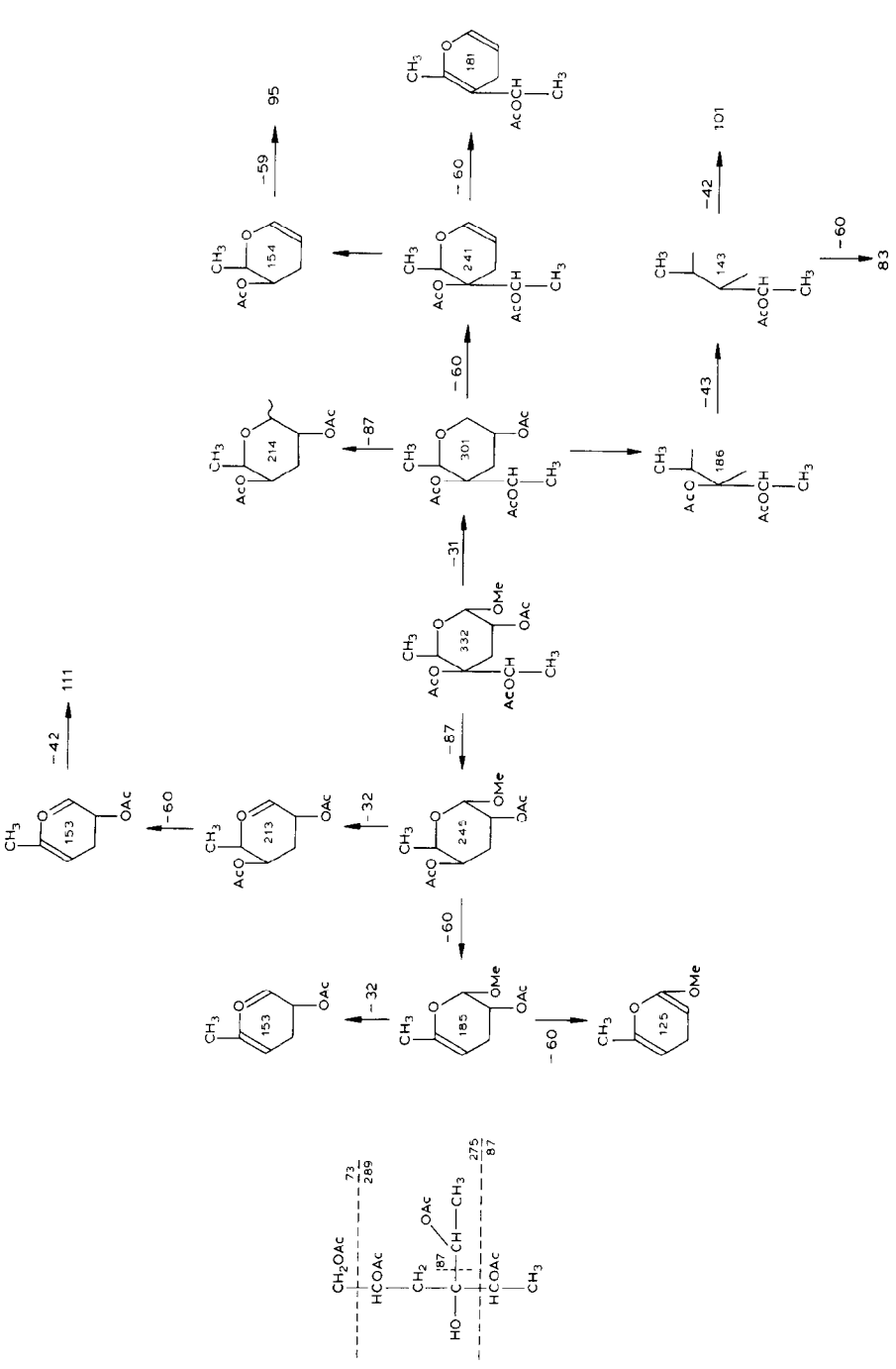


Fig. 1. ^{13}C-N.m.r. spectrum of a solution of yersiniose in D_2O.

the signal at 35.9 p.p.m. Four signals (68.5, 69.8, 75.3, and 75.8 p.p.m.) were observed in the region for carbon linked to oxygen. A signal at 75.7 p.p.m. appeared to be associated with a tertiary carbon because of its relatively long relaxation-time. The chemical shift (104.4 p.p.m.) for C-1 of the yersiniosyl residue in the ^{13}C-n.m.r. spectrum of the O-specific polysaccharide from *Y. pseudotuberculosis* VI serovar LPS[8] indicated the D configuration[9]. The mass-spectral data for yersinitol acetate (Scheme 1) also indicated yersiniose to be a 3,6-dideoxy-4-*C*-(1-hydroxyethyl)hexose. Fission of the C-1–C-2 and C-4–C-5 bonds gave characteristic ions of *m/z* 275 and 289, and *m/z* 73 and 87. The intense ions at *m/z* 215, 155, and 95 reflected a successive loss of three acetic acid molecules from a primary ion of *m/z* 275. Fragmentation of the ion of *m/z* 289 afforded ions of *m/z* 229, 187, and 127 due to the loss of two acetic acid molecules and ketene. The mass-spectral fragmentation of acetylated methyl yersinioside is shown in Scheme 1 and also accords with the structure assigned.

EXPERIMENTAL

Descending p.c. was performed on Whatman 3MM and Filtrak FN-3 papers, with 2 developments with 1-butanol–pyridine–water (6:4:3) and detection with alkaline silver nitrate or aniline hydrogenphthalate. Paper electrophoresis was carried out in a 25mM pyridine acetate buffer (pH 4.5) at 28 V/cm for 1 h. G.l.c. was performed on a Pye Unicam 104 instrument fitted with a flame-ionisation detector and a glass column (150 × 0.4 cm) packed with 3% of QF-1 on Gas-chrom Q (100–120 mesh); the flow rate of argon was 60 mL/min, and the temperature programmes were 175→225° and 110→225° at 5°/min for alditol acetates and acetylated methyl glycosides, respectively. G.l.c.–m.s. was performed on an LKB-9000 instrument, using the above column. I.r. spectra were recorded with an IR-20 Zeiss spec-



Scheme 1. Mass-spectral fragmentation of methyl yersinioside tetra-acetate and yersiniol tetra-acetate.

trometer. Optical rotations were measured with a Perkin–Elmer 141 polarimeter. N.m.r. spectra were obtained with a Bruker HX-360 spectrometer for solutions in D_2O (external MeOH). The chemical shifts were calculated using[9] the equation $\delta_{Me_4Si} = \delta_{MeOH} + 49.6$ for ^{13}C data.

Isolation and degradation of the lipopolysaccharide (LPS). — *Y. pseudotuberculosis* VI serovar (strain No 1553), kindly provided by Professor H. H. Mollaret (Pasteur Institute, Paris), was grown in a nutrient medium, and the LPS was isolated from dry bacterial cells by the phenol–water procedure[1]. The LPS (500 mg) was hydrolysed with 0.125M sulphuric acid (50 mL) at 100° for 0.5 h. The precipitate of lipid A was removed by centrifugation at 5000 r.p.m., and the supernatant solution was neutralised with $BaCO_3$, deionised with Amberlite IR-120 (H^+) resin, and concentrated *in vacuo* at 40°. The residue was poured into ethanol (20 mL) to yield a crude mixture of monosaccharides (250 mg). Repeated preparative p.c. then yielded yersiniose (30 mg), $[\alpha]_{578}^{20}$ +2° (*c* 0.5, water), R_{Rha} 1.21.

Yersiniose derivatives. — (*a*) To a solution of yersiniose (5 mg) in pyridine (0.4 mL) was added acetic anhydride (0.2 mL), and the mixture was kept overnight at room temperature and then worked-up conventionally, by extraction into chloroform, to yield yersiniose tetra-acetate (4.0 mg), $[\alpha]_{578}^{20}$ −20° (*c* 0.4, chloroform).

(*b*) A solution of yersiniose (5 mg) in methanolic M hydrogen chloride (0.5 mL) was boiled under reflux for 1.5 h and then co-concentrated with methanol, to give methyl yersinioside (5.5 mg), $[\alpha]_{578}^{20}$ −10° (*c* 0.3, water), a portion (1 mg) of which was acetylated as described above. Mass spectrum: *m/z* 332 (3%), 301 (8), 245 (29), 241 (5), 214 (4), 213 (9), 185 (93), 181 (4), 153 (78), 143 (100), 125 (15), 111 (100), 95 (15), 83 (28), and 82 (35%).

(*c*) To a solution of yersiniose (5 mg) in water (5 mL) was added sodium borohydride (5 mg), and the mixture was kept for 7 h in the dark. Excess of borohydride was then destroyed with acetic acid and the mixture was worked-up in the conventional manner to yield yersinitol tetra-acetate (5.5 mg). Mass spectrum: *m/z* 289 (0.6%), 275 (1.8), 229 (0.5), 215 (23), 187 (5), 173 (4), 155 (100), 113 (23), 95 (52), and 87 (8%).

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