CHARACTERIZATION OF 3-DEOXY-D-MANNO-OCTULOSONIC ACID AS A COMPONENT OF THE CELL WALL LIPOGLYCAN FROM ESCHERICHIA COLI (2101-R)

M. B. Perry and G. A. Adams
Division of Biosciences, National Research Council
Ottawa, Canada

Received January 11, 1967

Heath and Ghalambor (1962) showed that a 2-keto-3-deoxy-octonate (KDO) was a glycosidically bound component of the cell wall lipoglycan in strains of Escherichia coli. The compound has also been found in the lipoglycans from strains of Salmonella (Osborne, 1963) and Pasturella (Ellwood, 1966).

Ghalambor, Levine and Heath (1966) synthesized KDO by the reaction between oxalacetic acid and D-arabinose and showed that the major product, resolved from the reaction mixture as crystalline 2,4,5,7,8-penta-O-acetyl-KDO methyl ester, was identical in all respects with the KDO liberated by mild acid hydrolysis of the cell wall lipoglycan of E. coli 0111-B_4. It was concluded that the KDO was 3-deoxy-D-manno-octulosonic acid on the basis of the following criteria: (a) its mode of synthesis from D-arabinose and oxalacetic acid, (b) its degradation with ceric sulfate following borohydride reduction to give a 2-deoxy-heptose, (c) its enzymic degradation by 3-deoxyoctulosonate aldolase to yield pyruvate and D-arabinose and (d) the rate of liberation of β-formylpyruvate on periodate oxidation. Although

---

Issued as N.R.C. No. 9369

the chemical synthesis and enzymic cleavage evidence indicates that the hydroxyl groups on C_5 through C_7 of the KDO have the D-arabino configuration the hydroxyl group configuration at C_4 remains in doubt and thus the 3-deoxy-octulosonic acid may have either the D-manno or D-gluco configuration. Ghalambor et al., (1966), on the basis of the rate of liberation of β-formylpyruvate on periodate oxidation of KDO (Preiss and Ashwell, 1962), suggested that the hydroxy groups at C_4 and C_5 are in a cis relationship and thus confer the D-manno configuration on the KDO molecule.

In this communication we wish to record that both the natural KDO isolated from the lipoglycan of E. coli (2101-R), and synthesized KDO, having identical properties with those recorded by Heath and co-workers, on ceric sulfate oxidation following borohydride reduction, gave a 2-deoxy-heptose identical in all respects with 2-deoxy-D-manno-heptose synthesized in this laboratory, thus confirming the structure assigned by Heath for KDO as 3-deoxy-D-manno-octulosonic acid.

Materials and Methods

Paper chromatography was performed on Whatman No. 1 filter paper using the following solvent systems: (A) pyridine-ethyl acetate-water (2:5:5 top layer), and (B) butan-1-ol-pyridine-0.1N HCl (5:3:2). Compounds were detected using alkaline silver nitrate (Trevelyan et al., (1950)) and by the periodate-thiobarbituric acid method (Warren, 1960)). Rates of movement are quoted relative to D-galactose (R_{gal}).

Gas-liquid partition chromatography (G.L.P.C.) was done with a Pye Argon Chromatograph using glass columns (120 x 0.5cm) packed with (A) 10% neopentylglycol sebacate polyester on 100-120 mesh Chromosorb W, and (B) a mixture containing 1.5%

ethyleneglycol succinate polyester and 1.5% silicone oil (XF-1112) on 100-120 mesh Chromosorb W. Retention times are quoted relative to penta-O-acetyl-L-arabinitol (T_A) or methyl 2,3,4,6-tetra-O-methyl-α-D-glucoside (T_G).

Melting points were determined using a Fisher-Johns apparatus. Optical rotations were determined at 22° using a Perkin-Elmer 141 Polarimeter and infrared spectra were obtained on chloroform solution using a Perkin-Elmer 237B Infracord.

Natural KDO - Washed freeze-dried cells of E. coli (2101-R) on phenol extraction (Westphal et al., 1952) gave a lipoglycan which was hydrolyzed with N sulfuric acid for 10 h. at 95°. The neutralized hydrolyzate ($Ba(OH)_2$) was passed down columns of Rexyn 101(H^+) and RG6(OH^-) ion-exchange resins and the KDO was removed from the latter column with ammonium carbonate solution. The KDO, freed from neutral glycoses, was fractionated by chromatography on large filter paper sheets (solvent (B)) to yield chromatographically pure KDO.

Synthetic KDO - 2,4,5,7,8-Penta-O-acetyl-KDO methyl ester (m.p. 156°; $[\alpha]_D$ + 105° (c, 1.9 MeOH) (mixed m.p. 156° with authentic sample kindly supplied by Dr. E. C. Heath) was prepared according to the method of Ghalambor et al., (1966) and the free KDO was prepared from this derivative by mild alkaline hydrolysis.

2-Deoxy-D-manno-heptose - This glycose was synthesized as follows: (Sowden and Fischer, 1947)

1-deoxy-1-nitro-D-glycero-D-gala-heptitol

↓

hexa-O-acetyl-1-deoxy-1-nitro-D-glycero-D-gala-heptitol

(m.p. 150-151°; $[\alpha]_D$ - 9.7° ($CHCl_3$)

↓

D-manno-pentaacetoxy-1-nitroheptene-1

(m.p. 115°; $[\alpha]_D$ + 38.4° ($CHCl_3$)

↓

1-nitro-1,2-dideoxy-D-manno-heptitol pentaacetate

(m.p. 100°; $[\alpha]_D$ + 38° ($CHCl_3$)

↓

2-deoxy-D-manno-heptose

The 2-deoxy-D-manno-heptose was obtained cyrstalline from propan-2-ol-ether (6:1) mixture. The crystals were extremely hygroscopic and gave a poor m.p. M.p. ca. 52°; $[\alpha]_D$ + 51° (equilibrium value c, 0.6 water). The heptose after reduction with borohydride and acetylation with acetic anhydride containing 1% sulfuric acid gave crystalline 1,3,4,5,6,7-hexa-O-acetyl-2-deoxy-D-manno-heptitol having m.p. 104° and $[\alpha]_D$ + 36° (c, 1.1 chloroform).

Results and Discussion

Crystalline 2,4,5,7,8-penta-O-acetyl-KDO methyl ester (0.6g) was saponified, reduced with borohydride, and oxidized with ceric sulfate in the manner described by Ghalambor et al., (1966). The product was fractionated by paper chromatography (solvent (A)) and the component corresponding to a 2-deoxy-heptose (84mg) was collected. Similarly KDO from the lipoglycan after reduction and ceric sulfate oxidation gave a 2-deoxy-heptose.

The 2-deoxy-heptose from both the natural and synthetic KDO had the same chromatographic mobilities as authentic 2-deoxy-D-manno-heptose (R_{gal} 1.37 (solvent (A)) and R_{gal} 1.49 (solvent (B)) and were revealed as the characteristic red spot on the chromatograms treated with the periodate-thiobarbituric acid reagents.

The 2-deoxy-heptose (25 mg) from the synthetic KDO on reduction and acetylation gave crystalline 1,3,4,5,6,7-hexa-O-

acetyl-2-deoxy-D-manno-heptitol having m.p. and mixed m.p. 104° and $[\alpha]_D$ + 35° (c, 1 chloroform), and gave an infrared spectrum indistinguishable from that of an authentic sample. The above heptitol acetate and a sample of the same derivative prepared on a small scale from the 2-deoxy-heptose derived from the natural KDO both gave single peaks on G.L.P.C. analysis having the same retention time as 1,3,4,5,6,7-hexa-O-acetyl-2-deoxy-D-manno-heptitol (Gunner et al., (1961)) - T_A 3.87 (Column (A), 202°) and T_A 4.17 (Column (B), 202°).

The 2-deoxy-heptose specimens from the natural and synthetic KDO when examined as their O-(trimethylsilyl) derivatives (Sweeley et al., (1963)) in each case gave only G.L.P.C. peaks corresponding in retention times with those of the corresponding derivative of authentic 2-deoxy-D-manno-heptose. The following G.L.P.C. relative retention times were recorded: O-(trimethylsilyl) derivatives of equilibrated 2-deoxy-D-manno-heptose: T_G 1.52 (5%), 1.80 (50%), 2.89 (45%) Column (A), 162°) and T_G 1.42 (52%), 2.10 (48%) (Column (B), 162°).

All the above evidence points to the identification of the 2-deoxy-heptose derived from natural and synthetic KDO as 2-deoxy-D-manno-heptose. All the derivatives showed quite distinctly different chromatographic properties from the corresponding derivatives prepared from 2-deoxy-D-gluco-heptose. The new findings taken in consideration with earlier experimental evidence of Heath and co-workers support the positive identification of KDO as 3-deoxy-D-manno-octulosonic acid.

References

Ellwood, D.C., Biochem. J., 99, 55p (1966).

Ghalambor, M.A. Levine, E.M. and Heath, E.C.J., Biol. Chem., 241, 3207 (1966).

Gunner, S.W. Jones, J.K.N. and Perry, M.B., Can. J. Chem., 39, 1892 (1961).

Heath, E.C. Ghalambor, M.A., Biochem. Biophys. Res. Commun., 10, 340 (1963).

Osborne, M.J., Proc. Nat. Acad. Sci., Wash., 50, 499 (1963).

Preiss, J. and Ashwell, G.J., Biol. Chem., 237, 317 (1962).

Sowden, J.C. and Fischer, H.O.L., J. Am. Chem. Soc., 69, 1048 (1947).

Sweeley, C.C. Bentley, R. Makita, M. and Wells, W.W., J. Am. Chem. Soc., 85, 2497 (1963).

Trevelyan, W.E. Proctor, D.D. and Harrison, J.S., Nature, 166, 444 (1950).

Warren, L., Nature, 186, 237 (1960).

Westphal, O. Luderitz, O. and Bister, F., Naturforsch., 76, 148 (1952).