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CHEMICAL STRUCTURE OF ESCHERICHIA COLI LIPID A

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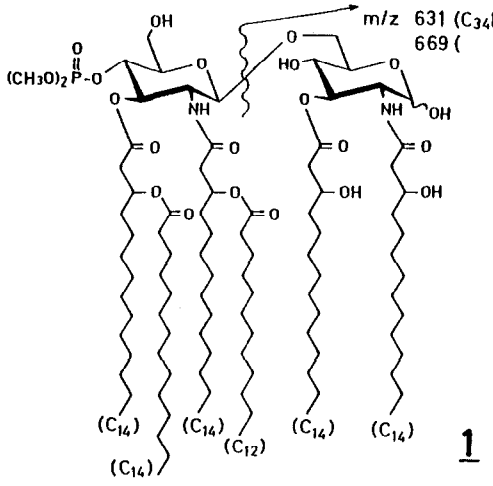
Summary : The chemical structure of *E. coli* lipid A was elucidated to be 2 by determination of the nature of the individual acyl groups bound to the two hydroxyl groups in positions 3,3' and the two amino groups of the D-glucosamine disaccharide phosphate backbone.

The lipophilic part of bacterial lipopolysaccharides (LPS) designated lipid A is responsible for the induction of unique endotoxic activities. Its chemical structure had been elucidated as a polyacylated β(1-6) disaccharide of D-glucosamine 1-α,4'-diphosphate.[1] Due to the heterogeneous character of bacterial LPS and lipid A, however, the positions of acylation had not been determined until recently when the structure of a homogeneous component of lipid A was investigated.[2] In this previous work we isolated the main component of *Escherichia coli* lipid A after removal of the glycosyl phosphate and methyl esterification. This compound (1) was shown to be a disaccharide 4'-monophosphate dimethyl ester acylated at the hydroxyl groups at positions 3 and 3' as well as at the two amino groups. As fatty acid components, 4 moles of 3-hydroxytetradecanoic and each 1 mole of dodecanoic and tetradecanoic acid were detected. It was further shown that two of the hydroxy acids were acylated at their hydroxyl groups to form 3-acyloxy acids.[2] However, the exact location of the individual acyl and acyloxyacyl groups could not be determined at that time. In the present communication, the distribution of acyl residues was studied and evidence is presented to elucidate the



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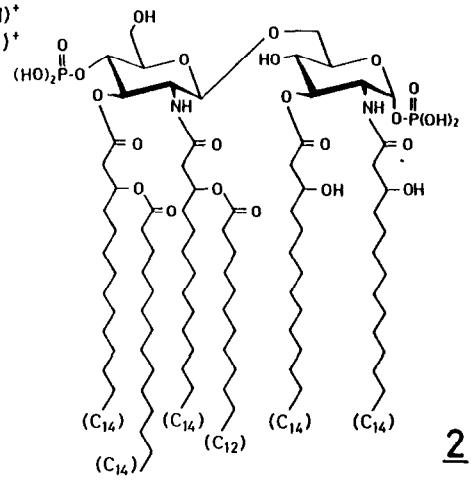

2

structure of *E. coli* lipid A as 2. The structural proposal is confirmed by our total synthesis described in the accompanying paper.[3]

For structural analysis, the peracetylated product (3) of 1 was treated with Et_3OBF_4 (in CH_2Cl_2 at room temperature) and acid (1N HCl in aqueous THF at room temperature). Subsequent GLC analysis for liberated fatty acid esters revealed the presence of ethyl 3-acetoxy- and 3-dodecanoyloxytetradecanoate showing that 3-dodecanoyloxytetradecanoic and one 3-hydroxytetradecanoic acid were bound to either one of the amino groups of the disaccharide.[4] In order to locate these N- and O-acyl groups on the disaccharide backbone, chemical degradations of 1 and 3 were attempted next. However, all procedures including acetolysis of the glycosidic bond in 3 and periodate oxidation of 1 failed to proceed or to give informative cleavage products.

The attachment site of acyl residues could, however, be deduced from an FD mass spectrum of 1, where various fragment ions were observed together with the quasimolecular peak at m/z 1784 (M+K). Among them, the distinct peaks at m/z 631 and 669 could be assigned as the protonated and plus potassium peaks of a fragment $C_{34}H_{64}NO_9$ (630) which are likely to be formed by cleavage of the glycosidic bond and to correspond to the reducing glucosamine residue bearing two 3-hydroxytetradecanoyl groups. Consequently, it can be concluded that both N-bound 3-dodecanoyloxytetradecanoyl and O-bound 3-tetradecanoyloxytetradecanoyl groups are located at the non-reducing glucosamine unit. Since the reducing glucosamine residue carrying the glycosyl phosphate moiety was already proven to have the α-configuration,[5] the structure of the main component of *E. coli* lipid A can be represented as 2. Qureshi et al. and Seydel et al. obtained similar results for lipid A's from other bacterial species.[6,7]

Biological tests of the recently synthesized lipid A precursor which contains 4 moles of 3-hydroxytetradecanoic acid but lacks the nonhydroxylated fatty acids already demonstrated that lipid A represents the endotoxic principle of LPS. Simultaneously, however, it turned out that the presence of acyloxyacyl moieties might be important for the expression of certain biological activities such as pyrogenicity.[8] Therefore, the structure 2 represents the final target for our purpose to construct a compound with full endotoxic activity chemically.

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References and Notes

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4) The formation of the bis-imino ether from 3 was followed by TLC. Though the reaction did not reach completion even after 48 hr, it was interrupted to avoid serious formation of by-products. The yields of 3-acetoxy- and 3-dodecanoyloxy acid esters reached 65 and 92% respectively as determined by GLC. A small amount (5%) of ethyl 3-tetradecanoyloxytetradecanoate was also detected. At present it cannot be decided whether this was formed by cleavage of the ester bond (position 3') or whether it is present as a minor amide-bound component.
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