The Structures of the Lipid A Moieties from the Lipopolysaccharides of Two Phytopathogenic Bacteria, *Xanthomonas campestris* pv. *pruni* and *Xanthomonas fragariae*

Alba Silipo,[a] Antonio Molinaro,*[a] Rosa Lanzetta,[a] Michelangelo Parrilli,[a] Buko Lindner,[b] and Otto Holst*[c]

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Lipopolysaccharides (LPSs) are vital and exclusive structural components of the outer membranes of all Gram-negative bacteria. They play an important role in the communication between the pathogen and both animal and vegetal host cells. Structurally, they comprise in their smooth (S) form three regions, namely the O-specific polysaccharide (or O-antigen), the core region and the lipid A (the endotoxic active moiety). In this paper, the structure of the lipid A moieties from two lipopolysaccharides of two phytopathogenic bacteria, *X. campestris* pv. *pruni* and *Xanthomonas fragariae*, is described. The sugar backbone is constituted by the typical bis(phosphorylated)β-(1′→6)-linked D-glucosamine disaccharide. Both lipid A fractions are remarkably heterogeneous with respect to the fatty acid chain length. The major species are hexacylated lipid A, with a symmetric [3+3] distribution in which the secondary fatty acids are exclusively ester-linked. The primary structure of these two complex glycolipids is herein elucidated by means of chemical degradation, MS spectrometry and 2D NMR spectroscopy.

Introduction

Xanthomonas campestris pv. *pruni*[1] (sin. *Xanthomonas arboricola* pv. *pruni*) is a Gram-negative bacterium that is the causative agent of bacterially induced leaf spots and cankers of several cultivated plants of the genus *Prunus* (i.e. peach, apricot, plum). Likewise, *Xanthomonas fragariae*[2] is the causative agent of the angular leaf-spot disease of strawberry, occurring in most of those geographical areas where these plants are agronomically important. In most cases, spreading of this bacterium represents a limiting factor for the cultivation, since it causes significant crop losses and, in the most severe epidemics, plant dieback and death.

Lipopolysaccharides (LPSs, endotoxin) are intricate macromolecules that form the main components of the outer membrane of the Gram-negative bacterial cell wall. They are built up of three genetically and structurally distinct regions which are covalently linked to each other: the O-specific polysaccharide (O-specific chain, O-antigen), the core oligosaccharide, and a glycolipid portion, termed lipid A, which anchors the molecule in the outer layer of the outer membrane. In human and animal pathogenic bacteria, lipid A possesses a rather conservative structure often consisting of a glucosamine (GlcN) disaccharide backbone which is phosphorylated at positions 1 and 4′ and acylated with primary 3-hydroxy fatty acids at positions 2, 3, 2′ and 3′.[3] Primary 3-hydroxy fatty acids may further be acylated by other fatty acids (secondary fatty acids). There are quite a number of LPSs known in which lipid A is additionally substituted by compounds that reduce its net charge, like 2-aminoethanol phosphate or 4-amino-4-deoxy-L-arabinopyranose.

It has been established that lipid A is the endotoxic principle of LPSs, expressing all the pathophysiological effects known to be induced by endotoxic active LPSs.[3] LPSs are known to be valuable chemotaxonomic and phylogenetic markers based on their compositions and structures of lipid A and the core regions.

The LPSs of plant-pathogenic bacteria have been shown to play a role in phytopathogenicity.[4] Despite the fact that a significant number of O-specific polysaccharides have been structurally characterised,[5] only a small body of data on the composition, structure and functions of lipid A of LPS from phytopathogenic bacteria is available.[6−11]

[a] Dipartimento di Chimica Organica e Biochimica, Università di Napoli, Complesso Universitario Monte Sant′ Angelo, Via Cintia 4, 80126 Napoli, Italy
E-mail: molinaro@unina.it
[b] Divison of Biophysics, Research Centre Borstel, Centre for Medicine and Biosciences, Parkallee 10, 23845 Borstel, Germany
[c] Divison of Structural Biochemistry, Research Centre Borstel, Centre for Medicine and Biosciences, Parkallee 22, 23845 Borstel, Germany
E-mail: oholst@fz-borstel.de

Since there is no information on the structure-function relationship of the lipid A moieties of the LPSs from *Xanthomonas campestris* pv. *pruni* and *Xanthomonas fragariae*, we initiated their characterisation and report now their structures.

Results and Discussion

Isolation and Compositional Analysis of Lipid A of LPS from *X. campestris* and *X. fragariae*

After extraction of the dried bacterial cell mass with hot phenol/water, both LPSs were found to be present in the water phases (yields: *X. campestris*, 0.270 g, 4.8% of dry cell mass; *X. fragariae*, 0.127 g, 6.4% of dry cell mass). Analysis of the LPS by SDS-PAGE showed a separation of the S-form LPS in a ladder-like pattern in both cases.[2,12]

After mild acid hydrolysis of both LPSs, lipid A fractions were obtained in yields of 6.5% (*X. campestris* and *X. fragariae*). Monosaccharide and methylation analyses of these showed the presence of terminal GlcN and 6-substituted GlcN residues, both with a D-configuration.

Fatty acid analysis by GC-MS of both lipid A preparations revealed the presence of linear, *iso*-branched (isopropyl end-group) and *ante-iso*-branched (*sec*-butyl end-group) acyl chains. In particular, (*R*)-3-hydroxydecanoic, *iso*-branched and *ante-iso*-branched (*R*)-3-hydroxyundecanoic [(*R*)-11:0(3-OH)], (*R*)-3-hydroxydodecanoic [(*R*)-12:0(3-OH)], *iso*-branched and *ante-iso*-branched (*R*)-3-hydroxytridecanoic [(*R*)-13:0(3-OH)], decanoic (10:0) and undecanoic (11:0) acids were revealed as ester linked fatty acids. Traces of [(*R*)-11:0(3-OH)], [(*R*)-13:0(3-OH)] and *iso*-branched and *ante-iso*-branched [(*R*)-12:0(3-OH)] were also detected. *iso*-Branched, *ante-iso*-branched [(*R*)-13:0(3-OH)] and linear [(*R*)-12:0(3-OH)] acids were identified as *N*-linked fatty acids. The branched position of the fatty acids was determined on the basis of MS fragmentation and comparison with standard fatty acids.

X*anthomonas fragariae* lipid A showed the same fatty acid composition and, in addition, possessed traces of *N*-linked (*R*)-3-hydroxyundecanoic acid.

In both lipid As, the phosphate content was stoichiometric with the GlcN content.

Analysis of De-*O*-acylated Lipid A

The lipid As from *X. campestris* and *X. fragariae* were de-*O*-acylated with anhydrous hydrazine and the products were examined by 2D NMR spectroscopy and mass spectrometry. Since both products give a very similar pattern of ions in the mass spectra and very similar ^{1}H NMR spectra (data not shown), only the results obtained on lipid A from *X. campestris* are presented in the following.

By means of ^{1}H, ^{31}P and ^{13}C NMR spectroscopy, a complete 2D analysis (COSY, TOCSY, ROESY, ^{1}H,^{13}C HSQC, ^{1}H,^{31}P HSQC) was carried out to characterise the sugar backbone of lipid A (Table 1). The chemical shifts and the coupling constants ($J_{H,H}$ vicinal coupling of about 10 Hz) proved the presence of a β-(1→6)-linked Glc*p*N disaccharide, both residues possessing the $^4C_1$ conformation. Two anomeric proton signals at 5.25 and 4.79 ppm (Figure 1) could be correlated with two anomeric carbon signals at 92.1 and 100.3 ppm, respectively, present in the ^{1}H,^{13}C HSQC spectrum (Figure 2), indicating one α- and one β-GlcN residue. This assumption was confirmed by a ^{1}H,^{13}C HSQC spectrum registered without decoupling during acquisition that showed a $^1J_{C,H}$ value of 173 Hz for α-GlcN residue (92.1/5.25 ppm) and 162 Hz for β-GlcN residue at 100.3/4.79 ppm. With the help of COSY and TOCSY spectra (Figure 2) each resonance of the two GlcN spin systems could be assigned. The carbon resonances of C2 and C2′ at 53.9 and 55.2 ppm, respectively, were diagnostic for the C−N linkage of the GlcN residues. The downfield shift of C6 of GlcN I at 67.1 ppm together with the *inter*-residual ROE contact of H1 of GlcN II at 4.79 ppm to $H6_b$ (3.61 ppm) of GlcN I confirmed the β-1→6 linkage between the two GlcN residues. The β-*gluco* and α-*gluco* configurations of GlcN I and GlcN II, respectively, were confirmed by a ROESY experiment that yielded *intra*-residual ROE connectivities from H1 to H3 and to H5 for GlcN II, whereas in GlcN I only an ROE connectivity between H1 and H2 was found. Signals attributable to the *N*-linked 3-hydroxy fatty acids were also detected (Table 1).

Table 1. ^{1}H and ^{13}C NMR resonances of major species of de-*O*-acylated lipid A of *Xanthomonas campestris*; the spectra were recorded at 600 MHz (^{1}H) and 150 MHz (^{13}C) in [D_6]DMSO at 60 °C

Position	δC	δH	δP
1	92.1	5.25	−1.58
2	53.9	3.65	
3	71.1	3.49	
4	71.0	2.98	
5	73.5	3.90	
6a/6b	67.1	3.86/3.61	
2/2′ N-H		7.27	
1′	100.3	4.79	
2′	55.2	3.46	
3′	73.5	3.90	
4′	71.1	3.82	3.0
5′	74.3	3.25	
6′a/6′b	60.8	3.62/3.50	
2/2′α_a/α_b	43.7	2.38/2.17	
2/2′β	67.4	3.79	
2/2′γ	36.7	1.38	
$(CH_2)_n$	28.9	1.27	
(CH_3)	13.6	1.15	

A ^{1}H,^{31}P HSQC spectrum was recorded in order to identify the phosphorylation sites. Two cross peaks were obtained. The ^{31}P resonance at 3.0 ppm, indicating a phosphomonoester group, correlates with a proton resonance at 3.82 ppm attributable to H4′; this cross peak established the first phosphate location at C4′ of GlcN II. Additionally, the α-anomeric proton of GlcN I correlates with a phosphate signal at −1.58 ppm, identifying the second phosphorylation site at C1 of GlcN I.

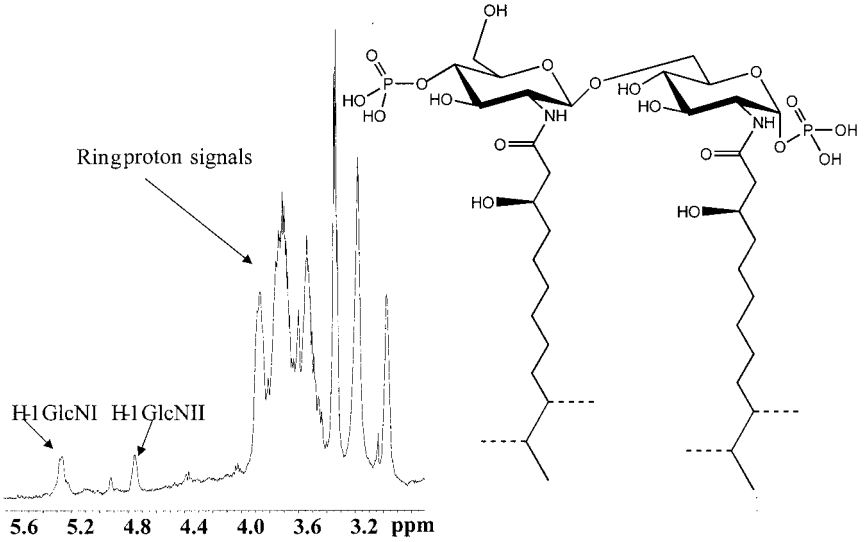

Figure 1. A portion of the 1D ^{1}H NMR spectrum of de-*O*-acylated lipid A of LPS from *X. campestris*; the spectrum was recorded in [D_6]DMSO at 600 MHz and 60 °C; inset: the lipid A molecules bearing linear and/or branched [(*R*)-12:0(3-OH)] and [(*R*)-13:0(3-OH)]; the dotted lines indicate methyl groups at the branching points and are present as a single substitution for each fatty acid

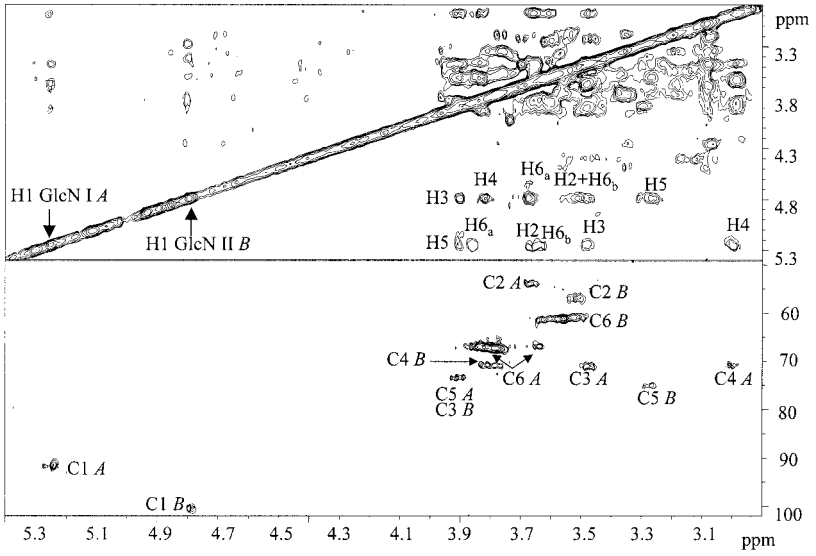

Figure 2. Sections of the TOCSY (top) and ^{1}H,^{13}C-HSQC (bottom) spectra of de-*O*-acylated lipid A of LPSs from *X. campestris*; both spectra were recorded in [D_6]DMSO at 600 MHz and 60 °C; GlcN I is labelled as A (*in italics*) whereas GlcN II is labelled as B (*in italics*)

The amide-bound fatty acids were definitively assigned by MALDI-TOF mass spectra of the de-*O*-acylated product. The negative-ion mass spectra revealed three molecular ions at *m*/*z* = 895.4, 908.7, and 922.5 corresponding to a bis(phosphorylated) disaccharide backbone carrying either two [(*R*)-12:0(3-OH)] or one [(*R*)-12:0(3-OH)] and one ([(*R*)-13:0(3-OH)], or two [(*R*)-13:0(3-OH)] moieties in the amide linkages, respectively. At low intensity monophosphorylated species were also detected. The positive ion MALDI-TOF spectrum revealed fragment ions originating from the cleavage of the glycoside linkage which represent the non-reducing end at *m*/*z* = 439.9 and 454.8 (B1 fragment, according to the nomenclature of Costello and Domon[13]). These latter ions were attributable to species bearing a phosphate group and one [(*R*)-12:0(3-OH)] or one [(*R*)-13:0(3-OH)] group.

Together with the results obtained from NMR measurements, these data proved the presence of a D-Glc*p*N-β-(1→6)-α-D-Glc*p*N backbone in both lipid As that is phosphorylated at position 1 and 4′ and *N*-acylated at C2 and C2′ by either [(*R*)-12:0(3-OH)] or [(*R*)-13:0(3-OH)].

Analysis of Intact Lipid A

The negative-ion ESI FT-ICR mass spectra of the underivatised intact lipid A moieties isolated from both strains are depicted in Figure 3. The spectrum of the lipid A from the LPS of *X. campestris* (top) comprises one intense group of

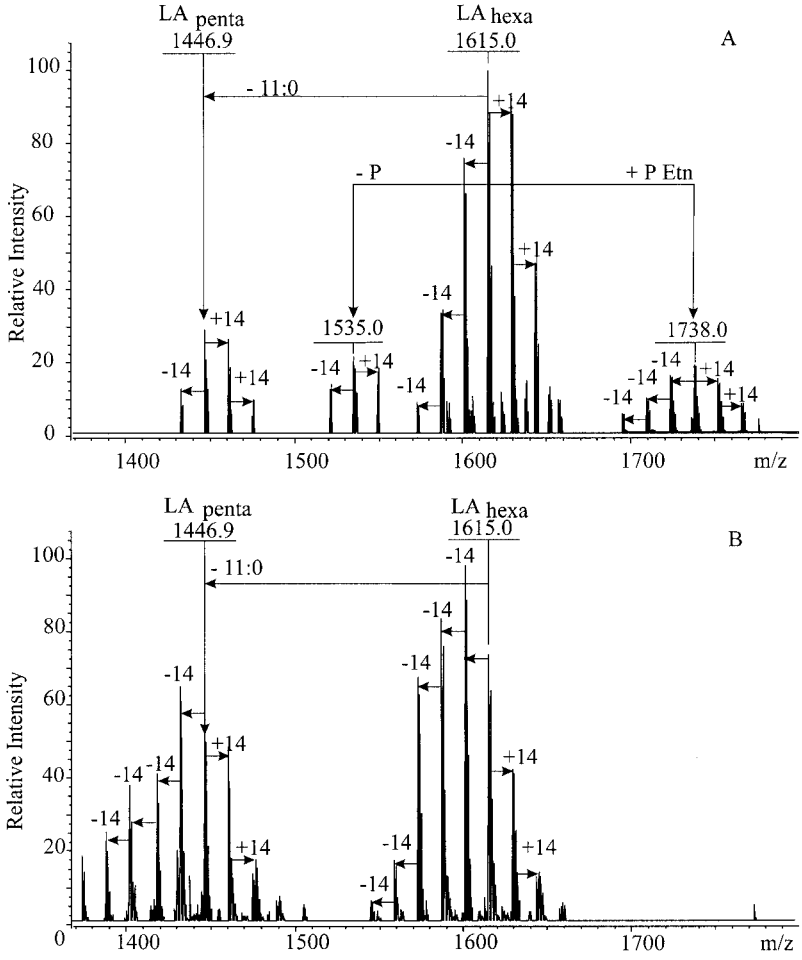

Figure 3. Charge-deconvoluted negative-ion ESI FT-ICR mass spectrum of de-*O*-acylated lipid A from *X. campestris* (A) and *X. fragariae* (B)

ions around 1615.0 Da with mass differences of 14 Da, indicating the presence of bis(phosphorylated) lipid A species carrying six fatty acid residues that differ in their chain lengths. Consistent with the results of the fatty acid analysis, these ions identified lipid A molecules carrying amide-linked (*R*)-12:0(3-OH) and/or (*R*)-13:0(3-OH), two different ester-linked 3-hydroxy fatty acids [(*R*)-10:0(3-OH), (*R*)-11:0(3-OH), (*R*)-12:0(3-OH), (*R*)-13:0(3-OH)] and two secondary fatty acids, 11:0 and/or 10:0. Furthermore, three groups of ions of minor intensity were detected, which could be attributed to hexaacylated species either missing one phosphate group (around 1535.0 Da) or carrying an additional *P*-Etn residue (ions around 1738.0 Da) and to pentaacylated species (ions around 1446.9 Da) lacking one secondary fatty acid (10:0 or 11:0). Non-labelled mass peaks refer to sodium- or potassium-adduct ions.

The spectrum obtained from lipid A of the LPS from *X. fragariae* shows identical bis(phosphorylated) lipid A species carrying six or five fatty acids. However, the masses of the most abundant peaks are 14 mass units lower, indicating that, on average, the fatty acid chain lengths are one CH_2 unit shorter than those from *X. campestris*. Furthermore, no monophosphorylated lipid A species and no species carrying an additional *P*-Etn were detected in this mass spectrum.

To determine the distribution of the acyl chains of the main hexaacylated lipid A, MS/MS experiments were performed in the positive ion mode. As an example, the fragment ions produced by infrared multi-photon dissociation (IRMPD) from the most abundant lipid A species at $m/z = 1616$ are shown in Figure 4. Besides the cleavage of the phosphate group linked to C1 ($\Delta m/z$ −98) and of a secondary fatty acid residue ($\Delta m/z$ −186), the spectrum comprises prominent B-fragment ions at $m/z = 806.5$ proving that the fatty acids are symmetrically (3+3) distributed, i.e. that both glucosamine residues carry three fatty acid residues. Additionally, two other B-fragment ions were observed ($\Delta m/z$ + and − 14) indicating that the selected ion at $m/z = 1616$ consists of isomers possessing different fatty acid combinations. The corresponding Y-fragments were also observed in very low intensity.

Thus, both lipid A species of LPSs from *X. campestris* and *X. fragariae* consist of a mixture of hexaacyl and pentaacyl types with a remarkable heterogeneity due to the fatty acids variability.

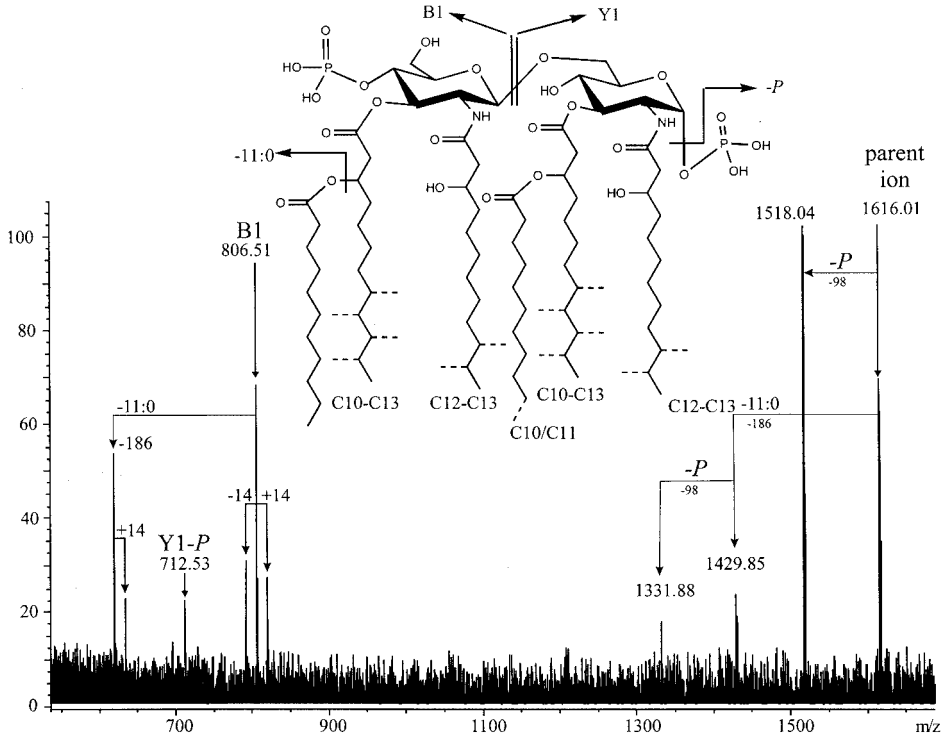

Figure 4. Positive-ion IRMPD-MS/MS mass spectrum of the most abundant hexaacylated lipid A species of *X. campestris*

In order to establish the secondary fatty acid location an aliquot of the intact sample was treated with 32% aqueous NH_4OH, at 20−22 °C for 16 h. This mild procedure selectively cleaves acyl ester and acyloxyacyl ester linkages, while it leaves acyloxyacyl amide linkages unaffected.[14] In the negative ion MALDI-TOF mass spectrum (not shown), three ions corresponding to completely de-*O*-acylated lipid A were identified, representing the same molecular species that had been obtained after mild hydrazinolysis. Thus, this hydrolysis is diagnostic of the exclusive presence of ester-bound acyloxyacyl moieties and allowed us to assign the position of the secondary fatty acids 10:0 and 11:0 at the 3-OH group of the ester-linked primary acyl chains. These results also confirmed the symmetric distribution of the acyl moieties on the disaccharide backbone.

Despite the low resolution of the 1D NMR spectra of intact lipid A (Figure 5), a complete 2D NMR analysis was considered worthwhile in order to elucidate other structural features. Starting from the anomeric and/or the amide protons in the COSY and TOCSY spectra, all resonances of each spin system could be assigned. Besides all the signals characteristic for the *N*-acylated GlcN disaccharide backbone, other characteristic resonances were identified (Table 2).

The downfield displacement of the chemical shifts of H-3 and H-3′ of the sugar spin-systems, present in the anomeric region at 5.00 and 4.98 ppm, respectively, is indicative of the *O*-acylation at these positions. The methyl and methylene signals of the fatty acids are present at 0.83 and 1.23 ppm, respectively. The H-3 protons of β-acyloxyacyl chains resonate at 5.05 ppm, whereas in the HSQC spectrum, the corresponding carbon signals appear at 70.1 ppm. In the COSY, TOCSY and HSQC spectra, these H-3 proton signals correlate with the diastereotopic α-methylene protons and the γ-methylene protons at 2.35/41.8 and 1.47/32.4 ppm, respectively.

The cross peak at 66.4 and 3.83 ppm was attributed to the H-3 proton of the β-hydroxyacyl chains. The corresponding signals for the α- and γ -CH_2 groups are observed at 2.30/42.2 and 1.36/36.5 ppm, respectively.

Taken together, the lipid A isolated from the LPSs of *X. campestris* and *X. fragariae* consists of a P→4-β-D-Glc*p*N-(1→6)-α-D-Glc*p*N-1→P backbone. In the main hexaacyl species, two unsubstituted 12:0(3-OH) and/or 13:0(3-OH) are linked to the disaccharide backbone in an amide linkage; furthermore, two primary fatty acids with different lengths [10:0(3-OH), 11:0(3-OH), 12:0(3-OH), or 13:0(3-OH)] are present in the ester linkage at C3 and C3′ of the GlcN residues. These acyl chains are further substituted at their 3-hydroxy group by 10:0 and 11:0. The pentaacyl species lack these secondary fatty acids (Figure 6).

Lipid A could be isolated from the LPSs of *X. campestris* pv. *pruni* and *X. fragariae* after mild acid hydrolysis and ultracentrifugation, indicating that the core-O-specific polysaccharide moieties are linked to the lipid A by an acid-labile constituent, i.e. 3-deoxy-D-*manno*-octulosonic acid, as is the case in most other LPS.[3] Both lipid As share the same backbone structure, P→4-β-D-Glc*p*N-(1→6)-α-D-Glc*p*N-1→P which is present in most of the lipid A structures characterised to date.[3] Also, as shown by compo-

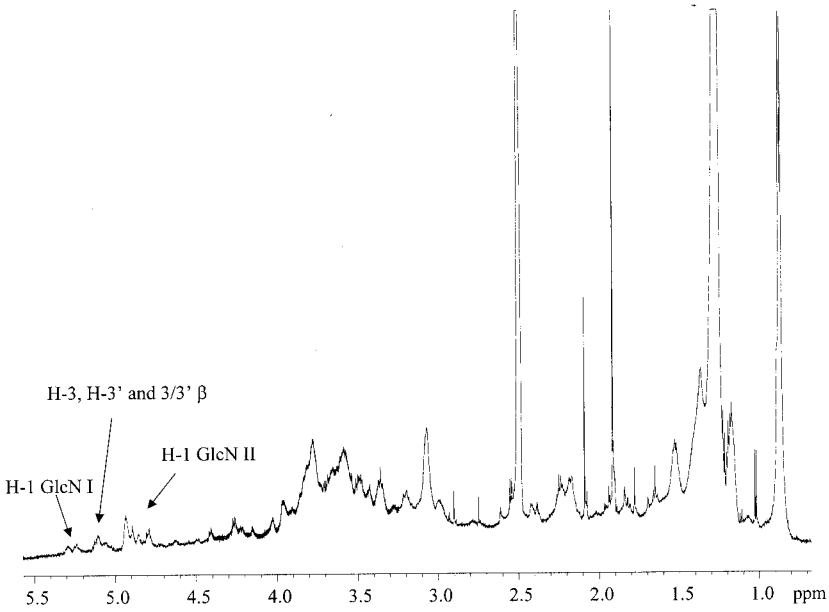

Figure 5. The 1D ^{1}H NMR spectrum of intact lipid A of LPSs from *X. campestris*; the spectrum was recorded in [D_6]DMSO at 600 MHz and 60 °C

Table 2. ^{1}H and ^{13}C NMR resonances of the major species of lipid A of *Xanthomonas campestris*; the spectra were recorded at 600 MHz (^{1}H) and 150 MHz (^{13}C) in [D_6]DMSO at 60 °C

Position	δC	δH
1	93.1	5.29
2	51.2	3.95
3	73.2	5.00
4	68.5	3.34
5	73.2	3.93
6a/6b	66.1	3.84/3.68
2 N-H		7.30
1′	99.9	4.83
2′	52.9	3.72
3′	73.1	4.98
4′	ND	4.11
5′	76.2	3.42
6′a/6′b	60.2	3.56/3.66
2′ N-H		7.60
2/2′α	42.2	2.30
2/2′β	66.4	3.83
2/2′γ	36.5	1.36
3/3′α	41.8	2.35
3/3′β	70.1	5.05
3/3′γ	32.4	1.47
$(CH_2)_n$	27.8	1.23
(CH_3)	12.8	0.83

sitional and mass spectrometrical analyses, both lipid A preparations represent mixtures of compounds which are mainly due to a remarkable variability in the fatty acid chain lengths. Slight structural differences were identified with regard to an additional *P*-EtN residue in lipid A of the LPS from *X. campestris* and, on average, shorter acyl chains in the lipid A of the LPS from *X. fragariae*.

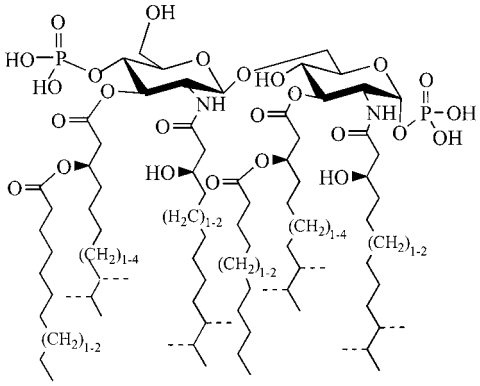

Figure 6. The structure of the lipid A from the LPSs from *X. campestris and X. fragariae*; the dotted lines indicate methyl groups at the branching points and are present as a single substitution for each fatty acid

Both structures represent a novel species of lipid A. A symmetric [3+3] distribution has been found in other lipid As previously, for example from the LPS of *Chromobacterium violaceum*, *Neisseria meningitidis* or *Shigella sonnei*.[3] However, in all these cases the acyloxyacyl groups are exclusively amide linked, whereas in the lipid A from LPSs of *X. campestris* and *X. fragariae*, these groups are exclusively ester linked.

From a phytochemical point of view, this chemical peculiarity in bacteria could play an important role for the bacterium in the infected host. In fact, plants have been found to have systems of innate immunity and the variability of the fatty acid could help the bacterium to evade the host's response.[4,9] Further studies are needed to confirm this hypothesis.

To the best of our knowledge, this is the first complete lipid A structure elucidated from the *Xanthomonas* genus.

Experimental Section

Cultivation of Bacteria and Isolation of LPS: *Xanthomonas campestris* pv. *pruni* type strain NCPPB416 and *Xanthomonas fragariae* type strain NCPPB1469 were grown in 200 mL of Wilbrink's medium on a rotary shaker at 150 rpm at 25 °C for 96 h. Cultures were centrifuged (20000 × *g*, 15 min), and the bacterial-cell pellets were washed twice with aqueous saline solution (0.8% NaCl) and freeze-dried. The dried cells of *X. campestris* pv. *pruni* (5.6 g) and of *X. fragariae* (2 g) cultures were suspended in 200 mL of ultrapure water and extracted with a hot phenol/water procedure .[15] Both the phenol and water phases were separately dialyzed against distilled water, then freeze-dried and analysed by SDS-PAGE (12% acryl amide) on a miniprotean gel system (Bio-Rad). Samples (4 μg each) were run at constant voltage (150 V) and stained with silver nitrate.[16] Pure LPSs were obtained in a yield of 4.8% (*X. campestris*, 0.270 g) and 6.4% (*X. fragrariae*, 0.127 g).

Preparation of Lipid A, De-*O*-acylated Lipid A and Dephosphorylated Lipid A: Free lipid A preparations were obtained by treatment of LPS with sodium acetate buffer (15 mg/ml) at pH 4.4 (100 °C, 2 h). The solutions were extracted three times with $CHCl_3$/MeOH/H_2O (100:100:30, v/v/v) and centrifuged (4 °C, 5000 g, 15 min); the organic phases containing the lipid A were dried under N_2 (yield 6.5% of LPSs). TLC experiments of lipid A were carried out on 20 × 20 cm silica gel 60 TLC plates (0.25 μm thickness) developed with $CHCl_3$/MeOH/H_2O (100:75:15, by vol.) containing 0.01% (v/v) acetic acid. Spots were visualised by spraying the plate with 10% (v/v) ethanolic H_2SO_4 and charring. Both lipid As were de-*O*-acylated with anhydrous hydrazine in THF at 37 °C for 1.5 h, followed by precipitation with cooled acetone and centrifugation (5000 × g, 15 min, 4 °C) (yield 50% of lipid A). Mild de-*O*-acylation was performed by treatment of lipid A preparations (200−500 μg) with 32% ammonium hydroxide (200 μL, 20 °C, 16 h).[14] Dephosphorylation of lipid A was carried out with 48% aqueous HF (4 °C, 48 h), followed by neutralisation, dialysis and lyophilisation.

Compositional Analysis: The content of organic bound phosphate was determined as described previously.[17] Monosaccharides were identified as acetylated *O*-methyl glycosides derivatives. Briefly, samples were treated with 1 M HCl/MeOH (85 °C, 24 h), dried under nitrogen, acetylated with acetic anhydride in pyridine (85 °C, 30 min) and analysed by GLC-MS.

The absolute configuration of the monosaccharides was obtained according to a published method.[18]

The total fatty acid contents were determined after acid hydrolysis of lipid A. Briefly, the lipid A was treated first with 4 M HCl (100 °C, 4 h) then with 5 M NaOH (100 °C, 30 min), and the pH of each sample was adjusted to slight acidity. The fatty acids were extracted with chloroform, then methylated with diazomethane and analysed by GLC-MS.

The ester-bound fatty acids were selectively liberated by base-catalysed hydrolysis with 0.5 M aqueous NaOH/MeOH(1:1 v/v, 85 °C, 2 h). The product was acidified, extracted with chloroform, methylated with diazomethane and analysed by GLC-MS.

The absolute configuration of the fatty acids was determined as described previously.[19] Briefly, the 3-hydroxy fatty acids were liberated by strong alkaline hydrolysis in 4 M NaOH (100 °C, 5 h), converted into the 3-methoxy acid L-phenylethylamides and analysed by GLC-MS. With the aid of authentic chiral 3-hydroxy fatty acids it was shown that they possessed the (*R*) configuration.

Gas Chromatography: All GLC analyses were performed with a Hewlett−Packard 5890 instrument equipped with an SPB-5 capillary column (0.25 mm × 30 m, Supelco). In methylation analysis the temperature program was 150 °C for 5 min, then 5 °C min^{-1} to 300 °C. In the analysis of the absolute configuration of GlcN the program was 150 °C for 8 min, then 2 °C min^{-1} to 200 °C, and finally 6 °C min^{-1} to 260 °C for 5 min. In fatty acid analyses the temperature program was 150 °C for 3 min, then 10 °C min^{-1} to 280 °C.

Mass Spectrometry: Matrix-assisted laser-desorption/ionisation time-of-flight (MALDI TOF) mass spectrometry was performed with a Bruker-Reflex II instrument (Bruker-Franzen Analytik, Germany) in linear and reflector configuration at an acceleration voltage of 20 kV and delayed ion extraction. Samples were dissolved in distilled water at a concentration of 10 μg/μL, and a 1 μL solution was mixed with 1 μL 0.5 M 2,4,6-trihydroxyacetophenone (Sigma−Aldrich, St. Louis, MO) in methanol as matrix solution. 0.5 μL aliquots were deposited on a metallic sample holder and analysed immediately after drying in a stream of air.

Underivatised native lipid A samples isolated from *X. campestris* and *X. fragariae* were analysed in the positive- and negative-ion mode by electrospray Fourier transform ion cyclotron resonance (ESI FT-ICR) mass spectrometry using an APEX II instrument (Bruker Daltonics, USA) equipped with a 7 Tesla actively shielded magnet and an Apollo ion source. Mass spectra were acquired using standard experimental sequences as provided by the manufacturer. Samples were dissolved at a concentration of about 10 $ng{\cdot}\mu L^{-1}$ in a 50:50:0.001 (v/v/v) mixture of 2-propanol, water, and triethylamine and sprayed at a flow rate of 2 $\mu L{\cdot}min^{-1}$. The capillary entrance voltage was set to 3.8 kV, and the dry gas temperature to 145 °C. The spectra, which showed several charge states for each component, were charge deconvoluted, and the mass numbers given refer to the monoisotopic molecular masses. Infrared multiphoton dissociation (IRMPD) of isolated parent ions was performed with a 35 W, 10.6 μm CO_2 laser (Synrad, USA). The unfocused laser beam was directed through the centre of the ICR cell. The duration of the laser irradiation was adapted to generate optimal fragmentation and varied between 10−80 ms. Fragment ions were detected after a delay of 0.5 ms.

NMR Spectroscopy: ^1H, ^{13}C and ^{31}P NMR spectra were recorded for solutions in [D_6]DMSO at 600, 150 and 243 MHz, respectively, with a Bruker AMX 600 spectrometer at 60 °C. ^1H and ^{13}C NMR chemical shifts are given in δ relative to dimethyl sulfoxide (^1H, 2.49 ppm; ^{13}C, 39.7 ppm). Aqueous 85% phosphoric acid in [D_6]DMSO was used as external reference for ^{31}P chemical shifts. Two-dimensional spectra [correlation spectroscopy (COSY), total correlation spectroscopy (TOCSY), rotating frame nuclear Overhauser enhancement spectroscopy (ROESY), and heteronuclear single quantum coherence (HSQC)] were recorded using the standard Bruker software. The homonuclear experiments were performed with 4096 data points in the F2 dimension and 512 experiments in F1. The data matrix was zero-filled in the F1 dimension to give a matrix of 4096 × 2048 points and was resolution-enhanced in both dimensions by a shifted sine-bell function prior to Fourier transformation. The TOCSY and ROESY experiments were performed with mixing times of 80 ms and 300 ms, respectively. The heteronuclear experiments were performed using pulse field gradient programs as *g*HSQC.

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