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Structure of a new 6-deoxy-α-D-talan from *Burkholderia* (*Pseudomonas*) *plantarii* strain DSM 6535, which is different from the O-chain of the lipopolysaccharide [1]

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Abstract

An *O*-acetylated homopolysaccharide of 6-deoxy-D-talose (6-deoxy-α-D-talan polymer) was isolated from *Burkholderia* (*Pseudomonas*) *plantarii* strain DSM 6535 by extraction with 2-propanol. The structure (**1**) of the trisaccharide repeating unit of the polysaccharide was established by studies of the intact and *O*-deacetylated polysaccharides using methanolysis, methylation analysis, ^{1}H and ^{13}C NMR spectroscopy, including 2D COSY, heteronuclear $^{13}C,^{1}H$ COSY, 1D NOE, and computer-assisted analysis of 1D ^{13}C NMR spectra. The remaining material after extraction of the biomass with 2-propanol showed to be a lipopolysaccharide with an O-specific polysaccharide chain having a different structure (**2**), which has been found previously in lipopolysaccharides of a number of other Gram-negative bacteria.

Ac
|
4
→3)-α-D-6dTal*p*-(1→2)-α-D-6dTal*p*-(1→2)-α-D-6dTal*p*-(1→

1

→4)-α-L-Rha*p*-(1→3)-β-D-Man*p*NAc-(1→

2

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1. Introduction

Burkholderia plantarii is a plant pathogenic, Gram-negative bacterium which is shown to be the causing agent of the rice seedling blight [1]. The bacterium initially classified to be *Pseudomonas plantarii* was later on transferred to the new genus *Burkholderia* covering several members of the rRNA homology group II of *Pseudomonas* [2,3].

A type-species of the genus *Burkholderia* is *Burkholderia cepacia* [4]. It was initially isolated as the causative agent of bacterial rot in onion bulbs [5], and shares with other plant pathogenic *Burkholderia* species and in particular with *Burkholderia plantarii*, the ability to form extracellular lipases/esterases [1]. These extracellular enzymes, secreted by the *Pseudomonas / Burkholderia* genera, gained growing interest as biological catalysts in organic synthesis, as detergent enzymes [6], and as digestives [7] for enzyme substitution therapy. After extraction of the biomass the exoenzymes are shown to be accompanied by large amounts of polysaccharide and lipopolysaccharide (LPS). By application of a newly introduced extraction protocol it was possible to separate the 6-deoxy-α-D-talan polysaccharide from the LPS and, thus, to gain detailed knowledge on two important surface antigens of *B. plantarii* which may also play an important role in plant–pathogen interaction. Interestingly, the polysaccharide extracted from *B. plantarii* is substantially different from an exopolysaccharide described for *B.* (*Pseudomonas*) *cepacia* comprising galactose, glucose, mannose, glucuronic acid, and rhamnose, with lesser amounts of uronic acid [8]. This exopolysaccharide, however, was found to be closely related to the serotype-specific polysaccharide antigens (SPA) isolated from *Actinobacillus actinomycetemcomitans* serotypes a and c [9,10] but being structurally different from the O-specific polysaccharide of *B. plantarii* strain 6535. The LPS isolated from this strain showed an identical structure when compared with the O-specific polysaccharides of *B. cepacia* serogroup O5 [11] and *P. aeruginosa* serotype Meitert X [12].

2. Results and discussion

The polysaccharide 6-deoxy-α-D-talan (poly-6dTal) and the lipopolysaccharide (LPS) were isolated by extraction of the biomass of a strain of *Burkholderia* (*Pseudomonas*) *plantarii* with 2-propanol. Sodium chloride was added to the cell free supernatant to yield a two phase system. The aqueous phase, which was found to contain 6-deoxy-α-D-talan and LPS, was applied to a TSK-butyl column to remove contaminating proteins. The lyophilized polysaccharide material was suspended in chloroform–methanol–water, and fractionated by centrifugation.

6-Deoxy-α-D-talan homopolymer.—The polymer 6-deoxy-α-D-talan was found to be soluble in 140:60:20 chloroform–methanol–water, a fact which also allowed to analyze the polymer by TLC. The native homopolymer (**1**) expressed an R_f-value of 0.51 in 100:100:30 chloroform–methanol–water, whereas the *O*-deacetylated homopolymer remained at the start (Fig. 1).

The ^{13}C NMR spectrum of the polysaccharide (Fig. 2, Table 1) contained 18 signals for sugar carbon atoms, including those for three anomeric carbon atoms (δ 98.2, 102.5, and 104.0) and three methyl groups (C-6) of 6dTal [δ 16.4 and 16.7 (2C)], and two signals for an *O*-acetyl group at δ 21.4 (Me) and 174.5 (CO). Therefore, the repeating unit of the polysaccharide included three residues of D-6dTal and one *O*-acetyl group.

In the ^1H NMR spectrum of the 6-deoxy-α-D-talan polymer **1** (Fig. 3, Table 2), inter alia, three signals for methyl groups (H-6) of 6dTal at δ 1.19, 1.25, and 1.29 (all d, $J_{5,6}$ 6.5 Hz) were present. Furthermore, in the anomeric region at δ 5.11–5.26 four signals were detected, which belonged evidently to three anomeric protons and one proton at a carbon atom carrying an acetoxy group. These four and most other signals in the spectrum were unresolved doublets. After *O*-deacetylation of **1** the four signals in the anomeric region were reduced to two signals representing the three anomeric protons of **3** (H-1 of **B** and **C** at δ 5.12, and H-1 of **A** at δ 5.05), whereas the additional skeleton protons in **3** were unresolved signals (Fig. 3).

Based on the $^1J_{C\text{-}1,H\text{-}1}$ coupling constant values, 169.6–172.9 Hz, determined from the gated-decoupling spectrum of the 6-deoxy-α-D-talan polymer, all three monosaccharide residues in the repeating unit are in the pyranoid form and α-linked [13,14].

The *O*-deacetylated 6-deoxy-α-D-talan **3** was studied by a computer-assisted ^{13}C NMR-based method

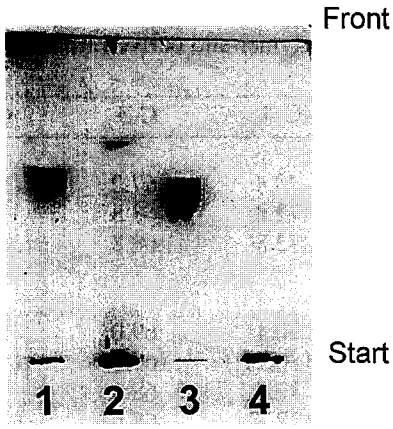


Fig. 1. TLC of polysaccharides isolated from *B. plantarii* strain 6535. Lane 1, crude extract isolated from the TSK-butyl column containing LPS and 6-deoxy-α-D-talan polymer **1**; lane 2, LPS after phenol–chloroform–petroleum ether extraction; lane 3, purified native 6-deoxy-α-D-talan polymer **1**; lane 4, 6-deoxy-α-D-talan polymer **3** after *O*-deacetylation of 6-deoxy-α-D-talan polymer **1**. Solvent system: 100:100:30 $CHCl_3$–MeOH–H_2O.

[15,16]. For this purpose, the ^{13}C chemical shifts database used in this method was extended to include the data for the glycosylated pyranose with the *talo* configuration. The chemical shifts for talose and 6-deoxytalose were available from the literature [17,18]; the glycosylation effects were calculated using published data (mainly [9,19]), and some missing effects for 2- and 4-substituted talopyranose taken the same as used in the database for mannopyranose and galactopyranose, respectively.

Only one structure was revealed that fitted with the experimental ^{13}C NMR spectrum (see below). This structure was characterized by the smallest value $S = 0.5$ among all possible linear structures of the polysaccharide with the given size and composition of the repeating unit. The analysis allowed also tentative assignment of the signals in the ^{13}C NMR spectrum of **3** (Table 1).

→3)-α-D-6dTal*p*-(1→2)-α-D-6dTal*p*-(1→2)-α-D-6dTal*p*-(1→

A B C

3

The established structure of **3** was in accord with the α-D-configuration of the three 6dTal residues determined from the gated-decoupling ^{13}C NMR spectrum (see above) and was additionally corroborated by methylation analysis. This analysis revealed two partially methylated 6dTal derivatives, 6-deoxy-2,4-di-*O*-methyltalose (*T* 11.07 min) and 6-deoxy-3,4-di-*O*-methyltalose (*T* 11.48 min) in a molar ratio

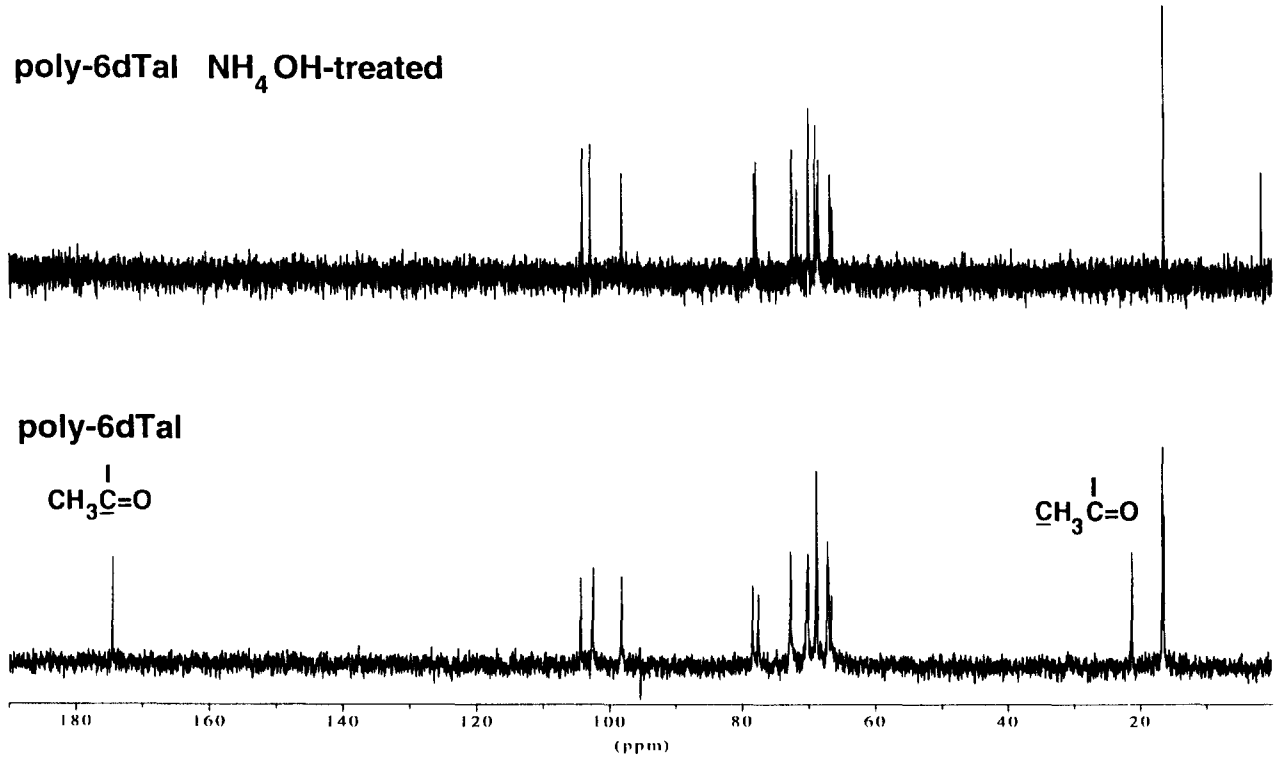


Fig. 2. ^{13}C NMR (90.6 MHz, D_2O, 330 K) spectrum of 6-deoxy-α-D-talan polymer **1**; upper spectrum after de-*O*-acetylation (**3**); lower spectrum native polysaccharide (**1**).

Table 1
^{13}C NMR chemical shifts of native (**1**) and *O*-deacetylated (**3**) 6-deoxy-α-D-talan polymer and derived fragments (**4**, **5**, and **6**) (δ in ppm) [a]

Compounds/residues	C-1	C-2	C-3	C-4	C-5	C-6
6-Deoxy-α-D-talan polymer (**1**)						
→ 3)-4-Ac-α-D-6dTal*p*-(1 → (**A**)	104.3	70.2	71.8	70.4	67.2 [c]	16.4 [d]
→ 2)-α-D-6dTal*p*-(1 → (**B**)	102.5	78.5	67.1 [c]	72.8	68.8	16.7 [d]
→ 2)-α-D-6dTal*p*-(1 → (**C**)	98.2	77.6	66.6 [c]	72.8	68.8	16.7 [d]
O-Deacetylated 6-deoxy-D-talan polymer (**3**) [b]						
→ 3)-α-D-6dTal*p*-(1 → (**A**)	104.0	70.1	71.8	70.1	69.0	16.5
	(104.3)	(70.1)	(72.0)	(70.1)	(69.0)	(16.5)
→ 2)-α-D-6dTal*p*-(1 → (**B**)	102.8	78.1 [c]	66.7 [d]	72.4 [e]	68.5 [f]	16.5
	(102.8)	(78.8)	(66.5)	(72.7)	(68.6)	(16.5)
→ 2)-α-D-6dTal*p*-(1 → (**C**)	98.1	77.9 [c]	66.4 [d]	72.6 [e]	68.7 [f]	16.5
	(98.2)	(78.8)	(66.5)	(72.7)	(68.6)	(16.5)
α-D-6dTal*p*-(1 → OMe (**4**)	102.3	70.4	66.4	72.9	67.9	16.4
2,3,4-Ac_3-α-D-6dTal*p*-(1 → OMe (**5**)	99.3	67.2	66.0	68.9	64.5	16.1
Disaccharide (**6**) [b]						
α-D-6dTal*p*-(1 →	99.3	70.7	66.3	72.9	68.8	16.4
	(99.4)	(70.4)	(66.4)	(72.9)	(68.7)	(16.4)
→ 3)-α-D-6dTal*p*-(1 → OMe	102.2	70.0	71.5	69.7	67.9	16.4
	(102.3)	(69.7)	(71.5)	(69.6)	(67.9)	(16.4)

[a] Chemical shifts for CO at δ 174.5 and Me at δ 21.4 in **1**; OMe: δ 55.6 in **4**, 55.2 in **5**, and 55.7 in **6**; OAc: δ 20.6, 20.7, and 20.9 (Me), and 169.6, 170.1, and 170.6 (CO) in **5**.
[b] Chemical shifts calculated by the published methods [15,16] are given in parentheses.
[c,d,e,f] Assignment could be interchanged.

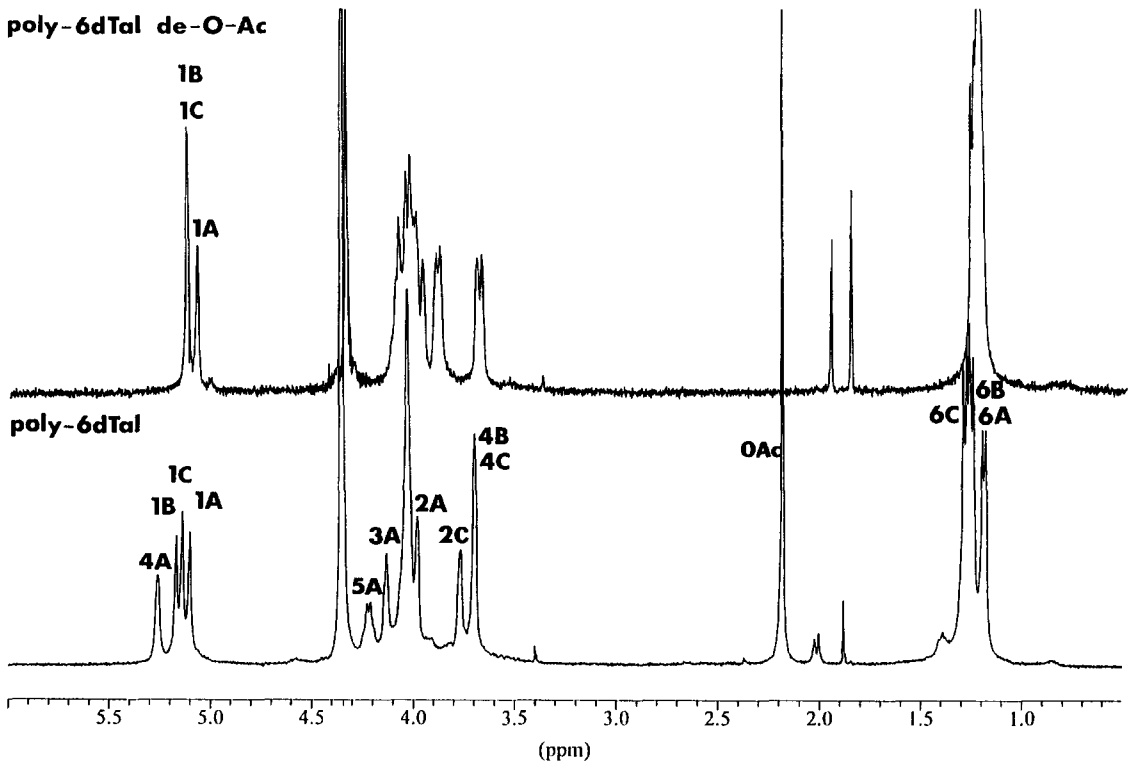


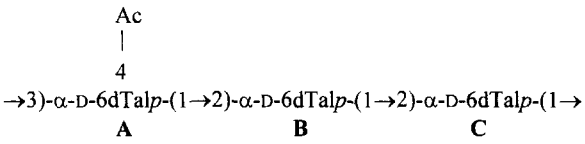
Fig. 3. ^1H NMR (360 MHz, D_2O, 330 K) spectrum of 6-deoxy-α-D-talan polymer; upper spectrum, after *O*-deacetylation (**3**); lower spectrum, native polysaccharide (**1**).

of 1:2, which were identified by GLC–MS of derived alditol acetates. This confirmed the pyranoid form of the sugar residues and showed that the polysaccharide is linear with one 3-substituted (unit **A**) and two 2-substituted (units **B** and **C**) residues of 6dTal in the trisaccharide repeating unit. In addition to the main products, two minor derivatives, 6-deoxy-2,3,4-di-*O*-methylhexose (*T* 9.24 min) and 6-deoxy-2,4-di-*O*-methylhexose (*T* 11.55 min), were identified. Most likely, the former was derived from 6dTal occupying the nonreducing end of the polysaccharide, whereas the origin of the latter derivative, which had the same mass spectrum as the corresponding derivative of 6dTal but different retention time, was not clarified.

The ^{1}H and ^{13}C NMR spectra of the intact polymer 6-deoxy-D-talan (**1**) were assigned using 2D COSY and heteronuclear $^{13}C,^{1}H$ COSY, respectively. Selective preirradiation of **B** H-1 at δ 5.18 resulted in a strong interresidue NOE on **C** H-2 at δ 3.77 that was in agreement with the (1 → 2) linkage between units **B** and **C**, and also in NOEs on **A** H-5,6 at δ 4.22 and 1.19, thus suggesting a conformation of the polysaccharide where the protons **B** H-1 and **A** H-5,6 are in close proximity. A likewise strong NOE on **A** H-3 at δ 4.14 was observed on selective preirradiation of **C** H-1 at δ 5.15 and confirmed the (1 → 3) linkage between units **C** and **A**. Finally, selective preirradiation of **A** H-1 at δ 5.11 caused an NOE on a signal at δ 4.03 which belonged evidently to **B** H-2, although unambiguous assignment could not be done because of a coincidence of a number of signals at this frequency.

A low-field position at δ 5.26 of the signal for H-4 of unit **A** in the ^{1}H NMR spectrum of **1** was due to the deshielding effect of the *O*-acetyl group and showed that this group was attached at C-4 of this unit (Fig. 3). As expected [20], *O*-acetylation caused also a marked upfield displacement of the signal for **A** C-5 from δ 69.0 (**3**) to 67.2 (**1**), whereas no effect was on **A** C-3, and only a small downfield displacement from δ 70.1 to 70.4 was observed for the signal of **A** C-4.

Therefore, 6-deoxy-D-talan **1** from *B. plantarii* has the following structure:



1

As judged by GLC–MS analysis of peracetylated derivatives, acidic methanolysis of the polysaccharide resulted in methyl glycosides of a 6-deoxyhexose and a disaccharide composed of two residues of 6-deoxyhexose. The latter displayed in the electron impact mass spectrum peaks of a glycosyl cation at *m/z* 273 and a biosyl cation at *m/z* 503. Both compounds were isolated from the methanolysate by HPLC and studied by NMR spectroscopy.

The ^{1}H and ^{13}C NMR spectra of the monosaccharide (**4**) and its peracetylated derivative (**5**) were assigned using 2D COSY and heteronuclear $^{13}C,^{1}H$

Table 2
^{1}H NMR data of native 6-deoxy-α-D-talan polymer (**1**) and derived fragments (**4** and **5**) (δ in ppm, *J* in Hz) [a]

Compounds/residues	H-1	H-2	H-3	H-4	H-5	H-6
6-Deoxy-α-D-talan polymer (**1**)						
→ 3)-4-Ac-α-D-6dTal*p*-(1 → (**A**)	5.11	3.99	4.14	5.26	4.22	1.19
→ 2)-α-D-6dTal*p*-(1 → (**B**)	5.18	4.03	4.03	3.70	4.03	1.29
→ 2)-α-D-6dTal*p*-(1 → (**C**)	5.15	3.77	4.03	3.70	4.03	1.25
α-D-6dTal*p*-(1 → OMe (**4**)	4.81	3.82	3.85	3.74	4.00	1.28
	$J_{1,2}$ < 1	$J_{2,4}$ 1.4	$J_{2,3}$ 3.3	$J_{3,4}$ 3.3	$J_{4,5}$ < 1	$J_{5,6}$ 6.6
2,3,4-Ac$_3$-α-D-6dTal*p*-(1 → OMe (**5**)	4.72	5.07	5.26	5.14	4.11	1.21
	$J_{1,2}$ 1.3	$J_{2,4}$ 1.0	$J_{2,3}$ 3.6	$J_{3,4}$ 3.8	$J_{4,5}$ 1.6	$J_{5,6}$ 6.4
α-L-6dTal*p* [b]	5.23	3.83	3.93	3.76	4.19	1.26
	$J_{1,2}$ 2.3	$J_{2,3}$ 3.5	$J_{3,4}$ 3.5	$J_{4,5}$ 1.6	$J_{5,6}$ 6.9	
→ 3)-α-L-6dTal*p*-(1 → [b]	5.26	4.11	4.07	3.92	4.31	1.23
	$J_{1,2}$ < 1	$J_{2,3}$ 3.2	$J_{3,4}$ 3.2	$J_{4,5}$ < 2	$J_{5,6}$ 6.7	

[a] Chemical shifts for OAc at δ 2.19 in **1**, OMe at δ 3.42 in **4**, and at δ 3.37 in **5**; for OAc at δ 1.97, 2.13, and 2.15 in **5**.
[b] Data from ref. [13].

COSY, respectively (Tables 1 and 2). Based on the ^1H chemical shifts and the $^3J_{H,H}$ coupling constant values, the *talo* configuration of **4** and **5** was suggested (cf. published data [9,17]). The position of the signal for C-5 at δ 67.9 showed that **4** had the α configuration (cf. δ 67.7 for C-5 in 6-deoxy-α-talopyranose, but δ 72.0 in 6-deoxy-β-talopyranose [17]). The optical rotation value for **5**, $[\alpha]_D$ + 75° (methanol), proved the D configuration of the monosaccharide (cf. $[\alpha]_D$ + 76° (methanol) for methyl 2,3,4-tri-*O*-acetyl-6-deoxy-α-D-talopyranoside [21]).

As judged by the ^{13}C NMR data (Table 1), the disaccharide **6** consisted of two residues of 6-deoxytalopyranose. The structure of **6** was established using a computer-assisted ^{13}C NMR-based analysis [15,16]. The analysis revealed only one structure, α-D-6dTal*p*-(1 → 3)-α-D-6dTal*p*-(1 → OMe (**6**), which was consistent with the experimental ^{13}C NMR spectrum (Table 1); it was characterized by a very small sum of squared deviation of the signals in the calculated and experimental spectra per one sugar residue, $S = 0.1$.

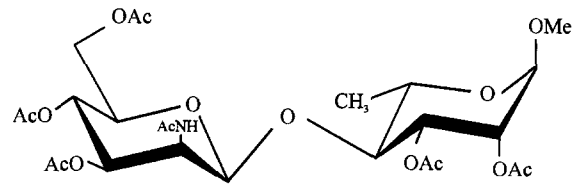
Lipopolysaccharide.—The lipopolysaccharide of *B. plantarii* was degraded with diluted acetic acid, and an O-specific polysaccharide was isolated by GPC of the carbohydrate portion on Sephadex G-50. Sugar analysis after acid hydrolysis showed that the polysaccharide contained rhamnose and 2-amino-2-deoxymannose. As judged by the ^{13}C NMR spectrum, it had a disaccharide repeating unit containing one residue each of these monosaccharides. The same spectrum displayed the polysaccharide moiety of an *O*-deacylated product prepared by alkaline degradation of the lipopolysaccharide (LPS-OH), suggesting that no acid-labile component was lost during the preparation of the O-specific chain by acid degradation of the lipopolysaccharide.

The ^{13}C NMR spectrum of the O-specific polysaccharide (Table 3) was found to be practically identical to that of the O-specific polysaccharides from lipopolysaccharides of *Pseudomonas aeruginosa* serotype Meitert X (serogroup O14 in the Lányi–Bergan classification scheme [12]), which has the structure **2** [22]. The same structure has been reported also for the O-specific polysaccharide of *Burkholderia* (*Pseudomonas*) *cepacia* serogroup O5 [11] and *Vibrio fluvialis* serovar 3 [23], and for the main chain of the O-specific polysaccharide of *Aeromonas salmonicida* [24].

→4)-α-L-Rha*p*-(1→3)-β-D-Man*p*NAc-(1→

2

In addition to the ^{13}C NMR data obtained for the polysaccharide (**2**), the repeating unit of the O-specific chain was isolated after methanolysis and peracetylation as disaccharide **7**. The ^1H NMR data (Table 4) were fully compatible with the structure proposed for the O-chain. Selective preirradiation of H-1 of ManNAc at δ 4.77 resulted in a strong interresidue NOE of Rha H-4 at δ 3.62 (8.3%), further supporting the (1 → 4) linkage in **7**.



7

Therefore, the polysaccharide chain of the lipopolysaccharide of *B. plantarii* has the structure **2** occurring in a number of other Gram-negative bacteria, but is different from poly-6d-Tal **1** isolated from the same strain. Interestingly, partially *O*-acetylated 6-deoxy-D-talan and 6-deoxy-L-talan with disaccharide repeating units have been described previously as serotype-specific antigens of *Actinobacillus actinomycetemcomitans* [9,10]. As in *B. plantarii*, these homopolysaccharides were biologically active and

Table 3
^{13}C NMR data [a] of the O-specific chain from LPS of *B. plantarii* strain DSM 6535

Residue	C-1	C-2	C-3	C-4	C-5	C-6	*Me*CO	Me*C*O
→ 3)-β-D-Man*p*NAc-(1 →	100.7	50.9	77.2	66.4	77.3	61.7	23.8	175.9
→ 4)-α-L-Rha*p*-(1 →	97.8	71.8	71.3	80.9	68.5	18.0		

[a] δ in ppm, 75 MHz, 343 K, D_2O, relative to acetone at δ 31.45.

Table 4
^{1}H NMR data of a peracetylated disaccharide derivative (**7**) isolated from the repeating unit in the O-specific chain in the LPS of *B. plantarii* strain DSM 6535 (δ in ppm, J in Hz) [a]

Residue	H-1	H-2	H-3	H-4	H-5	H-6(a)	H-6b
β-D-Man*p*NAc-(1 →	4.77	4.62	4.90	5.04	3.68	4.10	4.27
	$J_{1,2}$ 1.7	$J_{2,3}$ 4.0	$J_{3,4}$ 10.2	$J_{4,5}$ 9.9	$J_{5,6a}$ 2.8	$J_{6a,6b}$ 12.2	$J_{6b,5}$ 8.5
→ 4)-α-L-Rha*p*-(1 → OMe	4.57	5.21	5.13	3.62	3.78	1.00	
	$J_{1,2}$ 1.9	$J_{2,3}$ 3.6	$J_{3,4}$ 9.8	$J_{4,5}$ 9.5	$J_{5,6}$ 6.4		

[a] 360 MHz, $CDCl_3$, room temperature. Chemical shifts for NH: δ 5.56, $J_{2,NH}$ 8.5 Hz; OMe: δ 3.36; 5 OAc: δ 2.14, 2.10, 2.07, 2.06, and 2.03; NAc: δ 2.00.

differed from the O-specific polysaccharide chains of the corresponding lipopolysaccharides. The data on the biologically activity of 6-deoxy-D-talan described in this paper will be published elsewhere.

3. Experimental

Miscellaneous methods.—Optical rotation was measured with a Knauer chiral detector A1000. GPC was performed on a column (45 × 2.4 cm) of Sephadex G-50 (S) (Pharmacia) using a pyridinium acetate buffer (pH 4.5) and monitored with a Knauer differential refractometer. GLC was performed with a Varian Model 3700 chromatograph equipped with a capillary column of SPB-5 operating at 150 °C for 3 min, then starting a linear temperature gradient 150 → 320 °C at 5 °C/min. GLC–MS was carried out with a Hewlett–Packard Model 5989 instrument equipped with a capillary column of HP-1 under the same chromatographic conditions as in GLC.

TLC of the 6-deoxy-α-D-talan polymers **1** and **3** was performed on silica gel plates (Kieselgel 60, E. Merck) developed with 100:100:30 $CHCl_3$–MeOH–H_2O. The substances were visualized by dipping the developed plates in ethanol containing H_2SO_4 (15% by vol) and heating.

^{1}H and ^{13}C NMR spectra were obtained with a Bruker AM-360 spectrometer for solutions in D_2O at 60 °C with acetonitrile (δ_H 2.07, δ_C 1.7) as internal standard or in $CDCl_3$ at ambient temperature. Standard Bruker software was used in 1D NOE and 2D COSY experiments.

Growth of bacteria.—Strain *B. plantarii* DSM 6535 (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany) was grown in a 10 L Chemap fermenter in 8 L medium containing 40 g $NH_4H_2PO_4$, 28 g KH_2PO_4, 28 g K_2HPO_4, 8 g $MgSO_4 \times 7\ H_2O$, 0.16 g $CaCl_2$, 80 g yeast extract, and 600 g soybean oil fed continuously with a rate of 8–12 g/h into the fermentation. The pH value was adjusted to 6.5 by titrating the fermentation with aq 25% ammonia. Formation of foam during fermentation was controlled by adding silicone antifoam with the help of a foam control unit (Russell, Fife, Scotland).

The fermentation was run under the following conditions: aeration rate, 1 VVM; temperature, 30 °C; agitation, 1000 rpm. *B. plantarii* was cultivated until reaching a bio-drymass of 35–40 g/L. The fermentation broth was diluted under vigorous agitation with an equal volume of 2-propanol. After mixing, the biomass was removed by centrifugal separation (4 °C, 8000 × *g*, 30 min).

Extraction of 6-deoxy-α-D-talan polymer, LPS, and O-specific polysaccharide.—To the cell free supernatant was added 13% NaCl. After complete dissolution of the salt, the agitation was stopped to achieve phase separation between an aqueous lower phase containing polysaccharide, lipopolysaccharide, and protein, and an organic upper phase containing mainly lipids. The upper phase was removed and discarded whereas the lower aqueous phase was applied to 1 L TSK-butyl resin (E. Merck, Germany) which was pre-equilibrated with 20 mM NaH_2PO_4, containing 2.5 M NaCl, pH 6.5. The aqueous flowthrough of the column was collected and combined with additional 3–4 column-volumes of wash with the equilibration buffer.

The combined solutions were desalted by diafiltration and concentrated by a factor of 20 with a polysulfone hollowfiber crossflow filtration membrane with an exclusion limit of 10,000 Da (Fresenius, Germany). The concentrate was shock-frozen in an acetone-dry ice bath and subsequently lyophilized in a laboratory freeze-dryer (Heraeus, Germany).

Isolation and separation of 6-deoxy-α-D-talan polymer from lipopolysaccharide.—1.05 g of the lyophilized material from the TSK-butyl column were suspended in 220 mL 140:60:20 $CHCl_3$–MeOH–H_2O with continuously stirring for 1 h at room tempera-

ture. The mixture was centrifuged (4000 rpm, 15 min; Rotixa, Hettich) and the LPS-containing sediment was further purified as described below. The supernatant, containing the 6-deoxy-α-D-talan polymer (**1**), was concentrated on a rotary evaporator to near dryness.

To the concentrated material was added 20 mL 100:25:2 $CHCl_3$–MeOH–H_2O, whereby two phases appeared. Finally, 3 mL MeOH was added until both phases gave a homogeneous solution, which was directly applied to a silica gel column (4.8 × 6 cm; Kieselgel 60, mesh 230–400, E. Merck) and eluted stepwise with the following solvents A–D: A, 80:20 $CHCl_3$–MeOH; B, 50:50 $CHCl_3$–MeOH; C, 67.5:87.5:20 $CHCl_3$–MeOH–H_2O; D, 25:100:50 $CHCl_3$–MeOH–H_2O. The desired 6-deoxy-α-D-talan polymer **1** was found in fractions C and D (yield: 561 mg, 53.2 wt%).

The LPS-containing sediment was suspended in 20 mL water and the pH was re-adjusted to 7.5–8 with triethylamine. The resulting suspension was dialyzed excessively against water and lyophilized. This crude LPS was further purified by the phenol–chloroform–petroleum ether method [25].

Preparation of O-specific polysaccharide.—LPS was degraded with aq 2% HOAc at 100 °C for 2 h, a precipitate was removed by centrifugation and the supernatant was fractionated by GPC on Sephadex G-50 to give a high-molecular-mass O-specific polysaccharide. Alternatively, the lipopolysaccharide was degraded with 1 M NaOH for 2 h at 100 C, neutralized with 4 M HCl, and fractionated on Sephadex G-50 to give *O*-deacylated lipopolysaccharide.

Preparation of disaccharide **7**.—Lipopolysaccharide (220 mg) was methanolyzed with 2.5 mL 0.5 M methanolic HCl (85 °C, 30 min) and peracetylated in 1:2 Ac_2O–pyridine (70 °C, 30 min). The product was fractionated on a silica gel column (2.0 × 12 cm) eluted stepwise with $CHCl_3$–MeOH of increasing polarity. The product eluted in the fraction with 45:55 $CHCl_3$–MeOH to give pure **7** (yield: 70 mg).

Degradation of 6-deoxy-α-D-talan polymer, derivatization, and isolation of fragments **4**, **5**, *and* **6**.—Methanolysis of purified 6-deoxy-α-D-talan polymer (**1**, 110 mg) was performed in 5 mL 2 M methanolic HCl (60 °C, 1 h) for the isolation of monomeric and dimeric methyl glycosides. From this methanolysate, a 25 mg aliquot was taken in order to isolate compounds **4** and **6** by HPLC using a Zorbax-NH_2 column (20 × 250 mm, DuPont). The column was operated at a flow rate of 10 mL/min and eluted with a two step linear gradient composed of acetonitrile and water (solvent A, 970:30 acetonitrile–water; solvent B, 5:95 acetonitrile–water). The gradient started at 0% B for 5 min, then raised to 30% B (30 min), and finally to 50% B (15 min) with a further isocratic elution for an additional 15 min. Fractions were collected (Foxy, Colora) and the eluate was monitored with a chiral detector (A1000, Knauer) whereby D-configurated 6dTal mono- and dimers express a positive optical rotation value which can be easily monitored. Under these conditions **4** had a retention time of 34.5 min (yield: 7.8 mg) and **6** eluted at 65.5 min (yield: 0.7 mg).

Another aliquot of the crude methanolysate (≈ 50 mg) was acetylated with Ac_2O–pyridine (70 °C, 0.5 h), then fractionated using standard silica gel chromatography to give pure **5** (yield: 20 mg).

Chemical analyses.—Hydrolysis of the O-specific polysaccharide was carried out with 2 M CF_3CO_2H (100 °C, 6 h) and followed by GLC analysis of neutral and amino sugars as their alditol acetates.

Methylation of 6-deoxy-α-D-talan polymer was carried out with MeI in Me_2SO in the presence of solid NaOH [26]. Partially methylated sugars were derived by hydrolysis with 2 M CF_3CO_2H (100 °C, 2 h), conventionally reduced with $NaBH_4$, acetylated, and analyzed by GLC–MS.

O-Deacetylation of poly-6-dTal (**1**, 25 mg) was performed with aq 12.5% NH_3 for 16 h at 40 °C. The product was lyophilized twice to give **3** (20.6 mg), which was directly subjected to NMR analysis without further purification.

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