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A New Type of Glycolipid, 1-[α-Mannopyranosyl-(1α-3)-(6-O-acyl-α-mannopyranosyl)]-3-O-acylglycerol, from *Arthrobacter atrocyaneus*

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Abstract: The polar lipids from the bacterium *Arthrobacter atrocyaneus* LMG 3814T were analyzed and a novel type of glycolipid isolated. Its structure was elucidated by the combination of different NMR techniques and FAB-MS/MS in the positive and negative modes. The glycolipid was identified as 1-[α-mannopyranosyl-(1α-3)-(6-O-acyl-α-mannopyranosyl)]-3-O-acylglycerol. The main compound had 14-methyl-hexadecanoic acid at the glycerol moiety and 12-methyl-tetradecanoic acid at C-6 of the inner mannose unit. Additionally six minor compounds were also identified with different fatty acids. Under (+)-FAB ionization dominant daughter ions were observed through neutral loss of hydrocarbons generated from the acyl fatty acids. Negative-ion MS/MS allowed the identification of the phospholipids which consisted of five phosphatidylglycerols and seven cardiolipins. © 1997 Elsevier Science Ltd. All rights reserved.

INTRODUCTION

Bacteria of the genus *Arthrobacter* are coryneform, Gram-positive microorganisms which are widely distributed in soils[1]. The genus contains various organisms that function as biodegraders, for instance of chlorophenol[2], cyanide[3], and diphenyls[4], and is therefore of environmental interest. In particular, strains of the species *Arthrobacter atrocyaneus* are capable of degrading phosphonates[5].

The characterization and identification of coryneform bacteria is of great interest because of their biotechnological potential[6] and because conventional identification methods often fail for these organisms[7]. For this purpose, we have applied two chemotaxonomic methods, namely gas chromatographic analysis of the fatty acid methyl esters (FAME) of the glyco- and phospholipids and fast atom bombardment mass spectrometry (FAB-MS) of the intact lipids, to strains of the genus *Arthrobacter* and isolates from the environment. Lipids of Gram-positive bacteria have been studied for many decades[8] and it has been shown that *Arthrobacter* mainly produce saturated and branched fatty acids of the *iso*- and *anteiso*-series. The glycolipids contain mono/digalacto- and dimannopyranosyl lipids, while phosphatidylmyoinositols, phosphatidylglycerols and cardiolipins have been reported in the phospholipid fraction[9].

The analysis of the polar lipids of these strains by FAB-MS affords spectra which can serve as "fingerprints" and allows a rapid comparison between different strains. The spectra reveal compounds which are characteristic for taxa of the coryneform bacteria. Such compounds are characteristic of a group of organisms, thus functioning as biomarkers, and are very valuable for the detection of these groups in bacterial communities. It has been shown that these compounds can be readily analyzed using tandem mass spectrometric (MS/MS) techniques and the structures of a number of rare and novel lipids have been detected. Further, the combination of NMR spectroscopy, which affords the structure and configuration of the polar lipid core, and MS/MS techniques, which give the structure and position of the various fatty acids attached to this core, provides a fast, sensitive and unambiguous method for the structure elucidation of these biomarkers. Here these techniques are used to investigate the glycolipids and phospholipids produced by the bacterium *Arthrobacter atrocyaneus*. The structure elucidation of a novel type of glycolipid is detailed and may prove useful for characterization of this bacterial strain.

RESULTS

Glycolipids. Negative FAB-MS of the glycolipid fraction of *Arthrobacter atrocyaneus* showed one prominent and five minor deprotonated molecular ions at m/z 891 and m/z 863, 877, 889, 905 and 919 (Fig. 1).

Table 1. NMR data of the major glycolipid **1** (CD_3OD)

	1		Reference compound (Jansson and Widmalm 1992)	
C	^{13}C	^{1}H	^{13}C	^{1}H
1	69.32 t	3.44 dd J=10.4, 6.6		
		3.76 m		
2	68.52 d	3.99 m		
3	65.36 t	4.14 dd J=11.4, 4.9		
		4.10 dd J=11.4, 6.0		
1'	102.26 d	5.09 d J=1.5	102.97	5.11
2'	70.72 d	3.97 dd J=3.3, 1.9	70.97	4.06
3'	71.36 d	3.80 m	71.37	3.87
4'	67.83 d	3.61 dd J=9.4, 9.4	67.80	3.67
5'	73.55 d	3.75 m	74.14	3.75
6'	61.93 t	3.73 m	61.81	3.77
		3.85 m		3.87
1''	101.17 d	4.77 d J=1.8	101.69	4.73
2''	69.93 d	4.05 dd J=3.2, 1.8	70.44	4.06
3''	78.98 d	3.84 m	79.16	3.85
4''	66.66 d	3.77 m	67.05	3.77
5''	71.36 d	3.76 m	73.63	3.64
6''	64.10 t	4.39 d br J=11.8	61.93	3.78
		4.29 dd J=11.8, 5.8		3.88
1-CO	174.60 s			
6''-CO	174.85 s			
fatty acids:				
C-2	34.37 t	2.35 m		
C-3	25.12 t	1.63 m		
C-ω	19.32 q	0.85 t J=6.5		
C-(ω-1)	36.88 t	1.3 m		
		1.08 m		
C-(ω-2)	34.65 d	2.35 m		
C-(ω-3)	27.33 t	1.30 m		
(ω-2)-Me	11.46 q	0.86 d J=6.3		

NMR data of the reference compound, methyl α-mannopyranosyl-(1α-3)-mannopyranoside, are taken from the literature

The differences in mass between these suggested the presence of a homologous series of compounds arising from differences in the fatty acid composition. Indeed major carboxylate anions for C15:0 (m/z 241), C16:0 (m/z 255) and C17:0 (m/z 269) were observed, together with minor ones corresponding to C15:1 (239) and C17:1 (267). The fatty acids were identified from their fragmentation patterns in additional MS/MS experiments as 12-methyl-tetradecanoic acid (C15:0a), n-hexadecanoic acid (palmitic acid, C16:0), and 14-methyl-hexadecanoic acid (C17:0a), respectively[10] (data not shown). Further MS/MS studies of the ion m/z 891 showed that it contained two fatty acids, 12-methyltetradecanoic acid and 14-methylhexadecanoic acid, as ions corresponding to the neutral loss

of free fatty acid (m/z 649 and 621) and the corresponding ketene (m/z 667 and 639) were observed (Fig. 2). This leaves a mass for the core of the molecule of 379 amu. Such a mass can be explained by two hexose units connected to a glycerol backbone. This seemed to be correct since a neutral loss of 162 mass units, corresponding to a dehydrated hexose unit, from the deprotonated molecular ion was observed resulting in an ion at m/z 729 (Fig. 2). No information about the nature of the hexose units, their conncetion to each other or to the glycerol backbone could be drawn from the negative-ion mode mass spectra. Similarly the position of the acyl groups could not be assigned. As these problems could be solved by NMR spectroscopy, the glycolipid fraction was separated by preparative TLC.

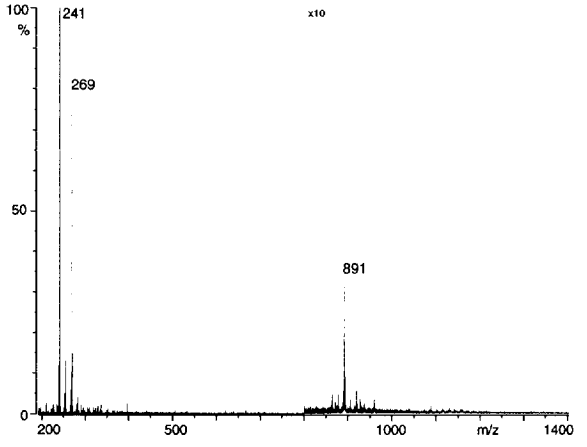

Figure 1: (-)-FAB-MS of glycolipid fraction of *Arthrobacter atrocyaneus*

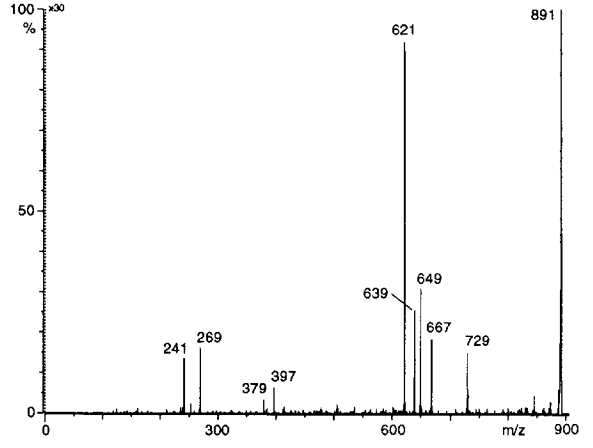

Figure 2: (-)-FAB-MS/MS of ion m/z 891

An almost pure fraction containing this compound was obtained that had impurities of less than 5%. The presence of spin systems corresponding to two hexoses, glycerol and fatty acid were readily identified from the 1D and 2D homonuclear ^{1}H COSY NMR spectra. 2D ^{1}H-detected one-bond ^{13}C-^{1}H correlation (HMQC)[11] gave the assignment of all protons and their directly-bonded carbons (Table 1), while the connectivities of all these carbons

and the sequential arrangement of the various subunits were determined from the ^{1}H-detected long-range ^{13}C-^{1}H shift correlation (HMBC, Fig. 3)[12].

The anomeric proton H-1' at δ_H5.09 displayed long-range couplings to carbons at δ_C71.36,73.55 and 78.98, while H-1" had couplings to carbons at 71.36, 78.98 and the methylene carbon at 69.32. The latter has long-range couplings with the geminal protons at 4.13 and 4.10 that were attached to the methylene carbon at 65.36 and hence belonged to the glycerol moiety. The central carbon of which was found via the COSY spectrum where the geminal protons at 4.10/4.13 and 3.44/3.76 both coupled with the central glycerol proton at 3.99. Both H-3 protons of glycerol displayed additionally $^{3}J_{CH}$ couplings to a carbonyl carbon at 174.60, thus indicating the presence of a fatty acid moiety at C-3 of glycerol. With the assigment of the glycerol resonances the anomeric proton at 4.77 was identified as H-1" and that at 5.09 as H-1'. This was confirmed by a $^{3}J_{CH}$ between C-1" and the glycerol geminal protons H-1A/B.

H-1" also displayed long-range couplings to 71.36 (C-5"), 78.98 (C-3") and a weak coupling to 69.93 (C-2") that had been assigned from the COSY and the HMQC spectra. The assignments of the remaining carbons, C-4" and C-6", of this moiety were confirmed by long-range couplings in the HMBC between H-2" and 66.66 (C-4"), and of H-6"A attached to C-6" to the same carbon, C-4". Both H-6" protons had $^{3}J_{CH}$ couplings to the carbonyl carbon at δ_C 174.85 which located one of the fatty acid esters at C-6". The protons and carbons of the second hexose moiety were assigned in a similar manner. This completed the signal assignments (Table 1) and the sequential arrangement of the moieties in the molecule (Fig. 3).

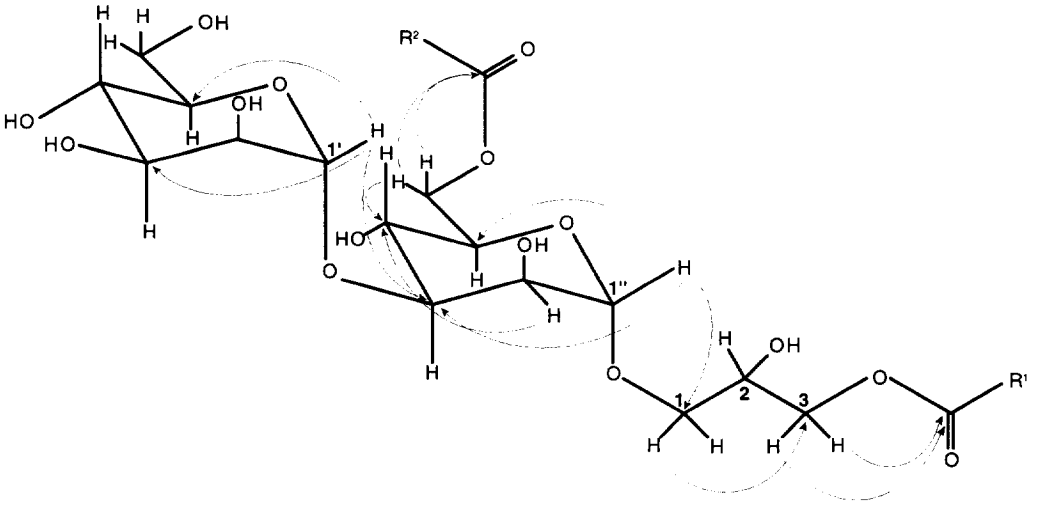

Figure 3: Schematic showing the multiple-bond couplings unambiguously identified in the ^{1}H-detected long-range ^{13}C-^{1}H shift correlation of **1**

Only the relative configuration of the sugars remained to be determined. H-4' of the first sugar had two large vicinal coupling constants of 9.4 Hz requiring the axial disposition of H-3', H-4' and H-5'. In contrast H-3' displayed only one large coupling constant and a smaller one of 3.3 Hz requiring H-2' in an equatorial position. Such an arrangement indicated that the terminal hexose had a mannopyranose configuration. It was connected to the second hexose by a 1α→3 linkage as $^{1}J_{CH}$ of C-1' was 171 Hz while a $^{1}J_{CH}$ of about 160 Hz is expected for a 1ß-linkage[13, 14]. This was corroborated by the absence of an NOE between H-1' and H-3' or H-5' and confirmed the equatorial orientation of H-1'. This line of argumentation is also applicable to the configuration at C-1", C-2" and

C-3". As H-2" does not display a $^4J_{HH}$ (W coupling) to H-4", the latter proton must be in an axial position[15]. Unfortunately H-4" and H-5" could not be resolved so the configuration at C-5" remained to be determined. Comparison of the ^{13}C NMR data of the glycolipid with literature data of methyl α-mannopyranosyl-(1α-3)-mannopyranoside (Table 1) identified the glycoside as α-mannopyranosyl-(1α-3)-mannopyranose[16]. Hence the NMR data unambiguously identified the glycolipid as 1-[α-mannopyranosyl-(1α-3)-(6-O-acyl-α-mannopyranosyl)]-3-O-acyl-glycerol.

The structure was confirmed by positive-ion FAB mass spectrometry and the position of the two different fatty acids was determined.

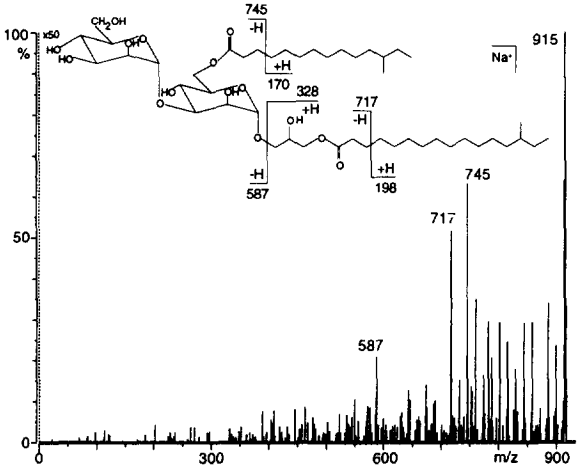

Figure 4: (+)-FAB-MS/MS of ion m/z 915. Main cleavages are indicated.

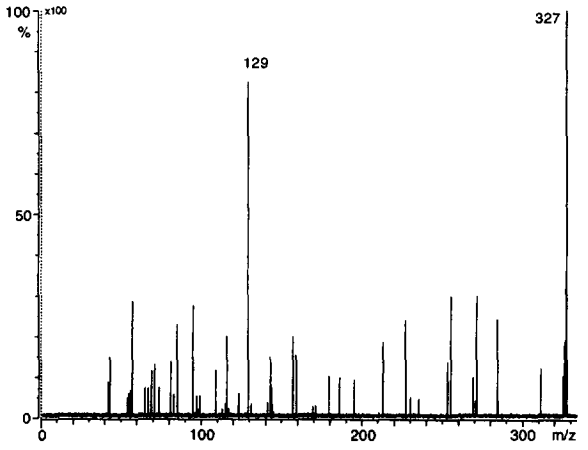

Figure 5: (+)-FAB-MS/MS of ion m/z 327

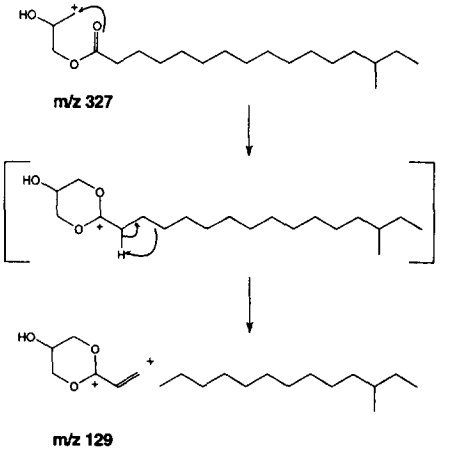

Figure 6: Proposed fragmentation pathway of ion m/z 327

Table 2. Polar lipids from *A. atrocyaneus*

No.	Type	Mass	R^1COOH[a]	R^2COOH	R^3COOH	R^4COOH
1	ADMMG[b]	892	C17:0a	C15:0a	-	-
2	ADMMG	864	C15:0a	C15:0a	-	-
3	ADMMG	878	C16:0	C15:0a	-	-
4	ADMMG	890	C17:1[c]	C15:0a	-	-
5	ADMMG	890	C17:0a	C15:1[c]	-	-
6	ADMMG	906	C17:0a	C16:0	-	-
7	ADMMG	920	C17:0a	C17:0a	-	-
8	PG[d]	694	C15:0	C15:0	-	-
9	PG	708	C16:0	C15:0	-	-
10	PG	722	C17:0	C15:0	-	-
11	PG	736	C16:0	C17:0	-	-
12	PG	750	C17:0	C17:0	-	-
13	cardiolipin	1296	C15:0	C15:0	C15:0	C15:0
14	cardiolipin	1310	C15:0	C15:0	C15:0	C16:0
15	cardiolipin	1324	C15:0	C15:0	C15:0	C17:0
16	cardiolipin	1338	C15:0	C17:0	C15:0	C16:0
17	cardiolipin	1338	C15:0	C15:0	C16:0	C17:0
18	cardiolipin	1352	C17:0	C15:0	C17:0	C15:0
19	cardiolipin	1352	C15:0	C15:0	C17:0	C17:0

[a]position of fatty acids: **1** - **7**: $R^1 = R'$, $R^2 = R''$; **8** - **19**: R^1COOH und R^3COOH at sn-1, R^2COOH and R^4COOH at sn-2 of glycerol; [b]ADMMG = acyldimannosylmonoacylglycerol, [c]position of the double bond could not be determined unambiguously; [d]PG = phosphatidylglycerol

The $[M+Na]^+$ ion at m/z 915 was analyzed by tandem mass spectrometry (Fig. 4) and gave dominant daughter ions by neutral loss of hydrocarbons generated from the acyl fatty acids. 12-Methyltetradecanoic acid led to a loss of 170 amu (resulting ion m/z 745), while 14-methylhexadecanoic acid gave rise to a loss of 198 amu (resulting ion m/z 717). The diagnostically important fragment ion at m/z 587 corresponds to a neutral loss of 328 amu arising from a dehydrated, monoacylated glycerol moeity containing 14-methylhexadecanoic acid. In fact the positively charged ion m/z 327 was observed in the normal FAB spectrum and this type of acylglyceride ion is well known to occur in (+)EI-MS[17] and (+)ESI-MS[18] of acylglycerols. It was further analyzed by MS/MS. The major daughter ion formed was m/z 129, corresponding to a neutral loss of 198 amu from a 3-methyltridecane residue (Fig. 5). The mechanism of this homolytic cleavage probably involves a cyclic transition state, in which the charge is stabilized over three atom centres (Fig. 6). Attack of the positively charged carbon of the acylglyceride type ion m/z 327 at the sn-2 carbonyl group leads to a six-membered ring from which the aliphatic residue is eliminated and the allylic cation m/z 129 is formed. A similar mechanism has been proposed for triacylglycerol fragmentation under (+)EI-ionization conditions[19].

These results indicated the molecule contained a lyso-glycerol moiety carrying 14-methylhexadecanoic acid. An ion at m/z 615 was not observed in the daughter ion spectrum confirming that 12-methyltetradecanoic acid was not bound at the glycerol unit. Thus the combined NMR and MS data identifies the major glycolipid as 1-[α-mannopyranosyl-(1α-3)-(6-O-(12-methyltetradecanoyl)-α-mannopyranosyl)]-3-O-(14-methylhexadecanoyl)-glycerol **1**. Biological tests of this lipid revealed no antibacterial, antifungal or cytotoxic activities[20].

Table 3. Fatty acid content (mean percentage of total) of the glycolipid (GL) and the phospholipid fraction (PL) of *A. atrocyaneus*

Fatty acid	GL [%]	PL [%]
dodecanoic acid	tr[a]	
12-methyltridecanoic acid	tr	tr
tetradecanoic acid	tr	tr
13-methyltetradecanoic acid	6.8	10.7
12-methyltetradecanoic acid	35.3	42.3
14-methylpentadecanoic acid	4.0	4.8
11-hexadecenoic acid		tr
palmitic acid	2.0	2.2
15-methylhexadecanoic acid	5.1	4.3
14-methylhexadecanoic acid	43.2	32.8
heptadecanoic acid		tr
12-octadecenoic acid?	tr	tr
octadecanoic acid	tr	tr
11-nonadecenoic acid	tr	tr
11-eicosanoic acid		tr
13-docosanoic acid		tr

[a]traces (less than 1% of total)

The remaining six minor glycolipids of this system (**2** - **7**) were only investigated by MS methods. The results are presented in Table 2. As the disposition of the substituents in the major compound corresponding to the ion at m/z 891 have been unambiguously identified, it is obvious that applying CID in the negative mode should result in the loss of fatty acid from the glycerol moiety more readily than that bound to the mannopyranosyl moiety. Hence the negative CID spectrum of the ion at m/z 877 clearly indicated palmitic acid was attached to glycerol (compound **3**), while for the ion at m/z 905 14-methylhexadecanoic acid was present (compound **6**). The ion at m/z 889 consisted of two isobaric glycolipids. One contains heptadecenoic acid (C17:1) and C15:0a (**4**) and the other C17:0a and pentadecenoic acid (**5**). In both compounds the C17-acid is located at the glycerol. These two glycolipids are the only ones containing unsaturated fatty acids. Their concentration were too low to allow determination of the positions of the double bonds and to detect them in the hydrolysate of the fatty acids (Table 3). For the remaining two compounds **2** and **7** only a single fatty acid moiety was present in both parts of the molecule.

Phospholipids. The phospholipid fraction of *Arthrobacter atrocyaneus* was also investigated by mass spectrometric techniques. It contained phosphatidylglycerols and cardiolipins which are widespread in coryneform bacteria. Five phosphatidylglycerols (**8-12**) and seven cardiolipins (**13-19**) could be identified by tandem mass spectrometry in the negative-ion mode[21,22] and their structures elucidated. Within these twelve phospholipids only saturated fatty acids were detected. The results are summarized in Table 2.

DISCUSSION

Glycolipids with an α-mannopyranosyl-(1α-3)-(6-O-acyl-α-mannopyranosyl)-3-O-acyl-glycerol backbone are described here for the first time. They are unusual with respect to the location of the fatty acids at the sn-1 of glycerol and the 6"-position of the inner hexopyranose moiety placing this new type of glycolipid into the class of lyso-glycerols.

To date dimannosyl glycolipids have only been reported from Gram-positive bacteria with high guanosin/cytosin content, for instance in *Arthrobacter spp.*[9,23], *Micrococcus luteus*[24], *Micrococcus lysodeikticus*[25]

and *Microbacterium lacticum*[26]. Although diacyl-α-mannopyranosyl-(1α-3)-mannopyranosylglycerols have been known for several decades, we have not found an unambiguous spectroscopic elucidation for the location of the two fatty acids at the glycerol moiety apart from the biosynthetic study of Lennarz and Talamo. For this reason it is difficult to conclude which of the reported dimannosyl glycolipids are identical with the new type from *Arthrobacter atrocyaneus,* and which are different. In the course of an ongoing study of coryneform bacteria we will try to address this question. Similarly it is still an open question to what extent this type of glycolipid is characteristic of the group of bacteria and, hence, to what extent these compounds can be considered as biomarkers. Clearly the confirmation of this type of compound as such could help identify these bacteria in microbial communities. Contrary to the glycolipids the phospholipids isolated from *Arthrobacter atrocyaneus* are widespread in coryneform bacteria and are not characteristic for this species or the genus *Arthrobacter.*

EXPERIMENTAL

The strain *Arthrobacter atrocyaneus* LMG 3814T (type strain) was obtained from the Laboratorium voor Microbiologie, Universiteit Gent, Belgium. It was grown at 37°C in 1 litre shake-flasks in a medium containing 20 g tryptone, 5 g yeast extract, 5 g sodium chloride in 1 litre deionized water and the biomass was harvested after 72 h in the late logarithmic phase.

Polar lipid fatty acid analysis. Lipids were extracted using a modified Bligh-Dyer procedure[27] as described previously[28]. All solvents were freshly distilled, all glassware used was rinsed with dichloromethane and gloves were used to reduce the risk of contamination of samples. Wet cells (2 g) were suspended in methanol, dichloromethane and phosphate-buffer (52.6 mL, 26.3 mL and 21.1 mL). They were treated for 15 min with an ultrasonic probe (Labsonic U, Braun, Germany) and kept overnight at room temperature. Additional methanol, dichloromethane and phosphate buffer (35.4 mL, 61 mL and 57 mL) were added, followed again by 5 min of ultrasonic treatment. The samples were centrifuged at 5860 g for 15 min to separate the phases. The dichloromethane phase was filtered through dry sodium sulphate and a hydrophobic filter. A reextraction was performed by addition of 25 ml of dichloromethane, centrifugation and filtration. This total lipid fraction was reduced in volume using a rotary evaporator and further fractionated using column chromatography (B&J Inert SPE, Silica, Burdick&Jackson, USA). The column was conditioned by overnight heating at 100°C and, after cooling to room temperature, with 10 mL of dichloromethane. The lipids were fractionated by sequential elution with dichloromethane, acetone and methanol which resulted in three fractions of different polarity: neutral-, glyco- (GL) and phospholipids (PL). The eluates were collected and dried under nitrogen. The GL- and PL-fractions were separately dissolved in 1 mL dichloromethane-methanol (1:1, v/v) and treated to a mild alkaline hydrolysis (1 M potassium hydroxide/methanol, 0.5 ml/2 ml, v/v, 40°C, overnight). The impurities were separated from the fatty acids by adding 2 ml hexane. After removal of the hexane phase, dichloromethane (1 ml), buffer (1 ml) and 6 M hydrochloric acid (180 µl) were added to the aqueous phase. The organic phase was separated, dried and the free fatty acids were methylated as described previously[28]. 1 mL n-octane including internal standard (n-hexadecane and n-tetracosane, 10 and 12 ng/mL respectively) was added to the dried fatty acid methyl esters, which were analyzed by gas chromatography and mass spectrometry.

Capillary gas chromatographic analyses were performed on a Hewlett Packard 5890 Series II gas chromatograph equipped with a HP Ultra 2 capillary column (50 m by 0.2 mm; film thickness 0.11 mm) and FID. Hydrogen served as the carrier gas. Injector temperature was set to 250°C and detector temperature was 300°C. The oven program was: 90°C for 2 min, 90°C to 280°C at 4°C/min followed by an isothermal period of 10 min. Polar lipid fatty acid methyl esters were identified by comparison with standards or GC-MS analysis.

Mass spectrometry. The GC-MS analyses were performed with a similar gas chromatograph to that described above for the analysis of the polar lipid fatty acids (same column and conditions but helium as carrier gas) connected to a HP 5989A quadropole mass spectrometer. The electron impact ion source was maintained at 200°C, while the quadropole temperature was 100°C. The electron energy was 70 eV.

Thin Layer Chromatography was performed on 20 x 20 cm plates coated with 1 mm silica gel 60 with a fluorescence indicator. The solvent system was ethyl acetate/methanol/formic acid 70/25/5 (v/v/v).

Fast Atom Bombardment (FAB) Mass Spectrometry. FAB-MS in the positive and negative mode was performed on the first of two mass spectrometers of a tandem high-resolution instrument in a $E_1B_1E_2B_2$ configuration (JMS-HX/HX110A, JEOL Tokyo) at 10 kV accelerating voltage with the resolution set to 1:1000. The JEOL FAB gun was operated at 6 kV with xenon. 3-Nitrobenzyl alcohol was used as matrix in the positive mode and a mixture of triethanolamine and tetramethylurea (Japanese matrix) in the negative mode.

Tandem Mass Spectrometry. Positive and negative daughter ion spectra were recorded using all four sectors of the tandem mass spectrometer. High energy collision-induced dissociation (CID) took place in the third field free region. Helium served as the collision gas at a pressure sufficient to reduce the precursor ion signal to 30% of the original value. The collision cell was operated at ground potential in the positive and negative modes. The resolution of MS2 was set to 1:1000. FAB-CID spectra (linked scans of MS2 at constant B/E ratio) were recorded with 300 Hz filtering on a JEOL DA 7000 data system.

NMR. All spectra were recorded at 300 K on a Bruker AM 600 NMR spectrometer locked to the major deuterium resonance of the solvent, CD_3OD. The value of the delay to optimise one-bond correlations in the HMQC spectrum and supress them in the HMBC spectrum was 3.45 ms and the evolution delay for long-range couplings in the latter was set to 70 ms. All spectra were recorded using the standard Bruker software package. All chemical shifts are given in ppm relative to TMS and couplings in Hz.

Other data. Infrared spectra were measured on potassium bromide using the diffuse reflected infrared Fourier transform (DRIFT) mode. Optical rotation was measured in methanol at different wavelengths.

1-[α-Mannopyranosyl-(1α-3)-(6-O-(12-methyl-tetradecanoyl)-α-mannopyranosyl)]-3-O-(14-methyl-hexadecanoyl)-glycerol. R_f 0.26 in ethyl acetate/methanol/formic acid 70/25/5 (v/v/v). IR (KBr): 3350 (br), 2920, 2715, 1735, 1600, 1460, 1365, 1250, 1070, 980, 770 cm^{-1}.

$$[\alpha]^{23°C} = \frac{\begin{array}{ccc} 589\ nm & 579\ nm & 546\ nm \\ +16.2° & +18.3° & +20.7° \end{array}}{} \quad (c=0.53, MeOH)$$

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