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Penta- and disaccharide lipid formation by *Nocardia corynebacteroides* grown on *n*-alkanes*

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Summary. *Nocardia corynebacteroides* SM1 synthesized di- and pentasaccharide lipids when grown on *n*-alkanes, especially under nitrogen limitation. Optimum conditions for their formation were pH 6.4–6.8, $NaNO_3$ as nitrogen source and yeast extract supplementation of the nutrient. A study of the time course of whole glycolipid production was carried out in a 20-l bioreactor. After extraction of the culture broth with organic solvents three main components could be isolated. Both ^1H and ^{13}C nuclear magnetic resonance spectroscopic and elemental analysis studies led to the identification of one novel pentasaccharide lipid and two trehalose corynomycolates. The oligosaccharide lipid showed significant surface and interfacial active properties.

Introduction

It is known that some microorganisms are able to synthesize surface active agents, in general during growth on hydrophobic substrates (Haferburg et al. 1986; Kosaric et al. 1987; Wagner 1987; Wagner and Lang 1988). These biosurfactants, with both hydrophilic and lipophilic structural moieties, seem to facilitate the uptake of hydrocarbons into the cells. Various surfactants can also be detected during growth on water-soluble substrates such as glycerol. Such compounds are able to reduce the surface tension between water and hydrocarbon phases.

In recent years various types of biosurfactants have been isolated and characterized. Besides the usual phospholipids, fatty acids and triglycerides the most commonly isolated microbial surfactants are the amino acid lipids, lipopeptides, lipoproteins, glycolipids and lipophilic polysaccharides. For the carbohydrate-containing lipids it is evident that either mono-, di- or polysaccharides are the hydrophilic backbones. Rhamnose-, sophorose-, cellobiose-, trehalose- and mannosylerythritollipids (Syldatk et al. 1985; Asmer et al. 1988; Frautz et al. 1986; Rapp et al. 1979; Kretschmer et al. 1982; Ristau and Wagner 1983; Kawashima et al. 1983) are examples of low molecular weight compounds, while Emulsan and Liposan are examples of high molecular weight lipophilic polysaccharides (Gutnick et al. 1981; Cirigliano and Carman 1984).

Oligosaccharide lipids are rare in the literature where only the acidic, dipyruvylated pentasaccharide lipid of *Mycobacterium smegmatis* ATCC 356 grown on glycerol (Saadat and Ballou 1983) has been reported. This oligosaccharide consists of glucose units and the hydrophobic moiety contains a mixture of long-chain fatty acids with 14–22 carbon atoms.

Here we present an investigation of the formation, isolation and characterization of another pentasaccharide lipid and of disaccharide lipids from *Nocardia corynebacteroides*, which was recently isolated from soil.

Materials and methods

Microorganism. Nocardia corynebacteroides SM1 was isolated from an oil-containing soil sample and identified by R. M. Kroppenstedt, Institut für Mikrobiologie, Technische Universität, Darmstadt, FRG.

* This paper is dedicated to Prof. Dr. Fritz Wagner, Technical University of Braunschweig, on the occasion of his 60th birthday

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Growth conditions. The bacterial strain was maintained on agar slants (Standard I-nutrient broth; Merck, Darmstadt, FRG) at 4° C and transferred at 2-month intervals. After inoculation the slants were incubated for 3 days at 27° C.

The basic medium used throughout these studies consisted of (g/l): $NaNO_3$, 2.0; KH_2PO_4, 1.0; $Na_2HPO_4 \cdot 2H_2O$, 2.5; $FeCl_3 \cdot 6H_2O$, 0.13; $MgSO_4 \cdot 7H_2O$, 0.75; $CaCl_2 \cdot 2H_2O$, 0.75; $MnSO_4 \cdot H_2O$, 0.2; $ZnSO_4 \cdot 7H_2O$, 0.02; yeast extract, 3.0; Mihagol S, 20.0. Composition of Mihagol S: 6.3% n-C_{10}, 76% n-C_{14}, 11.3% n-C_{15}, 6.3% n-C_{16}. Initial pH 6.6. Incubation temperature: 22° C.

Medium (100 ml in 500-ml erlenmeyer shake flasks) was inoculated with one platinum loop of the strain and incubated for 48 h at 100 rpm (Shaker KF4, Infors, München, FRG). Then 15 ml was added to 500 ml of the same medium in a 2-l shake flask. This cultivation was performed under the same conditions for 24 h.

For batch cultivation a bioreactor (type b20, Giovanola Frères, Monthey, Switzerland) equipped with an intensor system was used. Physiological activity was followed by the use of a pH electrode, a pO_2 electrode, and oxygen and carbon dioxide gas analysers (Unor and Oxygor, Fa. H. Maihak, Hamburg, FRG).

The conditions in the bioreactor were 1500 rpm; aeration rate, 0.6 (v/v/m); inoculation of 18 l fresh medium by 2 l culture broth; total incubation time, 80 h.

Analytical methods. Biomass was determined by centrifugation at 9600 *g* for 20 min after mixing 10 ml samples of the culture broth with 10 ml ethanol/*n*-butanol/chloroform (10:10:1, v/v/v) to remove residual *n*-alkanes. After washing with 10 ml water, the pellet was dried at 105° C (24 h) and weighed.

The ammonium-ion concentration in the supernatant was determined by the method described previously (Facwett and Scott 1960). The nitrate-ion concentration in cell-free culture liquid was followed using an enzymatic kit from Boehringer Mannheim (Mannheim, FRG). *n*-Alkane concentration was measured by the method described previously (Suzuki et al. 1969).

The glycolipid content was determined by the anthrone method, modified as described (Kretschmer et al. 1982) or by thin-layer chromatography (TLC; stationary phase: Chromarods SII) coupled with a flame ionisation detector using an Iatroscan TH10 (SES, Niederolm, FRG). Before measurement 10 ml of the whole microbial broth was extracted with CH_2Cl_2/CH_3OH (2/1, v/v) or ethyl acetate for isolation of the glycolipid. The TLC was conducted on TLC plates (no. 5554, Merck) which were developed horizontally using the solvent system $CHCl_3/CH_3OH/H_2O$ (60/30/2, v/v/v). Detecting reagents: anisaldehyde/sulphuric acid/acetic acid (1/2/100, v/v/v), 150° C; green spots in the case of glycolipids.

Surface tension was measured using the ring method in a Lauda Tensiomat (Fa. Lauda-Wobser, Königshofen, FRG) after previous emulsification of the glycolipid by ultrasonic treatment in water. Interfacial tension was measured in a similar way against *n*-hexadecane.

Purification and identification of the glycolipids. For the isolation of the glycolipids 20 l of the whole broth were extracted twice with 20 l ethyl acetate. After evaporation of the solvent to dryness the crude products were initially separated using medium pressure liquid chromatography (MPLC): Chromatography Pump 681 and Column B685, 460 × 49 mm (Büchi, Eislingen, FRG); stationary phase, Kieselgel 60, 70-230 mesh ASTM (Merck); loading, 5 g crude products; developing system, $CHCl_3/CH_3OH$ mixtures (100/0, v/v to 70/30, v/v; 5% steps, 800 ml per step). After this, the individual glycolipid fractions were purified by thick layer chromatography on silica gel plates (no. 7734, Merck); developing system 1, $CHCl_3/CH_3OH/H_2O$ (65/15/2, v/v/v); developing system 2, $CHCl_3/CH_3OH$ (60/30, v/v). Elution of the glycolipids was performed using $CHCl_3/CH_3OH$ (2/1, v/v).

The pure glycolipids were analysed by the following chemical and spectroscopic methods. Alkaline hydrolysis of the glycolipids for removal of fatty acids was carried out by refluxing with 1 *N* ethanolic sodium hydroxide solution. Acidic hydrolysis of the oligosaccharide (after alkaline hydroylsis) was performed by stirring in 0.5 *N* H_2SO_4 at 70° C for 4 h under a nitrogen atmosphere. All 1D and 2D COSY-90 ^1H (400.1 MHz) nuclear magnetic resonance (NMR) spectra were recorded at ambient temperature on a Bruker WM 400 NMR spectrometer locked to the major deuterium resonance of the solvent. The ^1H broad-band decoupled- and DEPT ^{13}C (100.6 MHz) NMR spectra were recorded on the same instrument as above. The lipid-free pentasaccharide (about 20mg) was dissolved in 0.6 ml D_2O and the glycolipid (about 25 mg) was dissolved in 0.6 ml $CDCl_3/CD_3OD$ (7/3, v/v).

Results

Shake flask experiments

Shake flasks were used to test the effects of various carbon and nitrogen sources, temperature and pH on the growth and formation of glycolipids by *N. corynebacteroides*. Table 1 contains data for *N. corynebacteroides* cultured in the presence of either water-soluble carbohydrates or wa-

Table 1. Influence of various carbon sources on the glycolipid production of growing cells of *Nocardia corynebacteroides* after 144 h

C-Source (2%)	Glycolipid (g/l)	Specific glycolipid formation (g/g biomass)
n-Decane	0.0	0.0
n-Undecane	0.4	0.15
n-Dodecane	0.8	0.30
n-Tetradecane	2.9	0.95
n-Pentadecane	2.8	1.00
n-Hexadecane	2.7	1.20
n-Octadecane	0.2	0.10
Mihagol L[a]	0.0	0.0
Mihagol M[b]	0.9	0.25
Mihagol S	2.9	0.95
Glucose	0.2	0.05
Fructose	0.2	0.10
Sucrose	0.1	0.09
Ethanol	0.0	0.00

Conditions: shake flasks, mineral salts medium with 3.0 g/l yeast extract; pH 6.6; temp. = 22° C

[a] Composition of Mihagol L: 86.1% n-C_{10}, 11.5% n-C_{11}, 2.4% n-C_{12}

[b] Composition of Mihagol M: 5.2% n-C_{10}, 33.1% n-C_{11}, 42.0% n-C_{12}, 19.7% n-C_{13}

Table 2. Influence of different nitrogen sources on the glycolipid formation of growing cells of *N. corynebacteroides* after 144 h

N-Source (0.2%)	Glycolipid (g/l)	Specific glycolipid formation (g/g biomass)
$(NH_4)_2SO_4$	1.4	1.20
$NaNO_3$	2.9	0.95
NH_4Cl	1.6	1.40
NH_4NO_3	1.7	1.10
$(NH_2)_2CO$	0.0	0.00
Glycine	0.2	0.20
Glutamic acid	0.15	0.15

Conditions: shake flaks; mineral salts medium with 3.0 g/l yeast extract; 2% Mihagol S; pH 6.6; temp. = 22 °C

ter-insoluble *n*-alkanes as sources of carbon and energy for growth and surfactant production. As can be seen the glycolipid production was absent using ethanol, *n*-decane and Mihagol L, because of the lack of growth. Among the other substrates *n*-C_{14} to *n*-C_{16} alkanes and Mihagol S led to the highest values with regard to volumetric and specific surfactant production. As the yields were similar to the technical grade $C_{14,15}$-*n*-alkane mixture, Mihagol S was used throughout the following studies.

The results of the influence of various nitrogen sources on glycolipid formation are presented in Table 2. As there were different changes in pH during the shake-flask cultivation caused by different counter ions and non-continuous pH adjustment, the yields should be interpreted with caution. Nevertheless urea, glutamic acid and glycine with zero or low values seemed to be unsuitable for glycolipid production whereas the inorganic nitrogen sources led to distinctly higher yields. A mineral salt medium containing $NaNO_3$ led to the highest glycolipid production with 2.9 g/l; a medium supplemented with NH_4Cl resulted in the highest specific surfactant production (1.4 g/l).

Studies on the influence of yeast extract concentration, pH and temperature are shown in Fig. 1. Figure 1a shows that yeast extract had a positive effect on the glycolipid production from *N. corynebacteroides*. The optimum concentration was found to be 3 g/l which was about 30% higher than the control medium without yeast extract.

After testing initial pH values in the range of 4 to 9 we observed that pH 6–7.5 allows optimum specific glycolipid production with Mihagol S as carbon source (Fig. 1b), and the best results were

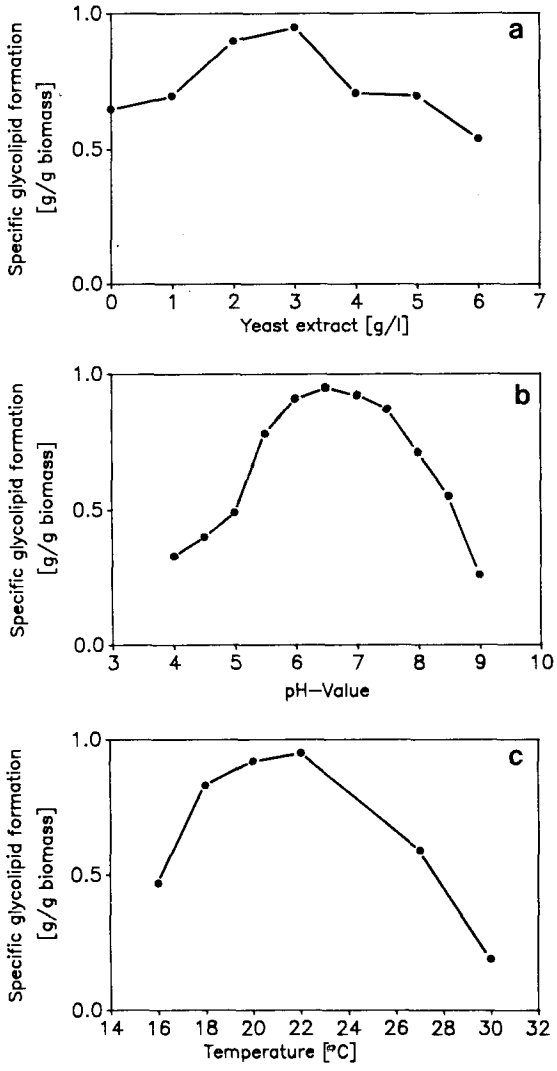


Fig. 1a–c. Influence of yeast extract, pH and temperature on glycolipid formation by *Nocardia corynebacteroides* after 144 h. All cultures were grown in shake flasks in mineral salts medium with 20 g/l Mihagol S. **a** pH 6.6, temp. = 22° C. **b** Temp. = 22° C, 3.0 g/l yeast extract. **c** pH 6.6, 3.0 g/l yeast extract

obtained at 22° C after 144 h cultivation (Fig. 1c).

In conclusion the results of shake-flask experiments on glycolipid production by *N. corynebacteroides* favoured the following cultivation conditions: supplementation of the mineral salt medium with 0.2% $NaNO_3$ and 0.3% yeast extract; pH 6.6; temp. = 22° C; Mihagol S as carbon source.

Bioreactor cultivation

Taking into account the above results a batch cultivation with automatic pH adjustment was per-

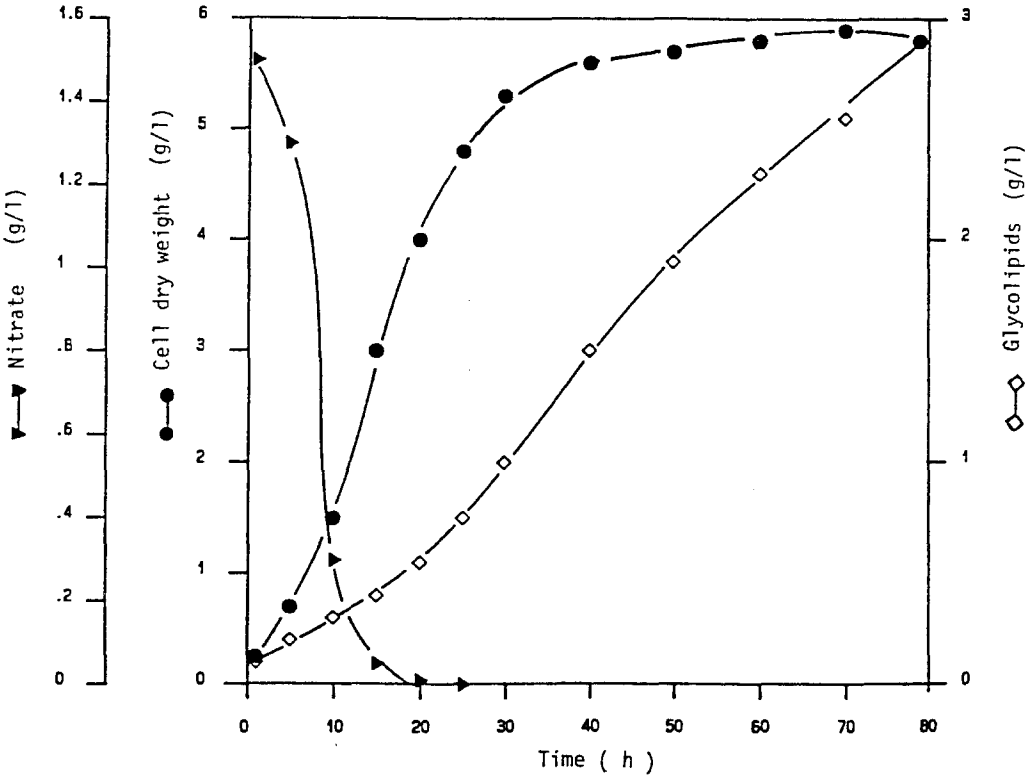


Fig. 2. Production of glycolipids with growing cells of *N. corynebacteroides* under *N*-limitation in a 20-l bioreactor (intensor system). Conditions: mineral salts medium including 2 g/l $NaNO_3$ and 3 g/l yeast extract; 100 g/l Mihagol S; pH 6.6; temp. = 22° C

formed using a 20-l bioreactor (intensor system). The results for growth and product formation are shown in Fig. 2. After nitrogen exhaustion the stationary phase of growth was reached after about 25 h. At this point the initial yield of whole glycolipids increased greatly until 75 h, resulting in 2.9 g/l after consumption of 52 g/l Mihagol S. The yield was 0.055 g glycolipid/g Mihagol S and the corresponding value for biomass formation was 0.48 g glycolipid/g biomass.

The qualitative composition of the crude organic extract was the same during the whole cultivation; only quantitative shifts were observed with increasing incubation time. Degradation of the cell-associated biosurfactants could not be detected throughout the cultivation. In the absence of the *n*-alkane substrate, but in the presence of all other nutrients, the glycolipids were not consumed by *N. corynebacteroides*.

Analysis of the crude organic extract

The qualitative analysis of the crude organic extract by TLC showed three glycolipids at R_f values of 0.78 (glycolipid TL-1), 0.48 (glycolipid TL-2) and 0.36 (glycolipid PL). The percentage content of the three components was estimated to be 2%, 6%, and 92%, respectively.

For both measurements of surface and interfacial tension and for structure elucidation studies the glycolipids of the crude extract were purified by MPLC and thick layer chromatography on silica gel.

Physico-chemical and structural characterization of pure glycolipids

The measurement of the physico-chemical properties showed that 4 mg/l of both TL-1 and TL-2 glycolipids reduced the surface tension of water from 72 mN/m to 36 and 32 mN/m, respectively, and the interfacial tension against *n*-C_{16} alkane from 43 mN/m to 17 and 14 mN/m, respectively. Employing the glycolipid PL, the surface and interfacial tensions were lowered to 26 mN/m and < 1 mN/m, respectively, at a concentration of 30 mg/l, showing that this compound may be a better surfactant than the other two.

The structures of the three glycolipids were elucidated by ^1H, ^{13}C NMR spectroscopy, ele-

mental analysis and by chemical methods. The two glycolipids, TL-1 and TL-2, were shown to be trehalose-6,6′-dicorynomycolates and trehalose-6-monocorynomycolate, which have been described previously (Rapp et al. 1979; Kretschmer et al. 1982).

The third glycolipid, PL, was identified as an oligosaccharide substituted with seven fatty acids and succinate. The structure of the sugar moiety was determined by investigation of the lipid-free carbohydrate produced by alkaline hydrolysis of PL. Inspection of the 2D ^{1}H COSY spectrum allowed the identification of five complete hexose spin systems, which were compatible with glucose units. This was confirmed by the results of a complete hydrolysis of the pentasaccharide as glucose was the only monosaccharide detected. The chemical shifts of all protons were determined from the COSY spectrum and are reported in Table 3, together with the magnitude of the coupling constants.

Shifts of H-6A and H-6B to a low field of ca. 4 ppm are characteristic of substitution at C-6, while ring substitution causes a low field shift of the adjacent ring proton. Hence the chemical shifts of the protons of two β-glucose units correspond to terminal moieties bound through C-1

Table 3. The ^{1}H data of the lipid-free pentasaccharide in D_2O

Chemical shifts (ppm)[a]	Sugar moieties				
H-1	5.22	5.20	4.70	4.54	4.44
H-2	3.84	3.67	3.38	3.32	3.55
H-3	4.08	3.88	~3.55	3.51	3.65
H-4	3.56	3.59	~3.40	3.67	3.92
H-5	3.85	3.96	3.46	3.87	3.67
H-6A	3.89–3.66	4.18	3.91	3.82–3.66	4.22
H-6B	3.89–3.66	3.89	3.73	3.82–3.66	3.88
Couplings (Hz)[b]					
(1–2)	3.8[c]	3.8[c]	7.9[d]	7.9[d]	7.8[d]
(2–3)	9.2	9.6	—	9.4	L
(3–4)	9.2	L	—	9.3	—
(4–5)	L	L	—	L	—
(5–6A)	—	~1.1	S	—	1.6
(5–6B)	—	M	M	—	M
(6A–6B)	—	11.5	L	—	11.6

[a] Chemical shifts were taken directly from the 2D ^{1}H COSY spectrum

[b] Coupling constants were determined from the 1D ^{1}H spectrum except in those cases where a letter is shown, when the magnitude of the coupling was assessed from the dimensions of the cross peaks in the 2D ^{1}H COSY spectrum: L = large coupling (>8 Hz); M = medium coupling (3–8 Hz); S = small coupling (<3 Hz)

[c] Characteristic coupling for α-glucopyranosyl moieties

[d] Characteristic coupling for β-glucopyranosyl moieties

Glc(1ß-3)Glc(1α-1α)Glc(6-1ß)Glc(6-1ß)Glc

Fig. 3. Structure of the lipid-free pentasaccharide produced by *N. corynebacteroides*

while the third β-glucose unit is substituted at C-6. One of the two α-glucose units possesses substitution at C-6, while the second has substitution at C-3 and a free CH_2OH group. The inter-ring linkage positions are shown by single underlining in Table 3. Comparison of the literature data for the ^{1}Hα (De Bruyn et al. 1975) and the ^{13}C chemical shifts of C-1α (Bock and Thøgersen 1982) in a comprehensive set of disaccharides in D_2O with those of the present work (^{1}H shifts: see Table 3, ^{13}C shifts: 93.81, C-1α $\times 2$) indicate that only an α,α-trehalose moiety is possible for the two α-glucose systems.

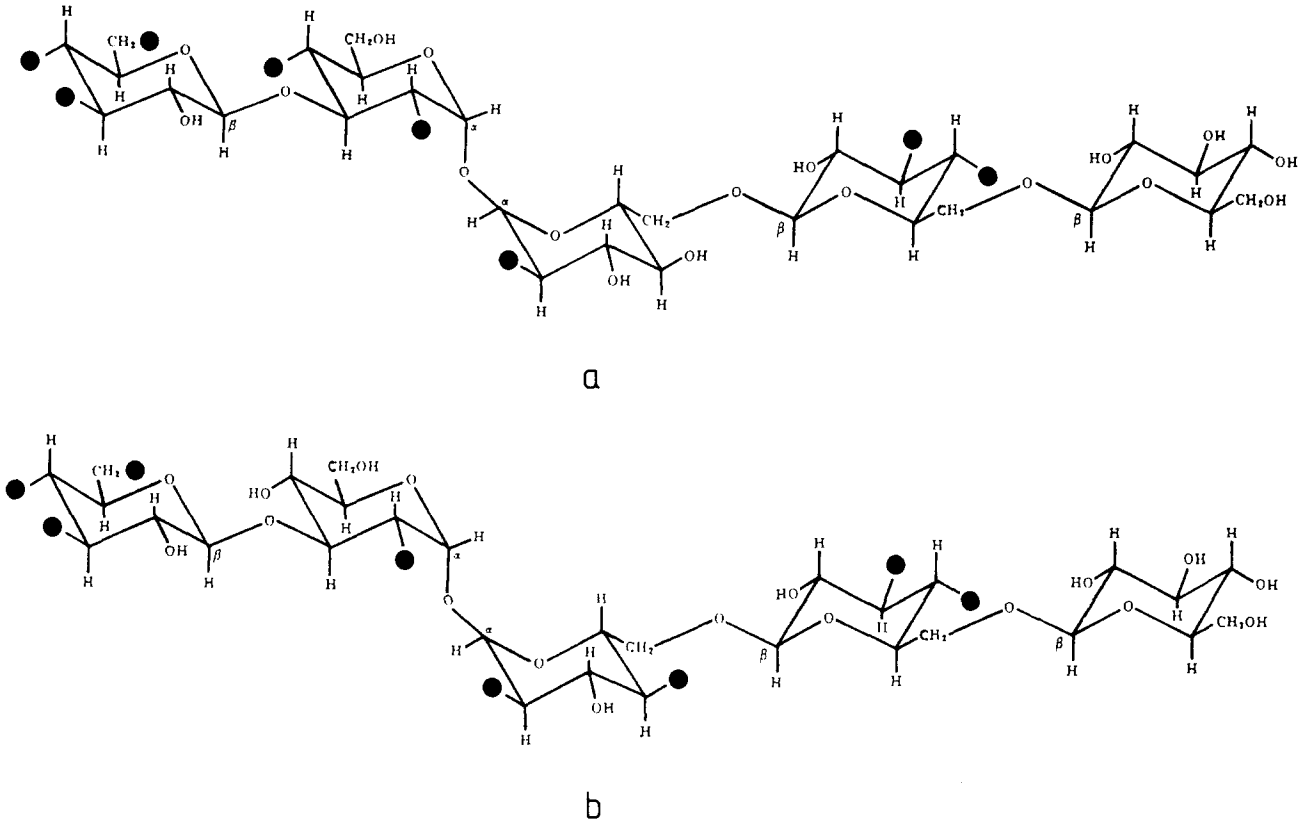
The presence of trehalose was confirmed experimentally as it was detected chromatographically after partial hydrolysis. Only the structure shown in Fig. 3 is compatible with all the NMR data. Both ^{1}H and ^{13}C NMR spectra of the native pentasaccharide lipid could only be obtained in a mixed solvent system of $CDCl_3/CD_3OD$ (7/3, v/v) where both the sugar and aliphatic side-chains gave sharp and well-resolved signals. The five sugar units were identified again in the 2D ^{1}H COSY spectrum. The chemical shift and coupling constant data are assembled in Table 4.

The position of the inter-ring linkages are more difficult to assess in the pentasaccharide lipid as the shifts are not as characteristic in the

Table 4. The ^{1}H data for the sugar moieties of the pentasaccharide lipid in $CDCl_3/CD_3OD$ (7/3, v/v)

Chemical shifts (ppm)	Sugar moieties				
H-1	5.34	5.32	4.58	4.36	4.30
H-2	4.82	4.87	3.53	3.73	3.29
H-3	4.07	4.00	5.13	4.90	3.47–3.23
H-4	4.94	3.52	4.91	5.37	3.43
H-5	3.87	3.73	3.91	3.91	3.26
H-6A	~ 3.56	3.80–3.65	4.04[a]	4.12[a]	3.81
H-6B	~ 3.56	3.80–3.65	3.59	3.91	3.77
Couplings (Hz)					
(1–2)	3.8	3.7	7.8	7.8	7.6
(2–3)	9.8	9.6	9.5	L	—
(3–4)	9.5	~9	9.5	—	L
(4–5)	~10	—	L	—	L
(5–6A)	—	—	S	—	—
(5–6B)	—	—	M	—	—
(6A–6B)	—	—	L	—	—

[a] One of the C-6 positions in these two β-glucopyranosyl systems is acylated, while the other is an inter-ring linkage



a

b

Fig. 4. The two possible molecular structures (**a**, **b**) of the native pentasaccharide lipid from *N. corynebacteroides:* ● = R = CH_3COO (2×), CH_3CH_2COO and $CH_3CH_2CH_2COO$ (3× in total; each of these residues shows three signals in the ^{13}C spectrum, with each signal being about half the intensity of each signal of the acetyl substituents), $CH_3(CH_2)_nCOO$ $n=6$ (2×), $HO_2CCH_2CH_2COO$ (1×)

mixed solvent and ring acylation causes significant effects. The acylation positions are readily found from characteristic low field shifts, shown underlined in Table 4. These data are compatible with two possible acylation patterns for the pentasaccharide lipid (Fig. 4) in which the only difference between the two is the position of one acylation site. Hence the latter is either at C-4 of glucose unit B (Fig. 4a) or C-4 of the middle glucose unit, C (Fig. 4b).

Information regarding the number and nature of the acyl substituents is available from inspection of the high field region, 2.7 to 0.8 ppm, of the 1D and 2D ^1H spectra and from the ^{13}C data. The structures of the major substituents follow from the 2D COSY spectrum and these are also shown in Fig. 4. The relative proportions of each system follows from integration of the 1D ^1H spectrum and from the intensities of the signals in the ^{13}C spectra. Integration of the 1D spectrum indicates that the major long-chain substituent is approximately eight carbon atoms in length. The only non-homologous acyl-substituent is succinate.

The elemental analysis (C: 52.4%, H: 7.1%, O: 40.5%) confirmed the structure proposal (C: 53.42%, H: 7.12%, O: 39.45%) with the molecular formula $C_{65}H_{104}O_{36}$.

Discussion

Nocardia corynebacteroides SM1 was found to produce an unusual pentasaccharide lipid with remarkable surface and interfacial active properties. Only one similar pentasaccharide lipid has been found and this was synthesized by *M. smegmatis* ATCC 356. This also contained two α-glucose and three β-glucose units but with other inter-ring linkage positions and the lipid appeared to be a mixture of long chain fatty acids with 14–22 carbon atoms (Saadat and Ballou 1983).

Besides the oligosaccharide lipid trehalose-mono- and dicorynomycolates were also detected in the case of *N. corynebacteroides*. From the literature it is known that *Rhodococcus erythropolis* is also able to form similar trehalose lipids and, in

addition, produces a third trehalose esterified in four positions with short chain fatty acids and succinate, when grown on *n*-alkanes under nitrogen limitation (Ristau and Wagner 1983). On comparison of the pentaglucose lipid of *N. corynebacteroides* and α,α-trehalose-2,2′-3,4-tetraester of *R. erythropolis*, it is conspicuous that one of the oligosaccharide lipid structures (Fig. 4a) contains an α,α-trehalose unit substituted at the same positions as the known trehalose tetraester. The similarity of the acyl groups in both lipids and the presence of succinate suggests that the trehalose lipid could be the biosynthetic precursor of the oligosaccharide lipid. This observation and the common occurrence of trehalose corynomycolates causes the supposition that *N. corynebacteroides* belongs to the genus *Rhodococcus*. However the pentasaccharide lipid has taxonomic significance.

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References

Asmer HJ, Lang S, Wagner F, Wray V (1988) Microbial production, structure elucidation and bioconversion of sophorose lipids. JAOCS 65:1460–1466

Bock K, Thøgersen H (1982) Nuclear magnetic resonance spectroscopy in the study of mono- and oligosaccharides. Annu Rep NMR Spectrosc 13:1–57

Cirigliano MC, Carman GM (1984) Isolation of a bioemulsifier from *Candida lipolytica*. Appl Environ Microbiol 48:747–750

De Bruyn A, Anteunis M, Verhegge G (1975) ^{1}H NMR study of the di-glucopyranoses in D_2O. Bull Soc Chim Belg 84:721–734

Facwett JK, Scott JE (1960) A rapid and precise method for the determination of urea. J Clin Path 13:156–159

Frautz B, Lang S, Wagner F (1986) Formation of cellobiose lipids by growing and resting cells of *Ustilago maydis*. Biotechnol Lett 8:757–762

Gutnick DL, Bayer EA, Rubinowitz C, Pines O, Shabtai Y, Goldmann S, Rosenberg E (1981) Emulsan production in *Acinebacter* RAG-1. Adv Biotechnol 3:455–459

Haferburg D, Hommel R, Claus R, Kleber HP (1986) Extracellular microbial lipids as biosurfactants. Adv Biochem Eng Biotechnol 33:53–93

Kawashima H, Nakahara T, Oogaki M, Tabuchi T (1983) Extracellular production of a mannosylerythritol lipid by a mutant of *Candida* sp. from *n*-alkanes and triacylglycerols. J Ferment Technol 61:143–149

Kosaric N, Cairns WL, Gray NCC (eds) Biosurfactants and biotechnology. In: Surfactant science series, vol 25, Dekker, New York

Kretschmer A, Bock H, Wagner F (1982) Chemical and physical characterization of interfacial-active lipids from *Rhodococcus erythropolis* grown on *n*-alkanes. Appl Environ Microbiol 44:864–870

Rapp P, Bock H, Wray V, Wagner F (1979) Formation, isolation and characterization of trehalose dimycolates from *Rhodococcus erythropolis* grown on *n*-alkanes. J Gen Microbiol 115:491–503

Ristau E, Wagner F (1983) Formation of novel anionic trehalose-tetraesters from *Rhodococcus erythropolis* under growth limiting conditions. Biotechnol Lett 5:95–100

Saadat S, Ballou CE (1983) Pyruvylated glycolipids from *Mycobacterium smegmatis*. J Biol Chem 258:1813–1818

Suzuki T, Tanaka K, Matsubara I, Kinoishita S (1969) Trehalose lipid and α-branched β-hydroxy fatty acid formed by bacteria grown on *n*-alkanes. Agric Biol Chem 33:1619–1627

Syldatk C, Lang S, Matulovic U, Wagner F (1985) Production of four interfacial active rhamnolipids from *n*-alkanes or glycerol by resting cells of *Pseudomonas species* DSM 2874. Z Naturforsch 40c:61–67

Wagner F (1987) Strategies for biosurfactant production. Fat Sci Technol 89:586–591

Wagner F, Lang S (1988) Nonionic and anionic biosurfactants: microbial production, structures and physico-chemical properties. Organized by ASPA (Syndicat National des Fabricants d'Agents de Surface et de Produits Auxiliaires Industriels). Président: L. Marcon. Proceedings of 2nd World Surfactants Congress, Paris, France, Paris, 24–27 may 1988, pp II 71–80

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