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Structural studies of the major glycolipid from *Saccharopolyspora* genus

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Abstract

A major glycolipid was isolated from the well characterized *Saccharopolyspora* species, *S. hirsuta, S. rectivirgula, S. erythraea* and one not completely identified strain (*Saccharopolyspora* sp.). On the basis of sugar and methylation analysis, specific enzymatic and chemical degradations of the carbohydrate moiety, its FAB mass spectrometry and NMR spectroscopy characterizations, the carbohydrate part was shown to be the glycerol linked dimannoside α-D-Man*p*-(1 → 3)-α-D-Man*p*-(1 → 1/3)Gro. The internal mannose residue is esterified at C-6 by one fatty acid residue, whereas another fatty acyl chain substitutes the primary methylene position of glycerol. The main fatty acyl residues are *anteiso*-branched heptadecanoic acid and the *iso*-branched fatty acids *iso*-17:0, *iso*-16:0, and *iso*-18:0, with the former species being predominant. The major glycolipid has potential value for taxonomic and diagnostic purposes, especially in the specific diagnosis of farmer's lung disease. © 1996 Elsevier Science Ltd.

Keywords: Glycolipid; Structure; *Saccharopolyspora*; Actinomycetes; Farmer's lung disease

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1. Introduction

The presence of a large number of saccharopolysporae spores in enclosed agricultural and industrial environments may cause significant health problems. The best known is hypersensitivity pneumonitis with the classical form 'farmer's lung' disease. It usually affects the middle and upper bronchi as well as lung parenchyma and represents an interstitial lymphocytic or granulomatous allergic reaction of pulmonary tissue. There has been some evidence that the sporactinomycetes can be involved in different forms of allergic alveolitis or similar diseases (e.g. mushroom worker's lung, humidified fever and the like) [1,2]. However, quantification of actinomycetes in humidified atmosphere and further clinical immunodiagnostic studies are required in order to confirm this information.

The former mono typing genus *Saccharopolyspora* comprises today several reclassified or new species of actinomycetes, found in many plant materials that have been stored or allowed to decay [3]. These organisms also produce various bioactive metabolites, and among them the antibiotic erythromycin.

The taxonomy of these microorganisms, as well as criteria used for the diagnosis of extrinsic allergic alveolitis and similar diseases, are complicated [1,4]. Currently, the members of *Saccharopolyspora* genus can be distinguished from other related actinomycetes by comparison of their polar lipid and menaquinone composition [3,5].

During the last decade, we have undertaken studies on the taxonomic value of glycolipids from actinomycetes and allied taxa [6–9]. Among the many groups of these microorganisms important in industry and medicine, the genus *Saccharopolyspora* was found to include a major glycolipid, absent in other related amycolate wall IV taxa and characteristic for these actinomycetes [9,10].

In this paper, we present the structure of the major glycolipid isolated from representatives of three most common species of *Saccharopolyspora (S. hirsuta, S. rectivirgula, S. erythraea)*, as well as from an incompletely identified strain, *Saccharopolyspora* sp. The potential value of this compound for taxonomic and diagnostic purposes is also presented.

2. Experimental

Organisms and growth conditions.—The following saccharopolysporal strains were used in these studies: *Saccharopolyspora hirsuta* ATCC 27875^T (previously designated K52, the type strain of the type species of genus *Saccharopolyspora*), *Saccharopolyspora erythraea* ATCC 11635^T (primarily labeled K600, producer of erythromycin) [10], *Saccharopolyspora rectivirgula* strains IMRU1258, LL-A-91 (formerly *Micropolyspora faeni*) [6] and *Saccharopolyspora* sp. LL-100-46 (formerly *Micropolyspora* sp.) [6], obtained from the Lechevaliers' Collection, Rutgers University, Waksman Institute of Microbiology, New Jersey, USA. The bacteria were cultivated on yeast extract-dextrose medium in submerged culture at 37 °C for 48 h as described before [6]. The cell mass was harvested during the stationary phase of the culture.

Extraction of lipids.—The wet cell mass of actinomycetes was extracted twice by stirring with 2:1 $CHCl_3$–MeOH at 30 °C for 12 h, and partially purified to remove non lipid material using a modification of the Bligh and Dyer procedure [11].

Separation and purification of the glycolipid fractions.—The crude lipid extract (200–250 mg) was fractionated on a column (1.8 × 45 cm) of activated silica gel (HI-Flosil 60–200 mesh) using $CHCl_3$ (400 mL), then acetone (600 mL) and finally MeOH (400 mL) as eluents. The eluates were monitored for glycolipids on Silica Gel H plates, using the solvent system 65:25:4 $CHCl_3$–MeOH–water. Further purification of the acetone eluates containing glycolipids, was performed by preparative TLC (30–40 mg of lipid per 20 × 20 cm Silica Gel F_{254} plate with 65:25:4 $CHCl_3$–MeOH–water as eluent). Glycolipid bands were located using UV light and orcinol [7]. The glycolipid fraction was eluted from the plate with 2:1 $CHCl_3$–MeOH and 1:1:0.2 $CHCl_3$–MeOH–water. Final purification of the fractions was achieved by repeated (up to 3 times) preparative TLC. The samples were checked for purity by two-dimensional TLC using two pairs of eluent systems: (a) 65:25:4 $CHCl_3$–MeOH–water in the first direction and 80:18:12:5 $CHCl_3$–AcOH–MeOH–water in the other; (b) 65:15:2 $CHCl_3$–MeOH–water in the first and then 65:10:20:10:3 $CHCl_3$–MeOH–acetone–AcOH–water in the second direction.

Analytical methods.—Total neutral sugars were determined with the phenol-sulfuric acid reagent [12]. Sugar analysis was carried out according to Sawardeker et al. [13]. Samples (0.4 mg) of each lipid fraction were hydrolyzed in 1 M HCl (0.5 mL) for 4 h at 100 °C. After reduction with $NaBH_4$ (20 mg, 16 h, 4 °C) and acetylation (1:1, Ac_2O–pyridine), the samples were analyzed by GLC–MS using a Hewlett-Packard 5971A gas chromatograph-mass spectrometer equipped with an HP-1 glass capillary column (0.2 mm × 12 m). A temperature gradient of 8 °C min^{-1} from 150 to 270 °C was applied. Analyses were also carried out on a Varian 2000 gas chromatograph fitted with a FID detector, and a glass OV-225 (2 m × 3 mm) column at 180 °C. Fatty acids were analyzed after methanolysis of the dried glycolipid (0.4 mg) with 1 M HCl in MeOH for 5 h at 80 °C. Samples, evaporated with a stream of N_2, were extracted with $CHCl_3$ and analyzed using GLC and GLC–MS under the conditions described above. HPLC analyses of glycolipids were performed on a Waters apparatus equipped with a M600E pump system and a M996 photodiode array detector, both controlled by a Millenium 2010 software. A Silica Gel Nucleosil 100-5 column (4 × 250 mm) was used with a gradient from 10% to 25% MeOH in $CHCl_3$.

De-O-acylation of the glycolipid.—For the analysis of the sugar moiety, glycolipid samples (2 mg) were subjected to alkaline hydrolysis with 5% NH_4OH (1 mL) for 3 days at room temperature. Neutralized samples (AcOH) were evaporated in a nitrogen stream and extracted with 1:1 $CHCl_3$–water. The water phase was lyophilized.

Digestion with α-mannosidase.—The oligosaccharide fractions from the de-*O*-acylated glycolipids were subjected to treatment with α-mannosidase (E.C. 3.2.1.24; Sigma) from *Canavalia ensiformis*. To the oligosaccharide (0.2 mg), dissolved in 0.2 M ammonium acetate buffer pH 4.9 (50 μL), the enzyme (3 μL) was added and the mixture was kept at 37 °C overnight. The presence of mannose and glycerol was checked by paper chromatography in 6:4:3 butanol–pyridine–water using periodate–benzidine detection.

Methylation analysis.—A sample of the oligosaccharide fraction (0.6 mg), obtained after de-*O*-acylation of the glycolipid, was methylated according to Hakomori [14] and purified with a Sep Pak C18 cartridge [15]. The product was hydrolyzed with HCOOH and H_2SO_4 (0.5 M, 16 h, 100 °C), reduced with $NaBD_4$ and peracetylated. Partially methylated alditol acetates were analyzed by GLC–MS as described above. The position of sugar *O*-acylation was established by methylation analysis carried out according to Prehm [16]. The glycolipid (0.5 mg) was solubilized in trimethyl phosphate (0.9 mL) and treated with 2,6-di-(*tert*-butyl)pyridine (0.15 mL) and methyl trifluoromethanesulfonate (0.15 mL) for 2 h at 50 °C in an ultrasonic bath. The product, purified on a Sep Pak C18 cartridge, was methylated again by the Hakomori method [14] using deuterated methyl iodide. The methylated product was hydrolyzed with 2 M trifluoroacetic acid at 120 °C for 2 h, reduced with $NaBD_4$, acetylated and analyzed by GLC–MS system as above described.

Determination of the absolute configuration of the sugar constituents.—The glycolipid (0.4 mg) was hydrolyzed with 1 M HCl for 4 h at 100 °C. The dried neutralized sample was dissolved in 0.2 M ammonium acetate buffer (pH 8.0) and treated with hexokinase (E.C. 2.7.1.1; Sigma) in the presence of ATP (2 mg), 0.02 M $MgCl_2$ and toluene, overnight at room temperature. Formation of mannose phosphate ester by hexokinase was checked by paper high voltage electrophoresis, followed by alkaline silver staining.

Mass spectrometry.—FAB mass spectra were recorded on a Fisons-VG type ZAB2-SEQ, double-focusing mass spectrometer, using a cesium ion gun (LSIMS). The native glycolipid was dissolved in 2:1 $CHCl_3$–MeOH and mixed in *m*-nitrobenzyl alcohol (NBA) as a matrix for FAB^+ or in triethanolamine (TEA) for FAB^-. The peracetylated glycolipid was dissolved in MeOH and mixed with a thioglycerol matrix. The B/E linked scans were scanned with argon as a collision gas. Peracetylation and perdeuteroacetylation of the glycolipid was carried out as described [17].

NMR spectroscopy.—NMR spectra were measured in $CDCl_3$ or in 6:4 $CDCl_3$–CD_3OD mixture at room temperature, using tetramethylsilane as a standard. Signal assignments in the ^1H- and ^{13}C-NMR spectra of glycolipids were performed using two-dimensional techniques, namely, homonuclear shift correlation (COSY), relayed COSY, phase-sensitive COSY and proton detected carbon-proton shift correlated (HMQC and HSQC) experiments. Typically, 200–400 free induction decays, each of 2 K size, were acquired for 2D NMR experiments. Additional confirmation of the assignments in crowded regions of ^{13}C NMR spectra was obtained from distortionless enhancement by polarization transfer (DEPT) spectra. Proton-detected NMR experiments were performed using Bruker AM360, AM500, and AMX500 spectrometers, and ^{13}C NMR spectra using Bruker AM-360 and MSL-300 spectrometers.

3. Results

Isolation and purification of the glycolipid components.—Crude lipids were obtained from four representatives of the best known species of saccharopolyspora *S. hirsuta* ATCC 27875^T, *S. erythraea* ATCC 11635, *S. rectivirgula* IMRU 1258 and LL-A-91

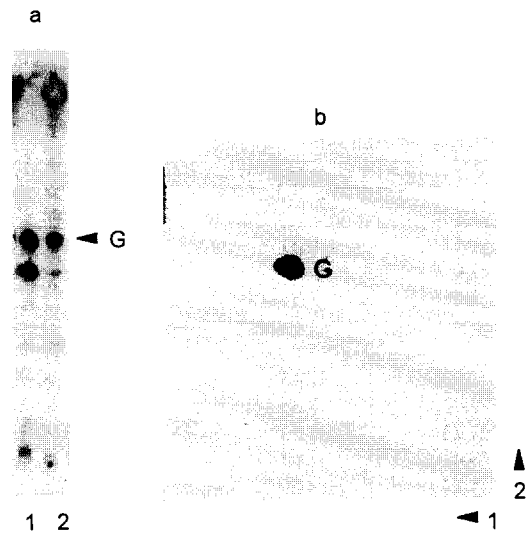


Fig. 1. (a) TLC of crude lipids from *Saccharopolyspora hirusta* ATCC 27875^T (1) and *Saccharopolyspora erythraea* ATCC 11635^T (2); in 65:25:4 $CHCl_3$–MeOH–water, detection with orcinol reagent. (b) Two dimensional TLC of the major glycolipid from ATCC 11635 after final purification; solvent system in the first direction as above, followed by 80:80:12:5 $CHCl_3$–AcOH–MeOH–water, detection with vanillin reagent. G, major glycolipid.

and one of the not completely identified strains *Saccharopolyspora* sp. LL-100-46. All total lipid extracts contained a major glycolipid component which was characterized by the same TLC-mobility, by positive reaction with orcinol but negative in staining for phosphorus and with ninhydrin reagents. From these crude lipids, the major glycolipid components (**1–5**) were isolated and purified by preparative TLC. The chromatograms are exemplified in Fig. 1. The purity was confirmed by two dimensional TLC in two pairs of solvents, and by HPLC. All studied compounds were eluted with the same retention time.

Glycolipid composition.—After hydrolysis (1 M HCl, 4 h, 100 °C) of the purified glycolipid, and its sugar analysis with paper and gas chromatography, Man and glycerol were identified in a 2:1 molar ratio, respectively. The results of the quantitative analyses of glycolipids **1–5**, including fatty acids composition, are given in Table 1.

Among the fatty acids, the most abundant was the *anteiso*-branched $C_{17:0}$ acid, amounting for 41–52% of the total. Other branched *i*-$C_{16:0}$, *i*-$C_{17:0}$, and some normal fatty acids, were also present in lower amounts. In glycolipids **1–5**, the amount of *ai*-$C_{17:0}$ fatty acid was very similar, but the relative amount of other fatty acids was strongly dependent on the bacterial strain. In glycolipid **5**, *ai*-$C_{17:0}$ seemed to be in equimolar ratio with *i*-$C_{16:0}$. In glycolipids **1–3**, the molar ratio of *i*-$C_{16:0}$, *i*-$C_{17:0}$ and *ai*-$C_{17:0}$ was 0.3:0.7:1, 0.4:0.6:1 and 0.4:0.3:1, respectively. In glycolipid **4**, the *anteiso*-$C_{17:0}$ fatty acid was again predominant, with, however, some very heterogeneous fatty acids remaining.

The enzymatic procedure used for the determination of the Man configuration revealed that the sugar present in all glycolipid hydrolysates was quantitatively phospho-

Table 1
Composition of glycolipids from *Saccharopolyspora hirsuta* (ATCC 27875^T), *Saccharopolyspora erythraea* (ATCC 11635^T), *Saccharopolyspora rectivirgula* (IMRU 1258, LL-A91) and *Saccharopolyspora* sp. (LL-100-46)

Characteristic		Glycolipid from strain:				
		ATCC 27875^T **1**	ATCC 11635^T **2**	IMRU 1258 **3**	LL-A91 **4**	LL-100-46 **5**
R_f value		0.51	0.51	0.51	0.51	0.51
Total sugar (%)		31.0	32.2	38.2	35.3	36.1
Mannose [a]		2.0	1.8	2.1	2.05	2.1
Glycerol [a]		1	1	1	1	1
Fatty acids: [b]	R_t [c]					
i 16:0	7.51	12.99	17.15	21.94	5.15	39.67
n 16:0	7.98	2.91	2.33	2.28	5.45	3.48
i 17:0	8.78	31.22	24.22	13.79	8.39	6.58
ai 17:0	8.89	49.44	48.97	52.12	49.73	40.97
n 17:0	9.22	1.23	4.09	1.25	5.29	1.32
i 18:0	9.75	0	0.74	0.73	12.11	2.20
n 18:0	10.42	2.21	2.50	7.89	13.88	5.88

[a] Molar ratio of Man is referred arbitrarily to a single glycerol residue.
[b] Abbreviations for the fatty acid methyl esters are explained by the following examples: *n* 16:0 for a normal, straight chain hexadecanoic acid, *i* 16:0 for *iso*-branched 14-methylpentadecanoic acid, *ai* 17:0 for *anteiso*-branched 14-methylhexadecanoic acid, etc.
[c] Retention time (R_t) in min, on HP-1 at 150–270 °C, 8 °C min^{-1}.

rylated by hexokinase. This means that Man has the D configuration, since the enzyme specifically phosphorylates the D enantiomers [18].

In order to isolate the sugar moiety, the glycolipid components were subjected to mild alkaline hydrolysis (5% NH_4OH, 20 °C). De-*O*-acylation was achieved within three days, and the reaction was monitored by the appearance of oligosaccharides and the disappearance of glycolipid spots on a TLC silica gel plate. When the oligosaccharide part of glycolipid **1** was treated with α-mannosidase, free Man and glycerol were obtained indicating that the Man residues had the α-anomeric configuration.

Table 2
Quasimolecular ions found in the FAB mass spectra from glycolipids of *S. hirsuta* (**1**) [a], *S. erythraea* (**2**), *S. rectivirgula* (**4**) and *Saccharopolyspora* sp. (**5**)

M	*m/z*	Relative abundance (%)			
	$[MNa]^+$ ($[M-H]^-$)	**1**	**2**	**4**	**5**
934	957 (933)	4.5 (4.0)	–	8	5
920	943 (919)	46.5 (48.0)	30	20	19
906	929 (905)	30.5 (31.0)	33	26	32
892	915 (891)	5.0 (13.5)	29	32	28
878	901 (877)	3.5 (3.5)	8	9	12
864	887	–	–	5	4

[a] Data for FAB^- quasimolecular ions in Glycolipid **1** are in brackets.

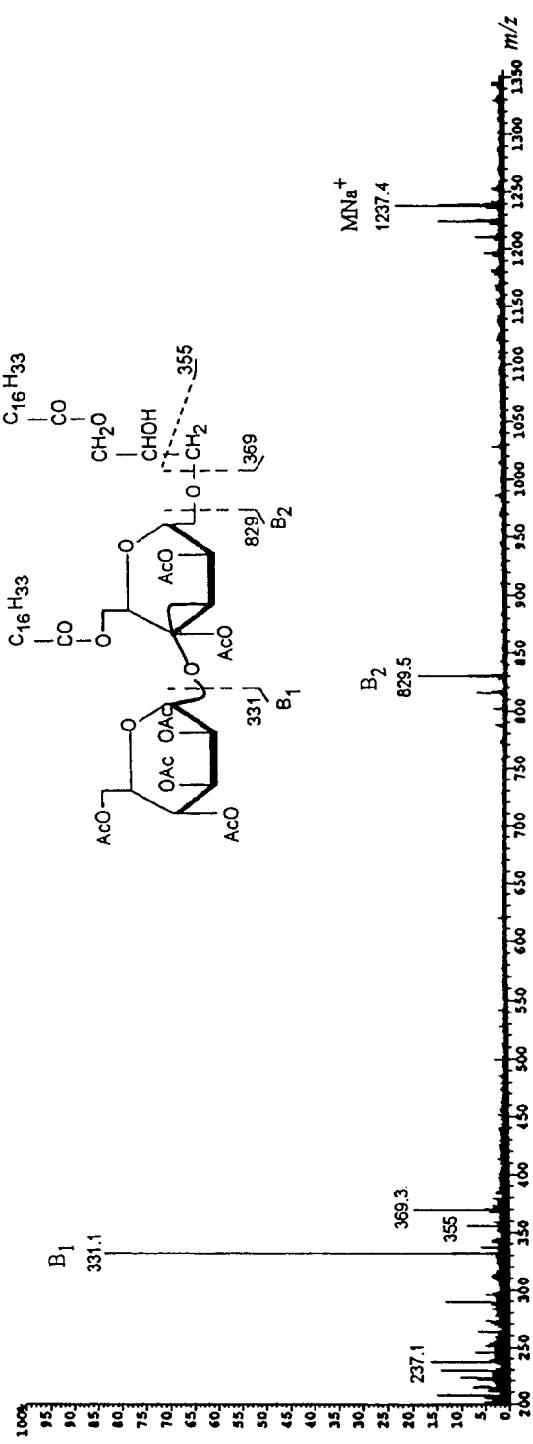


Fig. 2. Positive FAB mass spectrum and fragmentation pattern for the peracetylated major Glycolipid 1 from *Saccharopolyspora hirsuta* ATCC 27875^T.

Methylation analysis, performed on the oligosaccharides obtained from glycolipids **1** and **3**–**5**, resulted in two derivatives in equal proportions, namely, 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-mannitol and 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methyl-mannitol, in agreement with the presence of one terminal and one *O*-3-substituted Man residues. In non-alkaline conditions of glycolipid methylation by the method of Prehm [16], the fatty acyl substituent was not cleaved. Methylation of the product with deuterated methyl iodide resulted in a stoichiometric incorporation of a deuteromethyl group at C-6 of the internal Man residue, as proved by the presence of 1,3,5-tri-*O*-acetyl-6-*O*-deuteromethyl-2,4-di-*O*-methyl-mannitol in GLC–MS analysis.

Fast atom bombardment – mass spectrometry analysis (FABMS).—Positive as well as negative FAB mass spectra of native Glycolipid **1** revealed a mixture of molecules differing upon the length of the aliphatic chains. The fragments and their abundances are

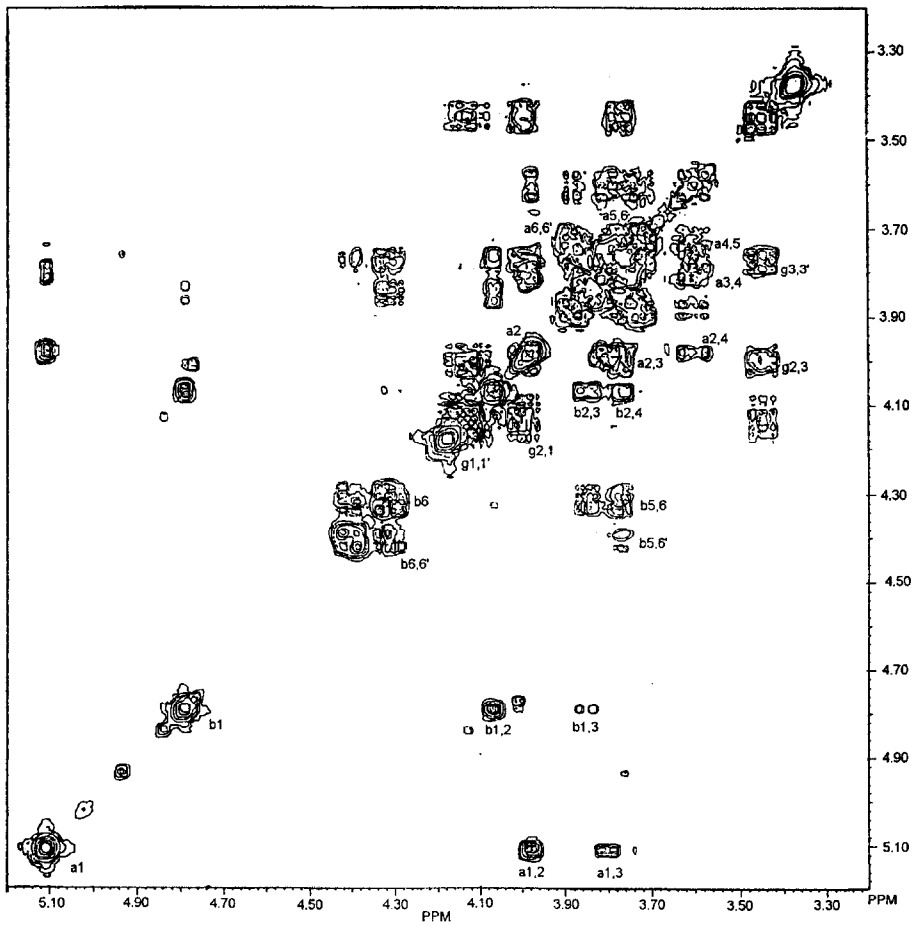


Fig. 3. ^{1}H COSY with relaxed coherence transfer (RCT) spectrum of the major Glycolipid **3** from *Saccharopolyspora rectivirgula* IMRU 1258; a, b, g refer to terminal mannose, internal mannose and glycerol residues, respectively.

tabulated in Table 2, and compared with glycolipids from other *Saccharopolyspora* differing in the length of the fatty ester chain. The masses in glycolipid **1** (from *S. hirsuta*) indicated a heterogeneity of the alkyl chain, with a main length of the chain in agreement with a $C_{17:0}$ ester for a molecular weight of 920. Shorter chains were present in 50% of the glycolipid molecule. The main molecular mass of glycolipid **1**, M = 920, is compatible with a structure of a dihexose condensed with glycerol and two C_{17} saturated aliphatic acids.

The sequence of this glycolipid has been determined with B_i ions on the FAB^+ mass spectrum of peracetylated molecules (Fig. 2), according to the nomenclature of Domon and Costello [19]. The B_1 ion (*m/z* 331) was found to be displaced by 12 mass units by deuteroacetylation, in agreement with the presence of 4 acetyl groups, indicating a non reducing terminal hexose without substituents. The B_2 ions (*m/z* 829, with 6 acetyl groups), pointed to an internal hexose residue with two acetyl groups, the third hydroxyl being esterified by a $C_{17:0}$ ester chain. NMR spectroscopy and methylation analysis allowed determination of the position of substitution as C-6. The fragments at *m/z* 369 (*m/z* 372 with CD_3: one acetylated hydroxyl group) and *m/z* 355 (*m/z* 358 with CD_3: one acetylated hydroxyl group) agreed with the presence of the monoalkyl ester of glycerol at the reducing end of the disaccharide molecule (Fig. 2).

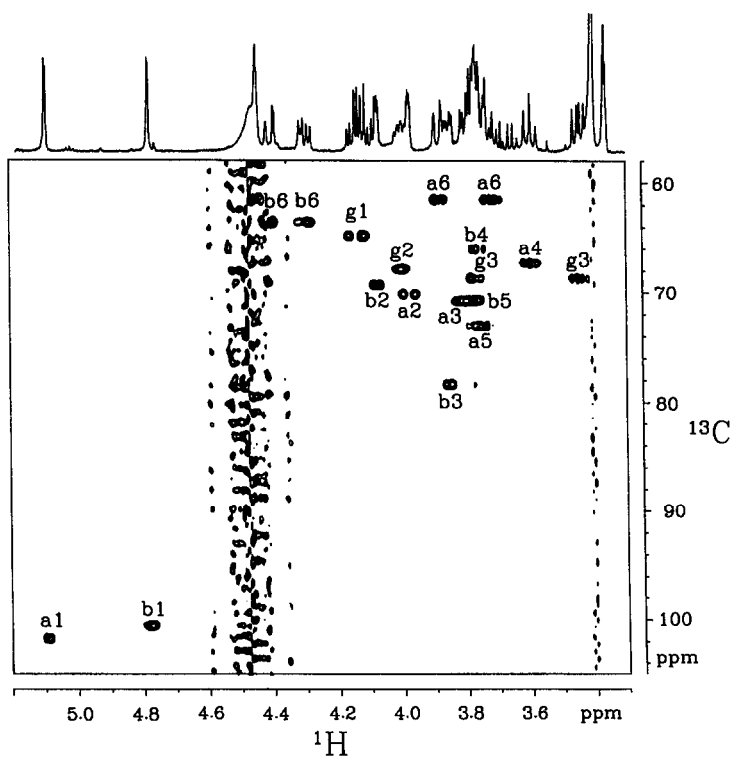


Fig. 4. Partial $^1H-^{13}C$ HSQC spectrum of the major Glycolipid **1** from *Saccharopolyspora hirsuta* ATCC 27875^T.

Table 3
^{1}H- and ^{13}C-NMR spectroscopy data for Glycolipid **3** from *Saccharopolyspora rectivirgula* [a]

	Chemical shift in residue (ppm)			
	α-D-Man*p*-(1 →	→ 3)-α-D-Man*p*-(1 →	Gro	Fatty acyl
Proton				
1	5.09 ($J_{1,2}$ 1.9)	4.78 ($J_{1,2}$ 1.8)		
2	3.98 ($J_{1,2}$ 1.8; $J_{2,3}$ 3.4)	4.07 ($J_{1,2}$ 1.8; $J_{2,3}$ 3.4)		
3	3.80 ($J_{2,3}$ 3.4; $J_{3,4}$ 9.5)	3.85 ($J_{2,3}$ 3.3; $J_{3,4}$ 9.3)		
4	3.59 ($J_{3,4}$ 9.3; $J_{4,5}$ 9.5)	3.75		
5	3.77	3.76		
6	3.88 ($J_{5,6}$ 1.8; $J_{6,6'}$ −11.4)	4.40 ($J_{5,6}$ 1.2; $J_{6,6'}$ −11.7)		
6′	3.72 ($J_{5,6'}$ 6.1; $J_{6,6'}$ −11.2)	4.31 ($J_{5,6'}$ 6.1; $J_{6,6'}$ −11.7)		
1			4.15 ($J_{1,2}$ 4.8; $J_{1,1'}$ 11.4)	
1′			4.13 ($J_{1',2}$ 5.8)	
2			4.00 ($J_{1',2}$ 5.8)	
3			3.45 ($J_{2,3}$ 6.7; $J_{3,3'}$ −10.4)	
3′			3.78 ($J_{2,3'}$ 4.4)	
CH_3				0.86
CH_2				1.27
				1.63 (3-CH_2)
				2.36 (2-CH_2)
Carbon				
1	102.43	101.23		
2	70.84	69.94		
3	71.37	79.08		
4	67.96	66.66		
5	73.67	71.48		
6	62.13	64.18		
1			65.41	
2			68.44	
3			69.24	
CH_3				11.46 (*ai*)
				19.25 (*ai*)
				22.67 (*i*)
CH_2				34.35 (C(2))
				25.14 (C(3))
				36.89 (*ai*)
				39.32 (*i*)
				27.34 (*ai*)
				27.66 (*i*)
				29.42–30.26
CH				28.21 (*i*)
				34.68 (*ai*)

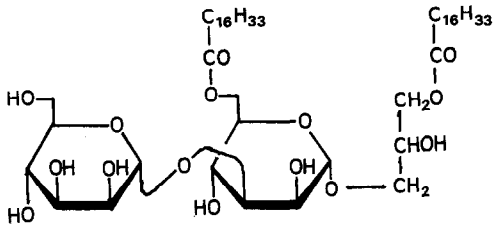
[a] Coupling constants (in Hz) are in parentheses.

These aliphatic chains have mainly a terminal methyl ethyl structure since the B/E linked scan of the $[MNa]^+$ (m/z 943) ion in the native compound showed fragmentation all along the alkyl chains, but cleavage of three carbon atoms was lacking, in agreement with a methyl ethyl chain end (structure *anteiso,* Table 2).

NMR spectroscopy.—The ^1H NMR spectra of glycolipids from *S. hirsuta* (**1**), *S. erythraea* (**2**), *S. rectivirgula* (**4**) and *Saccharopolyspora* sp. (**5**) were superimposable on the spectrum of glycolipid **3** from *S. rectivirgula.* Similarly, the chemical shifts of signals in ^{13}C NMR spectra were identical for all five glycolipids. However, some differences in intensity were observed in the upfield regions containing fatty acyl chain signals, in both ^1H and ^{13}C NMR spectra. This observation is in agreement with FABMS results and reflects different amounts of various fatty acids in compounds **1–5**. Assignment of proton signals was made using 2D-COSY and relayed-COSY for the representative glycolipid **3** (Fig. 3). ^{13}C NMR spectra were assigned using heteronuclear HMQC and HSQC experiments (Fig. 4). An additional DEPT experiment confirmed the assignments of the methylene groups of glycerol and hexoses as well as methine carbons of the branched fatty acyl chains. In ^{13}C NMR spectra of glycolipids **1–5,** two anomeric carbon signals were visible at 102.4 ppm and 101.2 ppm, as well as 13 well resolved resonances between 60 and 80 ppm for the remaining part of the two Man residues and for glycerol. In the high-field part of the spectrum (18–40 ppm), signals typical for fatty acyl chains were observed, including those very characteristic for branched *iso* and *anteiso* chains (Table 3). The ^1H NMR spectrum of glycolipid **3** (Table 3) also showed, at 5.09 and 4.78 ppm, two signals of equal intensity which could be assigned to anomeric protons with coupling constants of $J_{1,2}$ 1.9 Hz and $J_{1,2}$ 1.8 Hz typical for two α-mannose residues [20]. ^{13}C NMR chemical shifts for C-5 and C-6 (Table 3) indicated a substitution at C-6 in the internal, but not in the terminal hexose. Glycerol showed the signal of its CH group at 4.0 ppm, shifted up-field by 1 ppm in comparison to typical glycolipids where the CH group is substituted [21,22], consistent with a free hydroxyl group at C-2. From the ^1H NMR COSY spectrum performed in dimethyl sulfoxide, it was shown that CH_2 groups of glycerol had no cross peaks with free hydroxyl groups, which means that both CH_2 groups of glycerol are substituted. Instead, the methine group of glycerol has a cross peak with an hydroxyl group (data not shown). The ^{13}C NMR chemical shifts for the glycerol support further the presence of a free hydroxyl group at C-2.

4. Discussion

The structure of the major glycolipid component from various *Saccharopolyspora* strains, characterized by an identical TLC-mobility, is shown in Scheme 1. Such a glycolipid structure, containing an extra fatty acyl residue attached to the carbohydrate fragment, is not common among actinomycetes but was reported in a *Streptomyces* strain [23]. Fatty acids of the major glycolipid from *Saccharopolyspora* are mainly mono-methyl branched acids of the *iso* and *anteiso* series with a predominance of the *anteiso*-$C_{17:0}$ acid, and then of the *iso*-$C_{16:0}$ structure. Both were previously found



Scheme 1. Structure of the major glycolipid component (Glycolipid 1) from *Saccharopolyspora* genus.

among the five major fatty acids from whole cells of *Saccharopolyspora hirsuta* [24] and from other species of *Saccharopolyspora* [25].

It should be mentioned that in polar lipids from two new species, *Saccharopolyspora gregorii* and *Saccharopolyspora hordei,* some glycolipids were found [26]. On the basis of the TLC pattern presented in this paper, one could postulate that the major glycolipid of both species bears similarities with the structures analyzed in the present report. Also *Saccharopolyspora gregorii* bears another predominant, not yet characterized glycolipid found in *S. erythraea* (Fig. 1) and *Saccharopolyspora* sp. (LL-100-46). These data suggest that analyses of polar lipids, including glycolipids, might provide a valuable tool for the classification of *Saccharopolyspora* not only on the genus but also on the species level. Certain chemotaxonomic features appear to be conserved within *Saccharopolyspora* taxon, and entirely correlated with the actinomycete phylogenetic tree derived from ribosomal RNA sequences [27], whereby existing members might be recognized.

The major glycolipid from *Saccharopolyspora rectivirgula* was shown to react with sera of patients suffering from farmer's lung disease [28]. In this case, the glycolipid marker seems to be competitive to some protein antigens [29] which were recently prepared as reliable reagents in the specific diagnosis of this disease. Moreover, the glycolipid compounds studied in this paper appeared to be effective as immunoregulators of interferon synthesis [30] and tumor necrosis factor (unpublished data). The later cytokine was found to be important in hypersensitivity pneumonitis caused in mice by *Saccharopolyspora rectivirgula* [31,32].

Further studies are required in order to determine the role of the major glycolipid from *Saccharopolyspora* in immunological processes. In this respect, of particular relevance is a recent report which indicates that mycolic acid, a branched long chain fatty acid found in mycobacteria, could be bound by CD1-restricted $\alpha\beta^+$ cells, allowing mycolic acid presentation to T cell clones [33], suggesting that αTCR$^+$ cells could recognize a broader range of antigens than previously suspected, and especially non peptide structures.

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