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POLAR EPICUTICULAR LIPIDS OF *LYCOPERSICON PENNELLII*

BASIL A. BURKE, GWENDOLYN GOLDSBY and J. BRIAN MUDD

ARCO Plant Cell Research Institute, 6560 Trinity Court, Dublin, California 94568, U.S.A.

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Key Word Index—*Lycopersicon pennellii*; Solanaceae; wild tomato; trichomes; polar epicuticular glucolipids; 2,3,4-tri-*O*-acylglucose esters.

Abstract—Trichome exudates of *Lycopersicon pennellii* yielded nine 2,3,4-tri-*O*-acylglucose esters as α- and β-anomers from the polar fraction of the epicuticular lipid mixture. The esters were primarily derived from 2-methylpropanoic, 2-methylbutanoic, 3-methylbutanoic, 8-methylnonanoic and *n*-decanoic acids, together with trace quantities of 10-methylundecanoic and *n*-dodecanoic acids.

INTRODUCTION

Wild species of the genera *Solanum* and *Lycopersicon* (Solanaceae) have served as germ plasm in breeding of crop plants for resistance to insects and pathogenic organisms [1–6]. Investigations have shown that *S. berthaultii* Hawkes, the wild potato, exudes the volatile (*E*)-β-farnesane, an allomone which induces rapid dispersal of aphid colonies [7]. Similarly, the wild tomato, *L. hirsutum* f. *glabratum* C. M. Mull, exudes from its glanular trichomes a toxic factor, 2-tridecanone, which, along with other allelochemicals of the leaves, exhibits activity against the tomato fruit worm, *Heliothis zea* [8, 9]. In other areas, plant physiologists have been interested in these genera for other reasons, namely valuable traits such as drought resistance and salt tolerance. Thus, wild populations are continually being sought by plant breeders for highly producing, cultivated hybrids with effective insect resistance as well as salt and drought tolerance.

In the course of our studies we have investigated the chemistry of the trichome exudate of a greenhouse grown variety of a population of *L. pennellii* (Corr.) D'Arcy (formerly *S. pennellii*) whose native region is a narrow, extremely arid strip on the West slopes of the Andes in central Peru. *L. pennellii* is a prime target for breeding studies for several reasons. In its natural habitat it exhibits extreme drought tolerance and in the more arid areas exists where only cactus and bromeliads survive [10]. In addition, *L. pennellii* is a genetic source of resistance against several insect pests [3, 6]. Equally important is the fact that *L. pennellii* is fully crossable with the cultivated tomato, *L. esculentum* Mill. cv VF36.

We here report results of the chemical investigation of the polar component of the epicuticular/lipids from the trichome exudate of *L. pennellii*. A study of the accumulation of these lipids is reported elsewhere [11].

RESULTS AND DISCUSSION

The crude exudate from the trichomes of the leaves of *L. pennellii* was isolated by rinsing the surface of the leaves with chloroform [11]. An almost colourless solution resulted, which gave a tan coloured viscous gum upon removal of the solvent *in vacuo*. The polar lipids were separated from the nonpolar mix by partitioning the extract between methanol–water (3:1) and hexane, from which the polar lipids partitioned into the aqueous layer.

This mixture of lipids was significantly more polar than triacyl and diacylglycerols as judged from their chromatographic behavior. From a series of analyses by thin layer chromatography (TLC) utilizing spray reagents [11], it became apparent that the constituents of the mixture were not glycerides but glycolipids. Infrared spectra of the mixture as a film suggested ester (1749 cm^{-1}) and hydroxy (3470 cm^{-1}) functionalities. The nature of the sugar moiety was determined by a combination of hydrolyses, gas chromatography (GC) and specific enzymatic estimation as indicated below.

Using the conversion of glucose to glucose-6-phosphate by ATP in the presence of hexokinase, and then monitoring at 340 nm the subsequent reduction of NADP to NADPH by glucose-6-phosphate dehydrogenase, the glycolipid mixture was converted to the free sugar by acid hydrolysis, and the latter shown to be D-glucose (39%) by the enzymatic assay [12]. That glucose was the only sugar was demonstrated by a sequence of acid hydrolyses of the lipid mixture, borohydride reduction and subsequent acetylation to the glucitol acetate. This single sugar component was confirmed by direct GC comparison with authentic samples of glucitol, other alditols and with inositol.

The fact that about the same quantity of glucose was determined enzymatically after either mild alkaline hydrolysis (33%)—conditions which would not cleave dimers or oligomers of glucose—or after acid hydrolysis (39%), suggested that only esters of monomeric glucose was involved. It is expected that alkali hydrolysis will lead to partial decomposition of glucose, hence a lower value. No free glucose was observed in the unhydrolysed glucolipid mixture. Thus the material consists of a glucose moiety to which several acyl groups are attached as esters.

When the mixture of glucolipids was analysed by methanolysis and propanolysis followed by GC or GC/MS, the resulting fatty esters identified the fatty acids

which existed as glucose esters. All acids were bound to glucose since GC analysis of the diazomethane treated fresh leaf washings showed that there were no free acids in the extract. A total of eighteen glucolipids (**1–9**), each a 2,3,4-tri-*O*-acylglucose, were observed when the mixture of polar lipids was analysed by HPLC, and each component characterized after separation of the mixture by preparative HPLC on a reverse phase C_{18} column.

In addition to analysis of the mixture each of the separated components was reevaluated by HPLC and the constituent acyl groups defined by ^{1}H NMR and MS, and by alcoholysis followed by GC. The results of GC analyses of the mixture are shown in Table 1.

It should be noted that in estimating the esters, propanolysis proved superior to methanolysis as a method for a quantitative GC analysis of the more volatile C_4 and C_5 acids. In addition, the propyl esters of the 2- and 3-methylbutanoic acids were efficiently resolved on a capillary column whereas the corresponding methyl esters were not.

The first glucolipids identified, compounds (**1a** and **1b**), were chromatographically the most polar constituents ($\approx 10\%$) of the glucolipid mixture. These were eluted from the HPLC as two interconvertible anomers, an indication that C-1 of glucose was free to anomerise, and thus contained a free hydroxy group. Absorptions at 3470 and 1749 cm^{-1} in the IR spectrum showed hydroxy and ester absorptions, supporting the idea that compound **1** was a partially acylated glucose.

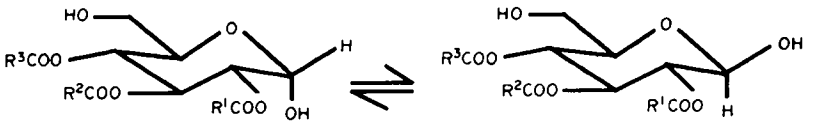
Table 1. Fatty acid composition of glucolipid mixture by propanolysis*

Propyl ester	% Fatty acid by wt†
2-Methylpropanoic	41.4 ± 3.3
2-Methylbutanoic	4.5 ± 2.1
3-Methylbutanoic	4.1 ± 0.7
8-Methylnonanoic	31.6 ± 2.3
n-Decanoic	10.4 ± 1.2
8-Methyldecanoic	1.2 ± 0.3
10-Methylundecanoic	0.8 ± 0.1
n-Modecanoic	3.4 ± 0.2

*Confirmed by GC/MS on methyl esters.
†Results of analyses of nine samples.

Although electron impact mass spectrometry was not very useful in identifying the compound, a positive DCI spectrum utilizing *iso*butane (*m/z* 373, $[M + H - H_2O]^+$) and ammonia (*m/z* 408, $[M + NH_4]^+$) demonstrated a M_r of 390 for compound **1**. The ^{1}H NMR spectrum of **1** was typical of a partially acylated glucose [13]. Peaks centered at δ1.13 (18 H, overlapping doublets, J = 6.9 Hz) and 2.4–2.6 (3H, overlapping heptets J = 6.9 Hz) signalled the presence of three *iso*butanoyl moieties. Their positions at C-2,3 and 4 of the glucose molecule followed from the values of the chemical shifts and coupling constants of H-2, H-3 and H-4 (δ4.88, *dd*, J = 9.8 and 3.7 Hz; 5.65, *t*, J = 9.8 Hz; and 5.02, *t*, J = 9.8 Hz respectively, for the α-anomer; and δ4.74, *dd*, J = 9.8 and 10.1 Hz; 5.37, *t*, J = 9.8 Hz; and 5.05, *t*, J = 9.8 Hz respectively, for the β-anomer) and of the corresponding values for H-1 (δ5.47, *t*, J = 3.7 Hz, α-anomer and 4.86, overlapping with the signal at 4.88, β-anomer). These signals for H-1 collapsed to doublets when the sample was shaken with D_2O. Assignments were confirmed by comparison with model compounds and by decoupling experiments. In particular, the presence of three axial protons strongly supports a glucose derivative. The above chemical shifts of the anomeric protons and those of the methylene protons of C-6 (centered at δ3.56 and 3.68) are indicative of hydroxy functionalities at these carbon atoms. Hence, only C-2,3 and C-4 bear ester groups. The integral of the H-3 signal (α-anomer and β-anomers) in this ^{1}H NMR spectrum established an anomeric distribution of 2α:1β in chloroform solution.

Methanolysis [14] of compound **1** gave methyl *iso*butanoate as the only methylate resulting from transesterification, cofirming that glucose was esterified with *iso*butanoate only. Acylation of compound **1** with acetic anhydride and pyridine gave mainly the expected α-anomer of the 1,6-diacetate, **10**. The NMR signals of **10** for the acetyl groups (δ2.17 and 2.09, each 3H, *s*, α-anomer; 2.22 and 2.09, each 3H, *s*, β-anomer), and also for H-1 (δ6.34, 1H, *d*, J = 3.6 Hz, α-anomer; 5.74 J = 8.1 Hz, β-anomer) and the two C-6 protons (4.15 and 4.25, each 1H, *m*) of the glucose moiety concurred. The mass spectrum was typical of a pentaacylglucose with the highest fragments at *m/z* 415 (100) $[M + H - AcOH]^+$ under DCI

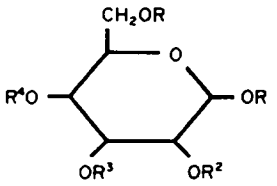


1 R¹, R², R³ = 3 x $(Me)_2CH$
2 R¹, R², R³ = 2 x $(Me)_2CH$, $MeCH_2(Me)CH$
3 R¹, R², R³ = 2 x $(Me)_2CH$, $(Me)_2CHCH_2$
4 R¹, R², R³ = 2 x $(Me)_2CH$, $(Me)_2CH(CH_2)_6$
5 R¹, R², R³ = 2 x $(Me)_2CH$, $Me(CH_2)_8$
6 R¹, R², R³ = $(Me)_2CH$, $MeCH_2(Me)CH$, $(Me)_2CH(CH_2)_6$
7 R¹, R², R³ = $(Me)_2CH$, $(Me)CHCH_2$, $(Me)_2CH(CH_2)_6$
8 R¹, R², R³ = $(Me)_2CH$, $MeCH(Me)CH$, $Me(CH_2)_8$
9 R¹, R², R³ = $(Me)_2CH$, $(Me)CHCH_2$, $Me(CH_2)_8$

with *iso*butane as reagent gas, and 492 (34) $[M+NH_4]^+$ and 415 (82) $[M+NH_4-NH_4OAc]^+$ with NH_3, in full agreement with structure **10** for the diacetate.

For complete structural confirmation, compound **1** was synthesized from glucose. First, glucose was converted to the 1,6-di-*O*-tritylglucose, **11**, with trityl chloride and pyridine. Acylation of the reaction mixture *in situ* with *iso*butanoyl chloride gave the triacylderivative, **12**, which after separation was converted to 2,3,4-tri-*O*-isobutanoylglucose by hydrogenolysis. This compound was identical to the naturally occurring compound.

Compounds **2–9** proved to be acyl homologues of compound **1**. Each showed ^{1}H NMR and mass spectra (Table 2) which indicated that they were 2,3,4-tri-*O*-acylglucoses with the anomeric C-1 and the methylene at C-6 bearing hydroxy groups. In general, the ^{1}H-chemical shifts for similar protons on the glucose moiety in this series of compounds varied by less than 0.02 ppm from the values of similar protons already mentioned for compound **1**. The signals for H-1 at 5.47 (1H, *t*, $J = 3.7$, α-anomer) and 4.86 (1H, *m*, β-anomer) each collapsed to a doublet when the hydroxy proton was exchanged with D_2O. Each acyl group showed the expected signals as follows: $(CH_3)_2CHCO$: δ1.13 (6H, overlapping *d*, $J = 6.5$ Hz) and 2.4–2.6 (1H, *h*, $J = 6.9$ Hz); $CH_3CH_2(CH_3)CHCO$: δ 0.85 (3H, *t*, $J = 6.7$ Hz) 1.13 (3H, *d*, $J = 6.5$ Hz), 1.59 (2H, *m*) and 2.4–2.6 (1H, *m*); $(CH_3)_2CHCH_2CO$: δ 0.91 (6H, *d*, $J = 6.7$ *Hz*) 1.40 (1*H*, *m*) and 2.41 (2H, *d*, $J = 7.0$ Hz); $(CH_3)_2CH(CH_2)_6CO$: δ 0.84 (6H, *d*, $J = 6.7$ Hz) 1.13 (2H, *m*) 1.23 (6H, *m*) 1.52 (2H, *m*) and 2.21 (2H, *t*, $J = 7.6$ Hz); $CH_3(CH_2)_8CO$; δ 0.86 (3H, *t*, $J = 6.9$ Hz) 1.23 (12H, *m*) 1.52 (2H, *m*) and 2.21 (2H, *t*, $J = 7.6$ Hz). The ^{13}C NMR spectrum of compound **4** is typical of these closely related esters, and allowed the assignments of major signals at δ61.08 (*t*), 68.63 (*d*), 68.8 (*d*), 69.5 (*d*), 71.19 (*d*) and 90.24 (*d*) for the α-anomer of the glucose moiety. Similarly, signals t δ18.65 (*q*), 18.80 (*q*), 18.87 (*l*), 18.93 (*l*), 33.95 (*d*), 33.97 (*d*), 176.23 (*s*) and 176.88 (*s*) were assigned to the *iso*butyryl groups. The 10 carbons of the *iso*decanoate group appeared at δ22.66 (2*q*), 24.85 (*t*), 27.15 (*t*), 27.94 (*d*), 29.48 (*t*), 33.79 (*t*), 38.92 (*t*) and 172.62 (*s*). Less intense signals for the minor amount of β-anomer were also observed. Compounds **2** and **3**, **6** and **7**, and **8** and **9** were inseparable pairs of compounds. Compounds within each pair differed in the replacement of the 2-methylbutanoyl group by a 3-methylbutanoyl moiety. The ratio of 2-methyl to 3 methylbutanoate in the mixture was 3:1.



10 R = MeCO; R^2, R^3, R^4 = 3 x $(Me)_2CHCO$
11 R = Trityl; R^2, R^3, R^4 = 3 x OH
12 R = Trityl; R^2, R^3, R^4 = 3 x $(Me)_3CHCO$

Positive ion DCI spectra were very informative in depicting the type of acyl group present. Indeed the ions *m/z* 89, 103 and 173 represented protonated C_4, C_5 and C_{10} saturated carboxylic acids arising from hydrogen rearrangement of the corresponding esters [15]. This mass spectral information was collated with data from ^{1}H NMR spectra to establish unequivocally the identity of the acyl groups of each of these esters, and to confirm the great structural similarity of this complex mixture of molecules.

Table 2. Results of positive ion DCI MS (200 eV) and GC analysis of fatty acid methyl esters (FAMES) present in individual glucolipids

Compound	*m/z*(% int.) *Iso*butane			NH_3		FAMES†
1	373	(8.5)	$[M+H-H_2O]^+$	408 (25.5)	$[M+NH_4]^+$	2-Methylpropanoate
	89	(100)	$[(CH_3)_2CHCO_2H_2]^+$	373 (100)	$[M+NH_4-NH_4OH]^+$	
2, 3	387	(11.4)	$[M+H-H_2O]^+$	422 (10.3)	$[M+NH_4]^+$	2-Methylpropanoate
	89	(100)	$[(CH_3)_2CHCO_2H_2]^+$	387 (23.9)	$[M+NH_4-NH_4OH]^+$	2-Methylbutanoate
	103	(58.6)	$[CH_3CH_2CH(CH_3)CO_2H_2]^+$*			3-Methylbutanoate
4	457	(6.7)	$[M+H-H_2O]^+$	492 (29.7)	$[M+NH_4]^+$	2-Methylpropanoate
	89	(100)	$[(CH_3)_2CHCO_2H_2]^+$	457 (18.5)	$[M+NH_4-NH_4OH]^+$	8-Methylnonanoate
4	457	(4.2)	$[M+H-H_2O]^+$	492 (25.4)	$[M+NH_4]^+$	2-Methylpropanoate
	89	(100)	$[(CH_3)_2CHCO_2H_2]^+$	457 (36.5)	$[M+NH_4-NH_4OH]^+$	*n*-Decanoate
	173	(100)	$[CH_3(CH_2)_8CO_2H_2]^+$			
6, 7	471	(16.0)	$[M+H-H_2O]^+$	506 (44.1)	$[M++NH_4]^+$	2-Methylpropanoate
	89	(70.4)	$[(CH_3)_2CHCO_2H_2]^+$	471 (37.0)	$[M+NH_4-NH_4OH]^+$	3-Methylbutanoate
	103	(84.8)	$[CH_3CH_2CH(CH_3)CO_2H_2]^+$*			2-Methylbutanoate
	173	(100)	$[(CH_3)_2CH(CH_2)_6CO_2H_2]^+$			8-Methylnonanoate
8, 9	471	(18.9)	$[M+H-H_2O]^+$	506 (64.5)	$[M+NH_4]^+$	2-Methylpropanoate
	89	(100)	$[(CH_3)_2CHCO_2H_2]^+$	471 (44.1)	$[M+NH_4-NH_4OH]^+$	3-Methylbutanoate
	103	(95.0)	$[CH_3CH_2CH(CH_3)CO_2H_2]^+$*			2-Methylbutanoate
	173	(83.0)	$[CH_3(CH_2)_8CO_2H_2]^+$			*n*-Decanoate

*Fragment also representative of $[(CH_3)_2CHCH_2CO_2H_2]^+$

†Results of methanolysis on each glucolipid separated by C_{18} HPLC.

Chromatographically the polarity of these compounds decreased from compound **1** to **9**, with the more polar β-anomer preceding the α-anomer during elution from reverse phase HPLC. After separation by HPLC, each anomer of a particular molecular species was observed to interconvert in acetonitrile/water as demonstrated by analytical HPLC. Despite this interconversion each component of the mixture, after separation by preparative HPLC, was analysed for acyl groups by methanolysis followed by GC, and by comparison with the appropriate standards. These GC results supported the assignments from mass and ^{1}H NMR analyses for the acyl moieties of these glucose esters.

As noted in Table 2, compounds **2** and **3**, **6** and **7**, and **8** and **9** were inseparable pairs of isomers under the conditions of purification by HPLC. These HPLC conditions successfully resolved the other compounds according to MW as well as polarity. Even compounds **4** and **5**, which differ only in the type of C_{10} acyl group present were successfully separated. The unseparated pairs of isomers, however, seem to lack the necessary chain extension of their acyl groups to be resolved on the C_{18} column. The ^{1}H NMR spectrm of each pair of components clearly showed signals characteristic of the designated acyl groups. Alcoholysis and GC results also supported the presence of these acyl groups. Finally, mass spectra confirmed the NMR results that the glucolipids represented by each inseparable pair were isomers differing only in the isomerism of the C_5 acyl groups and that they were not homologues differing in mass.

Except for compound **1** in which all three acyl substituents are the same, i.e. *iso*butanoate, it has not been possible, so far, to assign the precise position of each of the different acyl groups on the sugar moiety. In the case of triglycerides, this has proven difficult when the acyl groups are different. Despite these difficulties, the use of enzymes [16] on the one hand and mass spectrometry [17] on the other, has been effective in resolving the position of acyl groups. With these 2,3,4-triacylglucose esters, however, we are not aware of a specific hydrolase which can selectively remove one ester link while maintaining the integrity of the other positions; neither has mass spectroscopy been helpful in resolving our difficulty.

While the 2,3,4-tri-*O*-acylglucose esters described herein are relatively simple chemical structures, they are unprecedented as epicuticular lipids and have not been reported previously to occur in nature. They thus represent a new class of plant lipids. They are, however, closely related to the sucrose esters isolated from the leaves and cuticular waxes [18–20] of certain cultivars of tobacco also Solanaceae. These have been reported to be important contributors to tobacco flavor [13, 21] and are postulated precursors of the earlier isolated glucose tetraesters [22, 23]. A careful look at these glucolipids indicates a striking similarity with the well-known triglycerides. In fact, these 2,3,4-tri-*O*-acylglucoses may be viewed as substituted polar triglycerides, i.e. triglycerides with polar ends, each one containing at least one *iso*-butanoyl moiety. It is important to note, however, that no triglycerides were detected in the epicuticular lipids from *L. pennellii*.

Another interesting structural feature of the glucolipids is the presence of the intermediate chain C_{10} fatty acids. These are not common as natural esters, not even as triglycerides. Significantly, small quantities of similar C_{12} acids have also been observed during alcoholysis and have been characterized by GC and GC/MS. However, the corresponding glucolipids which were also detected by HPLC were not characterized.

Most natural glucosides or glucose esters are linked to the C-1 of glucose. In these triacylglucose derivatives C-1 and C-6 are always free. Although several hypotheses may be forwarded, no specific role has been assigned to these glucolipids. Nevertheless, the sheer quantity (up to 25% of leaf dry wt) [11] of these trichome exudates suggests a fundamental role in the biochemistry of the plant. Probably the most obvious function of these compounds is that they act as endogenous antidesiccants for *L. pennellii*, protecting the plant against loss of water to an extremely arid natural environment. The polar ends (C-6 and C-1) may be envisaged as prostheses for rapid transport of these molecules across the cuticle to the leaf surface [24]. Another immediately apparent role of these trichome exudates is in entrapment of insects which become physically ensnared in the sticky epicuticular product of plants grown in the field [8].

EXPERIMENTAL

^{1}H NMR and ^{13}C NMR were recorded at 300 and 74.5 MHz, respectively, using TMS as int. ref. and $CDCl_3$ as solvent. The multiplicity of the carbons in the ^{13}C expts were determined using the DEPT sequence software provided with the instrument using a J value of 140 Hz and θ value of 1.500 π. Direct probe inlet MS were recorded under positive ion DCI conditions (200 eV) using *iso*butane or NH_3 as ionizing gas. IR spectra were recorded as thin films. Unless otherwise specified, TLC was performed on reverse phase C_{18} silica gel plates.

Isolation and purification. Plant material, growth, isolation, and detection of the epicuticular lipids were as described previously [11] with the following modification for purification. The total epicuticular lipid was partitioned between hexane and MeOH–H_2O (3:1) to give a mix of polar lipids (8%) and nonpolar lipids (12%). The mix of polar lipids was evaluated by TLC (MeOH–H_2O, 7:3 and 4:1, successively) and by HPLC (Beckman) utilizing an ODS-2 C_{18} ultra-sphere reverse phase column (250 × 4.6 mm), at 210 nm, with gradient of MeCN–H_2O (1:1) for 6 min, then changing to MeCN–H_2O (4:1) over 2 min, and holding an additional 12 min before finally adjusting to 100% MeCN. Total run time was 25 min at a rate of 1 ml/min. Purification of the individual glucolipids was achieved by HPLC (Waters) using a μ-Bondapak C_{18} reverse phase semi-preparative column (300 × 7.8 mm), under isocratic conditions (MeCN–H_2O, 3:1) with a flow rate of 1.4 ml/min and detection at 210 nm. Fractions were collected with the following R_t (min) and wt (%) of total recovered): A, α- and β-anomer of compound **1**, 9.5 (16%); B, α- and β-anomers of **2** and **3**, 10.4 (25%); C, β-anomer of **4**, 22.4 (7%); D, α- and β-anomer of **4** and **5**, respectively, 23.6 (22%); E, α-anomer of **5**, 25.3 (8%); F, β-anomer of **6** and **7**, 26.7 (3%); G, α- and β-anomer of **6** and **7**, and **8** and **9**, respectively, 28.6 (11%); *J*, α-anomer of **8** and **9**, 30.6 (4%). The IR spectra of these compounds displayed identical functionalities: 3470 (-OH) and 1749 (ester C=O) cm^{-1}. The ^{1}H NMR and ^{13}C results are described in the text, and the MS results are represented in Table 2.

Glucose determination. Enzymatic determination of glucose content was accomplished using a glucose test kit (Sigma single assay vials 15-1). Production of NADPH was monitored at 340 nm [12]. Conditions for the preliminary base hydrolysis of the glucolipid mixture consisted of heating the mixture in a sealed vial in the presence of 5% KOH–MeOH at 35° for 30 min. Samples were then neutralized (2N HCl–MeOH), blown to

dryness with a stream of N_2 and resuspended in H_2O before enzyme analysis. The acid hydrolysis which preceded enzyme assay was accomplished by heating the glucolipid in the presence of 2 M aq. TFA for 1 hr at 100°.

GC analysis. Prepn of the crude polar lipid for sugar identification by GC was effected by first heating the polar lipid in 0.6 N HCl–MeOH (90°C for 1 hr) in a sealed reaction vial. Subsequent treatment of the mix (after neutralization) with $NaBH_4$ for 30 min (25°), followed by acetylation with Ac_2O–pyridine (1:1) at 25° overnight gave glucitol hexaacetate. Analysis by GC was conducted using a split injection capillary column (WCOT OV101, 25 m × 0.25 mm, 30:1 split ratio, He carrier gas flow 0.6 ml/min, FID det; inj and det temp 290°). Temp. prog. consisted of holding 1 min at 180° followed by increasing at 5°/min to 240° for 10 min. Standard acetates of alditols and inositols were used for comparison. Me esters for GC analysis of fatty acids present in the glycolipids, were prepd by the method of ref [14] which involves methanolysis of lipid samples first with NaOMe (1%) then with 2 N HCl–MeOH. Samples were extd into pentane (100 μl) and analysed by GC under similar conditions as described above for the analysis of glucitol acetate except with a temp. prog. beginning at 50° for 5 min then increasing to 180° at 10°C/min (det. and inj. temperatures at 230°C). Standard esters were used when appropriate. GC/MS data were acquired under similar conditions as the capillary GC. Propyl esters were prepared by established methods using *n*-propanol–benzene–H_2SO_4 (19:1:0.5). A silar 10C capillary column (25 m × 0.25 mn) was used for the analysis of esters. The temp. prog. was 50° for 8 min then 50° to 180° at 8°/min.

Acetylation of glucolipid. Acetylation of glucolipids individually purified by HPLC was effected by treatment with Ac_2O–pyridine (1:1). After removal of the reagents *in vacuo*, the product was purified (if necessary) by TLC (silica gel F-254, Me_2CO–hexane, 1:5) and characterized by ^1H NMR and CI MS. For example, compound **4** gave a diacetate (anomeric ratio 2α to 1β) with the following ^1H NMR ($CDCl_3$) spectrum for the α-anomer: δ0.85 (6H, *d*, $(\underline{CH_3})_2$CH-), 1.11 (6H, *m*, $(\underline{CH_3})_2$CHCO-), 2.18 (3H, *s*, $\underline{CH_3}$CO-), 2.21 (2H, *t*, J = 6.7 Hz, -$\underline{CH_2}$CO-), 2.49 (1H, *m*, $(\underline{CH_3})_2\underline{CH}$CO-), 4.09 (1H, *m*, Hm5), 4.09 and 4.22 (each 1H, *m*, 2X, H-6), 5.09 (1H, *dd*, $J_{1,2}$ = 3.5 Hz and $J_{2,3}$ = 9.9 Hz, H-2), 5.11 (1H, *t*, H-4), 5.54 (1H, *t*, $J_{2,3} = J_{3,4}$ = 9.9 Hz, H-3), 6.35 (1H, *d*, $J_{1,2}$ = 3.5 Hz, H-1). The signals for the β-anomer differed only in the following chemical shifts δ3.80 (1H, *m*, H-5), 5.32 (1H, *t*, $J_{2,3} = J_{3,4}$ = 9.9 Hz, H-3), and 5.71 (1H, *d*, $J_{1,2}$ = 8.3 Hz, H-1). DCI MS (NH_3), *m/z*: 576 (100) $[M + NH_4]^+$, 499 (88) $[M + NH_4 - NH_4OAc]^+$.

Synthesis of 2,3,4,-*tri-O-isobutanoyl-*D*-glucopyranoside.* Anhydrous glucose (1 mol), tritychloride (2 mols) and dried pyridine as solvent, were shaken vigorously and set aside with magnetic stirring for several days. To an aliquot (0.5 ml) of the above suspension was added excess *iso*butanoyl chloride in $CHCl_3$; this mixt. set aside overnight. The mixt. was dil. with 2% aq. $NaHCO_3$, extd with $CHCl_3$, dried (Na_2SO_4) and purified by prep. TLC (silica gel F-254, 1 mm, Me_2CO–hexane 1:5) to yield 1,6-di-*O*-trityl-2,3,4-tri-*O*-isobutanoyl-D-glucopyranoside. The ditrityl compound (14 mg) in dioxane (1 ml) was stirred overnight in a 10 ml flask with PtO_2 (16 mg) under an atmosphere of H_2. The resulting mixt was filtered through Celite, and after removal of the solvent *in vacuo*, the product was partitioned between hexane and MeOH–H_2O (3:1). The product of the polar layer gave essentially two bands by reverse phase C_{18} prep. TLC (MeOH–H_2O, 7:3). The major, higher R_f band was identical to the natural 2,3,4-tri-*O*-acylglucose, **1**, by NMR, MS and HPLC.

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