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TREHALOSE ESTERS FROM THE ASPEN FUNGUS *HYPHOZYMA LIGNICOLA*

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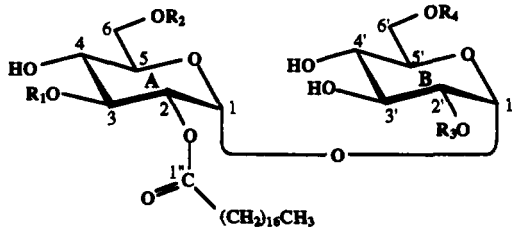
ABSTRACT.—Chemical investigation of the metabolites produced when a new fungus, *Hyphozyma lignicola*, isolated from blackgalls on aspen, was grown in malt extract liquid medium resulted in the isolation and identification of five trehalose esters **1–5**, which are unusual fungal metabolites. The major compound is 3,6-di-*O*-acetyl-2-*O*-octadecanoyl-α,α-D-trehalose [**2**]. The structures were established by the analysis of nmr and fabms data in combination with chemical degradation.

It has been observed that when the main stem of aspen trees (*Populus tremuloides* Michaux) carries a certain type of black gall of unknown origin, they tend to be resistant to decay- and stain-causing fungi (1). One possible explanation of this phenomenon may be that microorganisms associated with the black galls produce metabolites that are antagonistic to the decay- or stain-causing fungi. We have previously reported on the metabolites of fungi associated with aspen antagonistic to the wood-rotting fungus *Phellinus tremulae* (2,3) and to the blue-staining fungus *Ceratocystiopsis crassivaginata* (4). Recently a new species of fungus, *Hyphozyma lignicola* Hutchison, which is antagonistic to *C. crassivaginata*, has been isolated from the black galls of aspen (5). Our chemical investigation of the metabolites produced when *H. lignicola* is grown in malt extract liquid medium resulted in the isolation and identification of five trehalose esters **1–5**, representatives of an unusual type of fungal metabolites.

RESULTS AND DISCUSSION

H. lignicola was grown in malt extract/yeast extract liquid shake culture for 5 weeks. Extraction of the concentrated culture broth with EtOAc provided a crude mixture of metabolites as a glassy solid. Gel filtration of the crude extract on Sephadex LH-20 (MeOH) yielded three major fractions. Both tlc and nmr suggested that the first fraction, which accounted for about 75% of the crude extract, contained several related compounds. Indeed, acetylation of this mixture yielded a single product, indicating that these compounds share a common skeleton, differing from each other only in the position and degree of acetylation. Separation of the metabolites was achieved by Si gel chromatography utilizing several solvent systems.

Compound **2**, the major component of the extract, was obtained as a glass. Its



1 R_2=Ac, R_1=R_3=R_4=H
2 R_1=R_2=Ac, R_3=R_4=H
3 R_2=R_3=Ac, R_1=R_4=H
4 R_1=R_2=R_3=Ac, R_4=H
5 R_1=R_2=R_4=Ac, R_3=H

structure was established as 3,6-di-*O*-acetyl-2-*O*-octadecanoyl-α,α-D-trehalose [**2**] based on spectroscopic data in combination with chemical degradation. The ir absorptions at 3420 (br) and 1744 cm^{-1} suggested the presence of hydroxyl and ester groups. The ^{1}H-nmr spectrum displayed 14 signals between 5.4 and 3.3 ppm appropriate for two pyranose residues, signals for the 35 lipid chain protons (δ 2.22, t, 2H; 1.50, m, 2H; 1.20, br s, 28H; 0.80, t, 3H), and two acetyl singlets (δ 2.04, 2.02 ppm). The ^{13}C-nmr spectrum (Table 1) displays the appropriate signals for the 12 carbons in the two sugar residues, signals for a long lipid chain, and three ester carbonyls. A COSY experiment (Table 2) assisted by an HMQC (6) experiment clearly identified the two pyranose residues as two α-glucose residues, as indicated by the coupling constants of the relevant protons. $J_{2\text{-}3}$, $J_{3\text{-}4}$, and $J_{4\text{-}5}$ in each residue are all about 10 Hz, indicating these pairs to be trans diaxial. The coupling constants of the two anomeric protons (5.19 and 5.02 ppm) are each 3.5 Hz, indicating that both glucose units have the α configuration at the anomeric centers. The α configurations are also consistent with the ^{13}C-nmr shifts of the two anomeric carbons (91.80 and 94.75 ppm) (7). The identity of the lipid chain is based on the hrfabms data (Figure 1). The fabms fragment at 267.2723 amu ($C_{18}H_{35}O$) is attributed to a stearyl fragment.

The connectivities between the two glucose residues, the stearyl chain, and the two acetyls were established by an HMBC (6) experiment (Figure 2). The long-range H/C correlations from H-1 (5.19 ppm) to C-1′ (94.75 ppm) and from H-1′ (5.02 ppm) to C-1 (91.80 ppm) indicate that the two glucose residues are connected at C-1/C-1′ through a glycosidic linkage to form an α,α-trehalose moiety. The correlations from both H-2

TABLE 1. ^{13}C-nmr Data for Compounds **1–5**.[a]

Carbon	Compound				
	1	**2**	**3**	**4**	**5**
C-1	92.14, d	91.80, d	92.38,[b] d	92.27, d	91.33, d
C-2	72.35, d	70.32, d	72.50,[c] d	70.00, d	70.15,[b] d
C-3	70.34,[b] d	72.36,[b] d	70.86,[d] d	72.45,[b] d	72.23, d
C-4	69.79,[c] d	68.58, d	70.71,[d] d	68.70, d	68.64, d
C-5	69.67,[c] d	69.91,[c] d	70.40,[d] d	70.13,[c] d	70.15,[b] d
C-6	63.02, t	62.73, t	63.29, t	62.96, t	62.79, t
C-1′	94.71, d	94.75, d	92.41,[b] d	92.75, d	93.98, d
C-2′	71.40, d	71.37, d	72.10,[c] d	72.38,[b] d	71.33, d
C-3′	73.28, d	73.28, d	70.19,[d] d	70.38,[c] d	73.36, d
C-4′	70.46,[b] d	69.74,[c] d	70.10,[d] d	70.64, d	70.42, d
C-5′	72.16, d	72.30,[b] d	72.06,[c] d	72.28,[b] d	70.00,[b] d
C-6′	61.09, t	61.15, t	61.18, t	61.23, t	63.35, t
C-1″	173.70, s	173.12, s	173.74, s	173.08, s	172.83, s
C-2″	33.71, t	33.73, t	33.77, t	33.75, t	33.81, t
C-3″	24.53, t	24.67, t	24.55, t	24.68, t	24.74, t
C-4″–C-17″	31.75, 29.37, 29.53 (*n* C), 29.23, 29.18, 29.02, 22.50	31.75, 29.48, 29.53 (*n* C), 29.34, 29.18, 28.92, 22.51	31.77, 29.50, 29.55 (*n* C), 29.36, 29.20, 29.02, 22.52	31.76, 29.48, 29.53 (*n* C), 29.33, 29.19, 29.15, 28.93, 22.52	31.77, 29.33, 29.53 (*n* C), 29.19, 29.15, 28.95, 22.52
18″	13.87, q	13.89, q	13.90, q	13.90, q	13.90, q
OAc's	20.57, q	20.71, q 20.59, q	20.51, q 20.51, q	20.66, q 20.51 (2C), q	20.72, q 20.59, q 20.52, q
	171.79, s	171.64, s 171.26, s	171.48, s 170.65, s	171.45, s 171.17, s 170.64, s	171.59, s 171.36, s 171.28, s

[a]Recorded in 15% $CD_3OD/CDCl_3$ at 100 MHz.
[b–d]Assignments with the same superscripts may be interchanged within the same column.

TABLE 2. Nmr Data for Compound **2** (15% CD_3OD-$CDCl_3$).

Position	^1H (400 MHz)	COSY	Selected HMBC
1	5.19, d, 3.5	H-2	C-1′, C-2, C-3
2	4.80, dd, 10.2, 3.5	H-1, H-3	C-3, C-1″
3	5.37, t, 9.7	H-2, H-4	C-1, C-2, C-4, 2-OAc
4	3.52, t, 10.5	H-3, H-5	
5	4.12, ddd, 10.2, 4.0, 2.0	H-4, H-6a, H-6b	
6a	4.32, dd, 12.2, 2.0	H-5, H-6b	C-4, C-5, 6-OAc
6b	4.23, dd, 12.2, 4.3	H-5, H-6a	C-4, C-5, 6-OAc
1′	5.02, d, 3.4	H-2′	C-1, C-3′
2′	3.51, dd, 9.5, 3.4	H-1′, H-3′	
3′	3.77, t, 9.4	H-2′, H-4′	C-4′, C-5′
4′	3.42, t, 9.4	H-3′, H-5′	C-5′, C -6′
5′	3.54, dt, 9.5, 3.0	H-4′, H-6a′, H-6b′	
6′a	3.70, dd, 12.0, 3.6	H-5′, H-6b′	
6′b	3.62, dd, 12.0, 2.6	H-5′, H-6a′	
2″	2.22, t, 7.5; 2H	H-3″	C-1″, C-3″, C-4″
3″	1.50, m; 2H	H-2″, H-4″	
4″-17″	1.20, br s; 28H		
18″	0.80, t, 7.0; 3H	H-17″	C-16″, C-17″
OAc's	2.04, s; 3H		
	2.02, s; 3H		

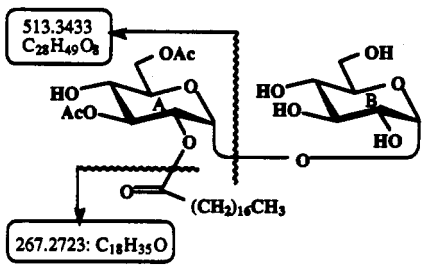


FIGURE 1. Hrfabms fragmentation pattern for compound **2**.

(4.80 ppm) and H-2″ (2.22 ppm) to the ester carbonyl at 173.12 ppm suggest that the lipid chain is attached to the oxygen on C-2. The correlations from H-3 (5.37 ppm) to an acetyl carbonyl carbon and from H_a-6/H_b-6 (4.32 and 4.23 ppm) to the other acetyl carbonyl indicate that the oxygens at C-3 and C-6 are acetylated. The fabms peak at *m/z* 715 is attributed to $[M+Na]^+$; the ion at 513.3433 amu, consistent with $C_{28}H_{49}O_8$, is due to a fragment arising from the loss of glucose residue B (Figure 1).

The identities of the two main fragments of compound **2**, trehalose and stearyl, are also supported by the results of alkaline hydrolysis. The major non-volatile products of

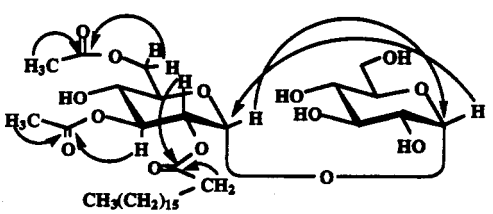


FIGURE 2. HMBC correlations for compound **2**.

alkaline hydrolysis of compound **2** are identified as α,α-D-trehalose and stearic acid, identical with authentic samples (tlc, ir, [α]D, and nmr).

Compound **1** was a foamy glass. Both the ^{1}H- (Table 3) and ^{13}C- (Table 1) nmr spectra suggest that compound **1** has only one acetyl group. The HMBC correlation from the two C-6 protons (4.30 and 4.22 ppm) to the acetyl carbonyl carbon (171.79 ppm) indicates that the oxygen on C-6 is acetylated. Thus compound **1** is 6-*O*-acetyl-2-*O*-octadecanoyl-α,α-D-trehalose. All of the observed HMBC and COSY correlations are consistent with the proposed structure. The fabms peak at 673 amu is attributed to $[M+Na]^+$, 42 units (C_2H_2O) less than that of compound **2**. The fragment arising from the loss of glucose residue B is 471 amu, also 42 units (C_2H_2O) less than that of compound **2**.

TABLE 3. ^{1}H-nmr Data for Compounds **1**, **3**, **4**, **5**[a].

Proton	1	3	4	5
H-1	5.17, d, 3.7	5.12, d, 3.6	5.17, d, 3.7	5.19, d, 3.5
H-2	4.68, dd, 10.1, 3.5	4.70, dd, 10.0, 3.5	4.83, dd, 10.3, 3.7	4.84, dd, 10.3, 3.5
H-3	3.93, t, 9.8	3.82, t, 10.0	5.27, t, 9.9	5.38, t, 10.0
H-4	3.38, t, 9.8	3.33, t, 9.5	3.48, t, 9.4	3.52, t, 10.4
H-5	4.03, ddd, 10.1, 4.3, 2.5	3.84	3.93	4.08, ddd, 10.1, 4.4, 2.2
H_a-6	4.30, dd, 12.2, 2.0	4.24, dd, 12.1, 5.8	4.28, dd, 12.0, 6.0	4.30, dd, 12.0, 2.3
H_b-6	4.22, dd, 12.2, 4.6	4.20 dd, 12.1, 2.9	4.21, dd, 12.0, 2.1	4.25, dd, 12.0, 4.4
H-1′	4.98, d, 3.6	5.12, d, 3.6	5.14, d, 3.7	5.03, d, 3.8
H-2′	3.46, dd, 9.7, 3.6	4.67, dd, 10.0, 3.5	4.70, dd, 10.2, 3.7	3.51, dd, 9.5, 3.8
H-3′	3.66, t, 9.7	3.86, t, 9.7	3.93, t, 9.5	3.76, t, 9.4
H-4′	3.41, t, 9.4	3.48, t, 9.4	3.49, t, 9.4	3.27, t, 9.9
H-5′	3.55, dt, 9.8, 3.5	3.62, dt, 9.6, 3.2	3.63, dt, 9.4, 3.7	3.71, ddd, 9.5, 6.2, 2.1
H_a-6′	3.68, dd, 12.0, 3.5	3.70, dd, 12.4, 4.3	3.70, dd, 12.2, 4.3	4.21, dd, 12.0, 6.1
H_b-6′	3.62, dd, 12.0, 2.8	3.66, dd, 12.4, 2.6	3.66, dd, 12.2, 2.7	4.16, dd, 12.0, 2.3
H-2″	2.28, m; 2H	2.30, m; 2H	2.25, t, 7.4; 2H	2.23, t, 7.5; 2H
H-3″	1.53, m; 2H	1.55, m; 2H	1.50, m; 2H	1.50, m; 2H
H-4″–H-17″	1.20, br s; 28H	1.20, br s; 28H	1.20, br s; 28H	1.20, br s; 28H
H-18″	0.80, t, 7.0; 3H	0.81, t, 7.0; 3H	0.80, t, 7.0; 3H	0.81, t, 7.0; 3H
OAc's	2.04, s; 3H	2.07, s; 3H 2.04, s; 3H	2.09, s; 3H 2.03, s; 3H 2.02, s; 3H	2.03, s; 3H 2.02, s; 3H 2.00, s; 3H

[a]Recorded in 15% $CD_3OD/CDCl_3$ at 400 MHz.

Compound **3** was also a glassy solid. Both the ^{1}H- (Table 3) and ^{13}C- (Table 1) nmr spectra suggest that compound **3** has two acetyl groups. The HMBC correlations from the C-6 protons (4.23 ppm) to the acetyl carbonyl carbon at 171.48 ppm and from the C-2′ proton (4.67 ppm) to the other acetyl carbonyl carbon (170.65 ppm) indicate that the oxygens on C-6 and C-2′ are acetylated. Therefore compound **3** is 6, 2′-di-*O*-acetyl-2-*O*-octadecanoyl-α,α,-D-trehalose. All of the observed HMBC and COSY correlations are consistent with the proposed structure. The fabms $[M+Na]^+$ peak is at 715 amu as in compound **2**. The fragment due to the loss of glucose residue B, however, is at 471 amu, indicating that there is one acetyl group in this residue.

Compound **4** was also a glassy solid. Both the ^{1}H- (Table 3) and ^{13}C- (Table 1) nmr spectra show that compound **4** has three acetyl groups. The HMBC correlations from the C-3 proton (5.27 ppm), the C-6 protons (4.23 ppm), and the C-2′ proton (4.70 ppm) to the three acetyl carbonyl carbons (171.45, 171.17, and 170.64 ppm) indicate that the oxygens on C-2, C-6, and C-2′ are acetylated. Therefore compound **4** is 2,6,2′-tri-*O*-acetyl-2-*O*-octadecanoyl-α,α-D-trehalose. All of the observed HMBC and COSY corre-

lations are consistent with the proposed structure. The fabms $[M+Na]^+$ peak is at 757 amu, 42 units more than that of compound **2**. The fragment due to the loss of glucose residue B is at 513 amu, indicating that there are two acetyls in this fragment.

Compound **5** was a foamy glass. Both the ^{1}H- (Table 3) and ^{13}C- (Table 1) nmr spectra show that compound **5** has three acetyl groups. The HMBC correlations from the C-3 proton (5.38 ppm), the C-6 protons (4.30 and 4.25 ppm), and the C-6′ protons (4.21 and 4.16 ppm) to the three acetyl carbonyl carbons at 171.59, 171.36, and 171.28 ppm, respectively, indicate that the oxygens on C-2, C-6, and C-6′ are acetylated. Therefore compound **5** is 2,6,6′-tri-*O*-acetyl-2-*O*-D-octadecanoyl-α,α-trehalose. All of the observed HMBC and COSY correlations are consistent with the proposed structure. The fabms $[M+Na]^+$ peak is at 757 amu as in compound **4**. The fragment due to the loss of glucose residue B is at 513 amu, indicating that there are two acetyl groups in this fragment.

The possibility that some acyl migration (8) has occurred during the separation process cannot be rigorously excluded. However, comparison of the tlc behavior of the pure components **1–5** with the crude extract suggests this is not the case.

A large number of trehalose esters, known as trehalose dimycolates (TDM), have been isolated from various species of bacteria belonging to the genera *Mycobacterium, Nocardia, Corynebacterium, Brevibacterium,* and *Arthrobacter* (9–11). However, unlike in compounds **1–5**, the long-chain acyl groups in trehalose dimycolates are almost exclusively located at the 6,6′ positions of the trehalose moiety. The lipid residues, with 30–90 carbons in most cases, normally have a side chain at the α position and a hydroxyl at the β position. It is commonly accepted that trehalose dimycolates are present primarily in the outer layer of the cell wall. It seems that the detergent properties of trehalose esters are used by bacteria for absorption of hydrocarbons from the medium. Many of these compounds have been found to be immunomodulators and antitumor agents. A large number of analogues with medium-sized lipid chains have been synthesized (12–14). Recently, a series of 2,3-di-*O*-acyl-trehalose derivatives has been isolated from *Mycobacterium tuberculosis* (15). Among fungal metabolites, however, trehalose esters appear not to have been reported previously. Since compounds **1–5** are produced by *H. lignicola* in large quantities, it seems possible that there trehalose esters play important biological roles in the organism. Preliminary experiments indicate that these compounds are not responsible for the antagonistic behavior of *H. lignicola* towards *C. crassivaginata* (P. Chakravarty and Y. Hiratsuka, Northern Forestry Center, Edmonton, personal communication).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All solvents used were distilled prior to use. ^{1}H-nmr spectra were recorded on either a Bruker AM400 or Varian Unity-500 spectrometer. All ^{13}C-nmr spectra were recorded on a Bruker AM400 spectrometer. All COSY and LR-COSY experiments were performed by using standard Bruker or Varian Unity programs. All HMQC and HMBC experiments were performed on a Varian Unity-500 spectrometer, with 2K data points in the F2 domain and 256 increments, and optimized for $^1J_{H/C}$=140 Hz and $^{2\text{-}3}J_{H/C}$=7 Hz. Details of culturing techniques have been described previously (16).

CULTURE CONDITIONS AND ISOLATION PROCEDURES.—*H. lignicola* cultures are deposited in the fungal culture collections at the Northern Forestry Center (NOF 1570), Forestry Canada, Edmonton, and the University of Alberta Microfungus Herbarium (UAMH 7002), Devonian Botanical Gardens, Edmonton, Alberta, Canada. *H. lignicola* was grown in malt extract/yeast extract liquid medium for 5 weeks (8 liters, shake culture, 125 rpm). The fine mycelium was removed by filtration to give a clear red broth, which was then concentrated to 1/10 volume under reduced pressure. The concentrated broth was extracted with EtOAc (500 ml×4). The combined organic extract was concentrated under reduced pressure to yield a foamy glass (12.8 g). A portion (6.23 g) of this extract was subjected to gel filtration chromatography on Sephadex LH-20 (MeOH) to yield three fractions (tlc). The first fraction (4.58 g) was chromatographed on Si gel (5–10% MeOH/CH_2Cl_2) to give three major subfractions. The second subfraction (2.25 g) contained mainly

compound **2**, which was further purified on a Si gel column [MeOH-Me_2CO-CH_2Cl_2 (2:40:60)] to give a foamy glass (50 mg from 80 mg). The third subfracton (0.23 g) contained mainly compound **1**, which was also further purified on Si gel (10% MeOH/CH_2Cl_2) to give a foamy glass (20 mg from 50 mg). The first subfraction (0.63 g) contained three major compounds (**3**, **4**, and **5**). Further chromatography of this mixture (100 mg out of 0.63 g) on Si gel [MeOH-Me_2CO-CH_2Cl_2 (2:25:75→2:30:70)] gave pure compound **4** (34 mg) and a mixture of compounds **3** and **5**, which were separated by Si gel chromatography (10% MeOH/CH_2Cl_2) to yield pure **3** (9.4 mg) and **5** (30 mg).

3,6-Di-O-acetyl-2-O-octadecanoyl-α,α-D-trehalose [**2**].—Obtained as foamy glass: $[\alpha]_D$ +97° (*c*=0.055, $CHCl_3$); ir ($CHCl_3$) λ max 3600–3100, 2923, 2853, 1744, 1367, 1237, 1111, 1049, 1017 cm^{-1}; fabms *m/z* (rel. int.) $[M+Na]^+$ 715 (4), 513 (39), 453 (14), 267 (32), 229 (20), 187 (48), 169 (33), 127 (100); hrfabms *m/z* 513.3433 ($C_{28}H_{49}O_8$, Δmmu=0.6), 267.2723 ($C_{18}H_{35}O$, Δmmu=−3.5); ^1H nmr see Table 2; ^{13}C nmr see Table 1.

Alkaline hydrolysis of compound **2**.—A solution of compound **2** (200 mg) in 95% EtOH (10 ml) and 5% NaOH (10 ml) was refluxed for 3 h. After cooling, the mixture was diluted to 100 ml, acidified to pH 5 with dilute HCl, and extracted with $CHCl_3$. Concentration of the $CHCl_3$ extract yielded stearic acid (76 mg, 93%), which was identical with the authentic sample. The aqueous solution was concentrated to dryness and acetylated with Ac_2O/pyridine/DMAP to produce α,α-D-trehalose octaacetate, which was also identical with the authentic sample (tlc, ir, $[\alpha]_D$, and nmr).

3-O-Acetyl-2-O-octadecanoyl-α,α,-D-trehalose [**1**].—Obtained as foamy glass: $[\alpha]_D$ +120° (*c*=0.16, $CHCl_3$); ir ($CHCl_3$) λ max 3600–3100, 2923, 2853, 1733, 1368, 1247, 1149, 1048, 1002 cm^{-1}; fabms *m/z* (rel. int.) $[M+Na]^+$ 673 (2), 471 (33), 443 (5), 267 (14), 187 (17), 155 (30), 119 (100); ^1H nmr see Table 3; ^{13}C nmr see Table 1.

6,2′-Di-O-acetyl-2-O-octadecanoyl-α,α-D-trehalose [**3**].—Obtained as foamy glass: $[\alpha]_D$ +103° (*c*=0.088, MeOH); ir ($CHCl_3$) λ max 3590, 3470, 3463, 2923, 2852, 1734, 1238, 1050, 1010 cm^{-1}; fabms *m/z* (rel. int.) $[M+Na]^+$ 715 (6), 471 (26), 453 (8), 267 (21), 205 (85), 187 (58), 169 (23), 127 (99), 109 (100); ^1H nmr see Table 3; ^{13}C nmr see Table 1.

3,6,2′-Tri-O-acetyl-2-O-octadecanoyl-α,α-D-trehalose [**4**].—Obtained as foamy glass: $[\alpha]_D$ +105° (*c*= 0.535, $CHCl_3$); ir ($CHCl_3$) λ max 3600–3100, 2924, 2853, 1745, 1370, 1234, 1150, 1099, 1048 cm^{-1}; fabms *m/z* (rel. int.) $[M+Na]^+$ 757 (4), 513 (15), 485 (2), 471 (2), 453 (8), 267 (16), 205 (65), 187 (48), 169 (20), 145 (40), 127 (83), 109 (89), 97 (100); ^1H nmr see Table 3; ^{13}C nmr see Table 1.

3,6,6′-Tri-O-acetyl-2-O-octadecanoyl-α,α-D-trehalose [**5**].—Obtained as foamy glass: $[\alpha]_D$ +82° (*c*=0.256, $CHCl_3$); ir ($CHCl_3$) λ max 3600–3200, 2923, 2853, 1744, 1368, 1237, 1147, 1040, 1009 cm^{-1}; fabms *m/z* (rel. int.) $[M+Na]^+$ 757 (15), 513 (24), 485 (2), 471 (2), 453 (11), 267 (21), 229 (15), 205 (17), 187 (66), 169 (26), 145 (34), 127 (99), 109 (100); ^1H nmr see Table 3; ^{13}C nmr see Table 1.

3,4,6,2′,3′,4′,6′-Hepta-O-acetyl-2-O-octadecanoyl-α,α-D-trehalose (peracetylated 2-O-octadecanoyl-α,α-D-trehalose).—Obtained as foamy glass: $[\alpha]_D$ +117° (*c*=0.52, $CHCl_3$); ir ($CHCl_3$) λ max 2925, 2854, 1754, 1368, 1224, 1038 cm^{-1}; fabms *m/z* (rel. int.) $[M+Na]^+$ 925 (0.02), 555 (3), 331 (14), 267 (12), 169 (94), 109 (100); ^1H nmr (400 MHz, $CDCl_3$) δ 5.49 (t, 9.8, 2H), 5.30 (d, 3.9, 2H), 5.05 (m, 4H), 4.24 (m, 2H), 4.00 (M, 4H) ppm; ^{13}C nmr (100 MHz, $CDCl_3$) δ 172.46 (s), 170.55 (s), 170.48 (s), 169.86 (s), 169.75 (s), 169.61 (s), 169.52 (s), 169.47 (s), 92.10 (d), 92.00 (d), 69.99 (d, 2C), 69.86 (d), 69.65 (d), 68.55 (d), 68.44 (d), 68.19 (d), 68.14 (d), 61.76 (t), 61.55 (t), 33.94 (t), 31.90 (t), 29.66 (t, *n*C), 29.59 (t), 29.44 (t), 29.33 (t), 29.24 (t), 29.11 (t), 24.90 (t), 22.66 (t), 20.64 (q), 20.61 (q), 20.56 (q, 5C), 14.08 (q) ppm.

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