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Enzyme-catalyzed regioselective transesterification of peracylated sophorolipids

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Abstract—Regioselective transesterifications and hydrolysis of peracylated sophorolipid (SL) derivatives catalyzed by lipases was investigated. This study is the first evaluation of the lipase-catalyzed reactions on the non-lactonic SL derivatives. Four lipases, namely from porcine pancreas (PPL, Type II), *Candida rugosa* (AYS, TypeVII), *Pseudomonas cepacia* (PS-30), and *Candida antarctica* (Novozym 435, carrier fixed lipase fraction B) were used in anhydrous THF or in phosphate buffer (pH=7.4, 0.2 M). It was confirmed from the detailed spectral analysis of the products that transesterification failed to furnish any free hydroxyls on the sophorose ring. Instead, transesterification took place on the methyl ester located at the carboxylic end of the 17-hydroxyoctadecenoic acid chain attached to the C-1′ position of the sophorose ring. It is proposed that in absence of the lactonic structural motif, the binding of the peracylated non-lactonic SLs in the lipase binding pocket takes place such that the carboxyl group of the octadecenoic acid, not the sophorose sugar, is preferentially accessible to the active site.
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1. Introduction

Sophorolipids, dimeric sophorose sugars (2′-*O*-β-D-glucopyranosyl-β-D-glucopyranose) linked β-glycosidically to a hydroxy-fatty acid, are amphipathic biomolecules produced by the yeast *Candida bombicola* (formerly *Torulopsis bombicola*)[1] or *Candida apicola*[2] from simple sugars and lipid substrates. Native sophorolipid is a complex mixture of up to 14 different compounds with the macrolactone and the sophorose glycoside being the major constituents of such mixtures.[3a–c] The fatty acid portion (17-hydroxyoctadecenoic acid) forms a 1′,4″ macrocyclic lactone ring (lactonic SLs) or has a free carboxylic end (acidic SLs) (Fig. 1). These molecules, because of their structure, naturally act as biosurfactants finding applications in the petroleum, pharmaceuticals and food processing industries where they can be used to reduce surface tension, stabilize emulsions, and promote foaming.[4] Compared to their synthetic counterparts, biosurfactants offer some distinct advantages: they can be produced from renewable resources or even industrial waste, they are biodegradable and non-toxic, they are environmentally friendly and are effective under extreme conditions in small quantities and are structurally diverse.[5a–c] In addition, biosurfactants can enhance the emulsification of hydrocarbons and therefore have the potential to solubilize hydrocarbon contaminants and increase their availability for microbial degradation.[6a–c]

There has been considerable interest in the physiological properties of sophorolipids, which have shown exciting potential in the treatment of a host of disorders. SLs have been reported to have caused differentiation and protein kinase C inhibition in the HL60 leukemia cell line.[7] Additionally they are useful as immunomodulators for Parkinson's disease, Alzheimer's disease, psoriasis, AIDS treatment, as well as for antiviral immunostimulation.[8] Consequently, there has been a great deal of interest in the synthesis of novel SL derivatives. To date, however, the primary strategy identified for the 'tailoring' of SL structure has been during in vivo formation by the selective-feeding of lipophilic substrates. For example, changing the co-substrate from sunflower to canola oil resulted in a large increase (50–73%) of the lactonic portion of SLs.[9] Interestingly, using oleic acid (alone or with glucose) increased the fraction of non-acetylated 1′,4″ sophorolipid lactone.[10] Unsaturated C-18 fatty acids such as oleic acid may be incorporated unchanged into sophorose lipids.[11]

A limited number of studies on selective in vitro enzymatic modifications of the natural SLs have been reported.[10,12a–d] Deacetylation of the 6′,6″-diacetylated lactonic SLs has been reported to result in formation of the 6′-hydroxy compound upon incubation with the enzymes acetylesterase,[10] cutinase,[12b] or lipases[12a] (from *Candida antarctica*-B, *Candida rugosa*, *Humicola* sp., porcine pancreas,

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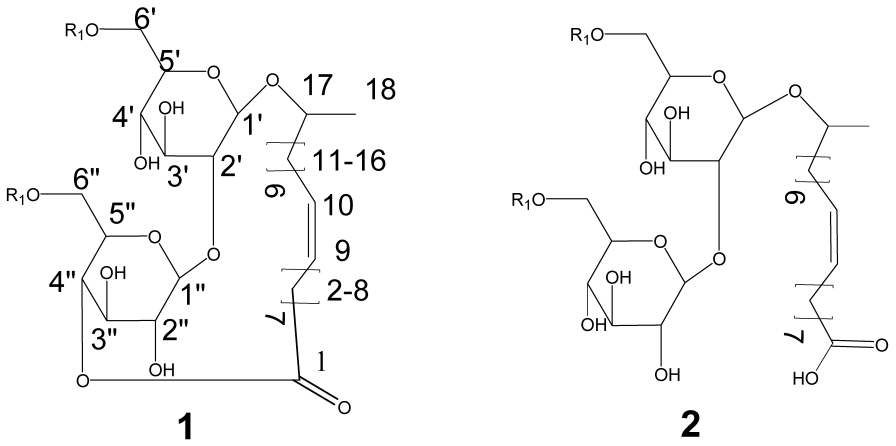


Figure 1. Classes of sophorolipid: the 1′, 4″ lactonic sophorolipid (lactonic SLs, **1**) and the acidic sophorolipid (acidic SLs, **2**) where R_1 is the acetyl group.

Pseudomonas sp., and *Mucor miehei*), while keeping the lactone ring intact.[12c,d] Enzymatic conversion of SLs to glucose lipids has also been reported using glycosidases, which released one glucose molecule from the disaccharide lipid.[13] Highly regioselective lipase Novozym-435 (*C. antarctica*-Lipase B)-catalyzed acylations of the C-6′ and C-6″ hydroxyl groups, in the non-lactonic SL methyl ester, have also been described for synthesis of well-defined sophorolipid analogs.[14a,b] Such derivatives are essential for evaluation of their bioactivities and for preparation of glycolipid-based polymers.[14a,b]

Lipase-catalyzed transformations provide a useful mean for the selective modification of sophorolipids and its derivatives since the glycosidic bonds between the glucose moieties and many acetyl groups may also be targets for hydrolysis under acidic or basic hydrolytic conditions. Utility of lipases in catalyzing selective transformations in lactonic sophorolipids has been previously demonstrated.[12c] Lipases, in addition to having a large fatty acid binding site are activated at the hydrophobic hydrophilic interfaces, which are likely to be present in the sophorolipids solutions. Our interest in lipases arises from their ability to catalyze reactions in non-aqueous solvents under relatively mild conditions with high chemo-, regio- and enantioselectivities, to accept a broad spectrum of substrates, and their biocompatibility.[15a–c]

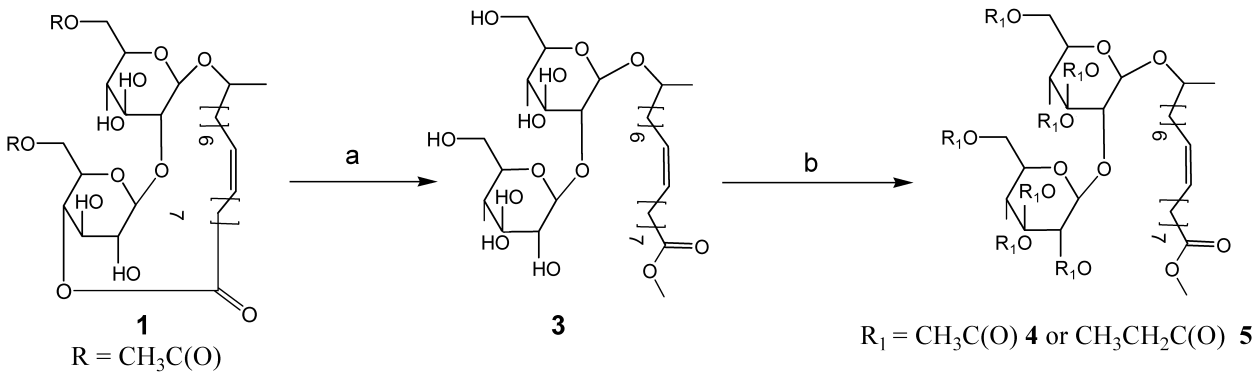
In this report, we describe and discuss regioselective transesterifications and hydrolysis of peracylated sophorolipid derivatives catalyzed by lipases. This study is the first evaluation of the lipase-catalyzed reactions on the peracylated non-lactonic SL derivatives. Four lipases, namely from Porcine pancreas (PPL, Type II), *C. rugosa* (AYS, TypeVII), *Pseudomonas cepacia* (PS-30), and *C. antarctica* (Novozym 435, carrier fixed lipase fraction B) were used in anhydrous THF or in phosphate buffer (pH=7.4, 0.2 M). Literature reports attest to the binding of the lactonic SLs in the lipase binding pocket such that the sophorose moiety is accessible to the active site.[12b] The data presented in this report suggests that in peracylated sophorolipids the absence of the 1′,4″-macrolactone ring results in its binding in the lipase active site such that the carboxyl end of the octadecenoic acid chain rather than that on the sophorose head group is preferentially accessible.

2. Results and discussion

Scheme 1 shows how the peracylated sophorolipids were synthesized from the corresponding naturally occurring lactonic sophorolipids (**1**). Methanolysis of the sophorolipid macrolactone with freshly prepared 0.022N sodium methoxide resulted in formation of the sophorolipid methyl ester (SL-Me) (**3**). Structural assignment of the methyl ester (**3**) was previously described using ^{1}H NMR, ^{13}C NMR, ^{1}H–^{1}H COSY and ^{1}H–^{13}C HETCOR spectral data.[14a] Since the naturally occurring SLs are a mixture of as many as 14 different compounds,[10] the use of the methyl ester intermediate provides a useful alternative to the otherwise cumbersome purification procedures while preserving the structural motif of the natural acidic SLs. The resulting SL-Me ester was acylated using acetic and propionic anhydride in dry THF in presence of dimethylamino pyridine (DMAP) as the catalyst to give the peracetylated and perpropionated sophorolipid methyl esters **4** and **5**, respectively. The structures of the peracetate and perpropionate **4** and **5**, respectively, were confirmed from their respective ^{1}H NMR and ^{13}C NMR spectral data. For example, the proton NMR spectra of **4** showed multiple new singlets at ~2.0 ppm which integrated for additional 21 hydrogens compared to 2 hydrogens of C-2 H signal at 2.3 ppm (Table 2). The ^{13}C NMR spectrum also showed additional resonance signals for the acetoxy carbonyls ($-C$(O)CH_3) between 169.2–170.6 ppm and the methyl ester carbonyl carbon (C-1) appeared at 174.2 ppm (Table 2). The structure of the perpropionate **5** was also established from its NMR spectral data and the assignments in the proton NMR were made using ^{1}H–^{1}H COSY correlations (Table 3). For example, in the ^{1}H–^{1}H COSY NMR spectrum, the propionyl methyl (C(O) CH_2CH_3) proton resonance signals in **5** at 1.01–1.17 ppm showed cross peaks to the methylene protons (C(O) CH_2CH_3) signals at 2.21–2.39 ppm. The integral ratio of the methyl protons (1.01–1.17 ppm, 21H), in the propionyl group, to the resonance signal of the C-9H and 10H signal (5.35 ppm, 2H) in **5** was used to confirm complete acylation of the SL-Me ester.

2.1. Screening of lipases

The screening for lipases that would accept the peracylated



Scheme 1. Synthesis of peracylated SL-Me ester: (a) 0.022N sodium methoxide, reflux, 30 min; (b) acetic anhydride or propionic anhydride, DMAP, dry THF (**4**, R_1=$CH_3C(O)$; **5**, R_1=$CH_3CH_2C(O)$).

SL-Me esters as substrates were carried out in a 50 mL round bottom flasks using 1:1 substrate to lipase ratio (w/w). The transesterification reactions were carried out in dry THF in presence of 1-butanol and 2-methylpropanol for 72 h. The hydrolysis reactions were evaluated by incubating the lipases with the substrates in phosphate buffer (pH=7.4, 0.2 M) for 72 h. Since the size of the scissile fatty acid binding pocket in lipases is known to vary considerably,[16] four lipases screened, namely, Novozym-435 (immobilized preparation from *C. antarctica*), PS-30 (from *P. cepacia*), PPL (from porcine pancreas) and AYS (from *C. rugosa*) exhibited different specificity for the SL substrates (Table 1). While no activity was seen for PPL and PS-30 lipases in any of the tested reaction media, formation of a single product was observed (different R_f values compared to the substrate) within 72 h upon incubation of the peracylated SL-Me ester substrates (**4** and **5**) with the lipase from *C. antarctica* (Novozym-435). The lipase AYS did not catalyze transesterifications in THF although was able to catalyze the hydrolysis of **4** and **5** in aqueous phosphate buffer (pH=7.4, 0.2 M). Interestingly, the specificity of the lipases Novozym-435 and lipase AYS was same for the peracetylated (**4**) and perpropionated (**5**) substrate, i.e. the corresponding products formed had the same R_f and same structures, confirmed from their NMR data as discussed in following sections.

2.2. Lipase-catalyzed regioselective transesterifications

Enzyme catalyzed deacetylation in aqueous buffer of 6′,6″-diacetylated lactonic SLs has been reported to give the corresponding 6′-hydroxy lactonic SL.[12a] It has been proposed, based upon 3D models, that the lactonic SL fit well in the binding pocket of several lipases and that while the 6″-OAc is buried inside the macrolactone structure the 6′-OAc is well accessible to the active site of the lipase.[17] The lipase catalyzed deacylation reactions, however, have only been reported with lactonic sophorolipids and no investigation of the acidic SLs has been reported to date.

Table 1. Screening of commercially available lipases for peracylated SL-Me esters transesterification after 72 h incubation

Media	Substrate **4** (**5**)[a] conversion (%)	
	Lipase AYS	Lipase Novozym
THF/*n*-butanol	NR	83 (77)
THF/*iso*-butanol	NR	58 (55)
Buffer (pH=7.4, 0.2 M)	91 (54)	85 (90)

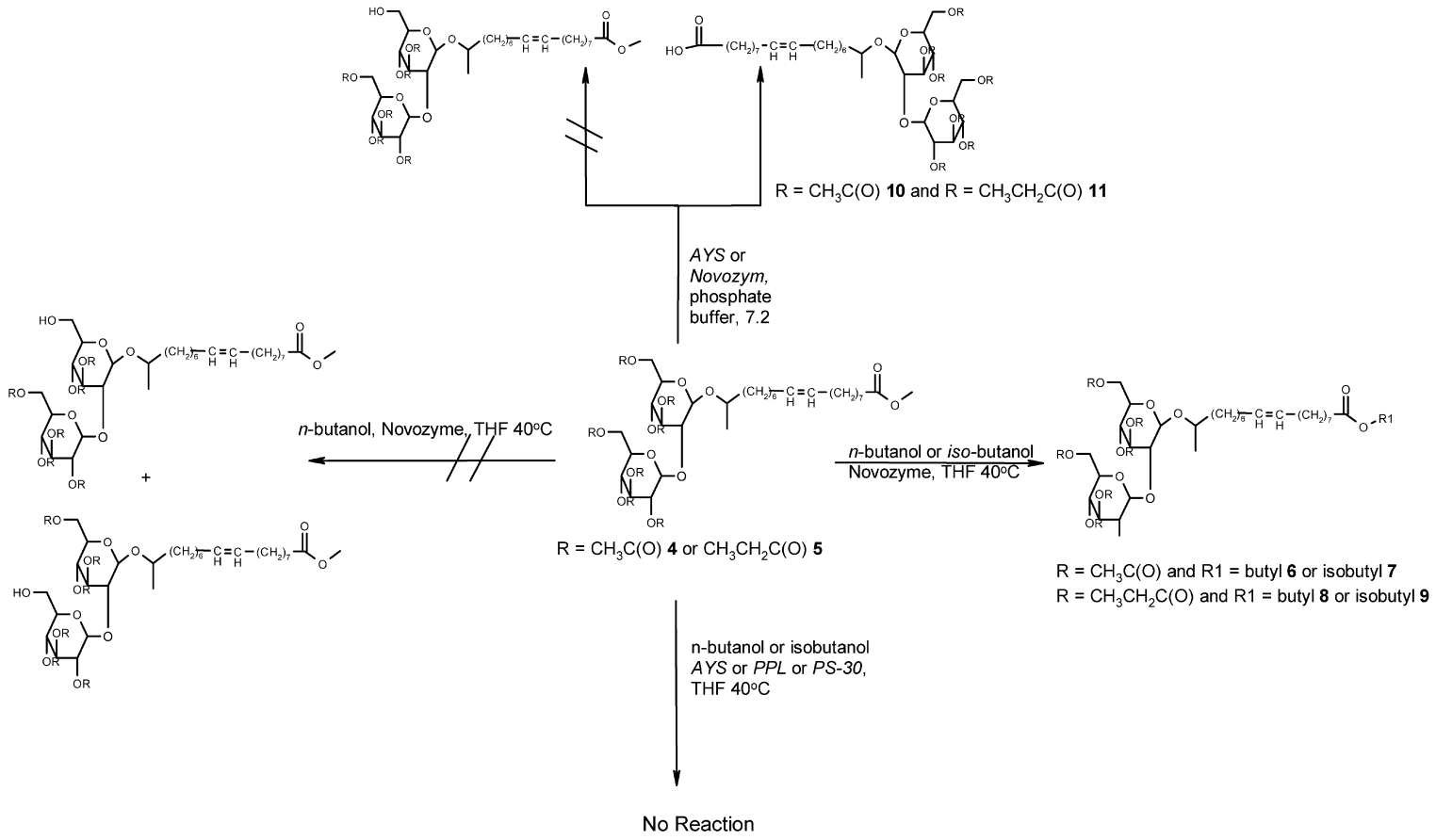
NR—no conversion observed.
[a] The value in parenthesis are conversions for perpropionate **5**. No reactions were observed in absence of lipase.

This is the first report on investigation of the lipase catalyzed transesterification/hydrolysis of peracylated acidic SLs. Since the only lipase that accepted peracylated SL-Me esters as substrates in transesterification reactions was Novozym-435, it was the only lipase used in further studies (Table 1). The transesterifications of the peracylated compounds **4** and **5** were conducted using excess 1-butanol and 2-methylpropanol in dry THF (Scheme 2). The product of the reaction was isolated by column chromatography. Upon comparison of the R_f values of the starting materials with the product, which had a higher value, the deacylation of one or more ester groups on the sophorose seemed unreasonable. The deacetylated product should have had R_f value lower than the starting material because of the resulting free hydroxyl group(s). It was confirmed from the detailed spectral analysis of the products that alcoholysis failed to furnish any free hydroxyls on the sophorose ring. Instead, transesterification took place on the methyl ester located at the carboxylic end of the 17-hydroxyoctadecenoic acid chain attached to the C-1′ position of the sophorose ring.

2.3. NMR spectroscopy

Detailed structural analysis of the products isolated from the transesterification reactions was undertaken using ^{1}H-, ^{13}C- and 2D NMR spectral data (Fig. 2, Tables 2 and 3). The ^{1}H NMR spectra of the products **6**–**11** when compared to the respective starting compounds **4** and **5**, varied little and because of a relatively short width of the spectra (0–10 ppm) unambiguous assignments were not possible (Tables 2 and 3). For example in proton NMR spectrum of product **6**, the integral value of the resonance attributed to the acetoxy methyl protons (1.98–2.08 ppm, 21H) was unchanged relative to the integral of the H-9 and H-10 vinylic protons (5.35 ppm, 2H). Although the sharp singlet for protons of the methyl ester between 3 and 5 ppm was absent in its proton NMR spectrum, structure of the product **6** could not be ascertained unambiguously from its ^{1}H NMR spectrum.

Bisht et al.[14a] had previously carried out detailed NMR



Scheme 2. Lipase-catalyzed hydrolysis and transesterification reactions of peracylated sophorolipid methyl esters.

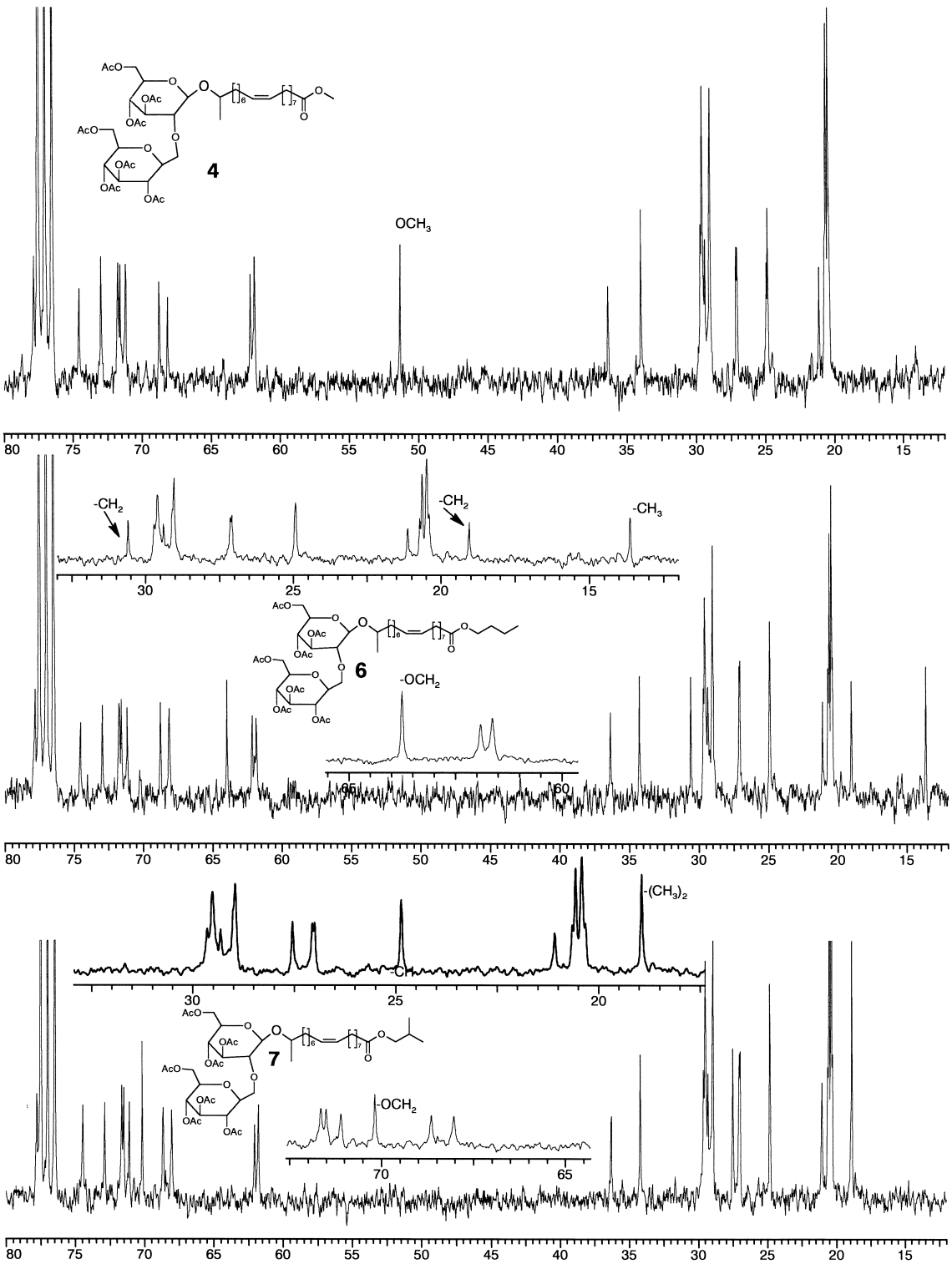


Figure 2. ^{13}C NMR spectra of peracetyl sophorolipid methyl ester and its derivatives.

analysis of the SL-methyl ester and noted limited utility of the ^{1}H NMR spectra in establishing the structure and assignment of various resonances.[14a] The ^{13}C NMR assignments were used to assign resonances in the proton spectra, utilizing correlations obtained from a $^{1}H-^{13}C$ HETCOR spectrum. In this paper, we will restrict ourselves to spectral information concerning the identification of the position of the various acyl groups. In particular, we will focus on the position of the transesterification reaction in the peracetate **4** upon its incubation with lipase Novozym in dry THF containing 1-butanol and extend the argument, in light of data observed, to products **7**–**9**.

In the ^{13}C NMR spectrum of the peracetylated sophorolipid methyl ester **4** (Fig. 2), the methyl carbon ($C(O)OCH_3$) resonates at 51.4 ppm. Upon comparing the ^{13}C NMR

Table 2. Assignment of ^{1}H and ^{13}C signals of sophorolipid-Me ester (**3**) and peracetylated sophorolipid derivatives (**4**, **6**, **7**, and **10**)

Position	**3**		**4**		**6**		**7**		**10**	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1	–	175.9	–	174.2	–	173.7	–	173.8	–	178.8
2	2.31, 2H, t, *J*=7.4 Hz	35.3	2.30, 2H, t, *J*=7.3 Hz	34.0	2.31, 2H, t, *J*=7.4 Hz	34.0	2.31, 2H, t, *J*=7.4 Hz	34.2	2.34, 2H, t, *J*=7.3 Hz	33.8
3, 15	1.60, 4H, bs	25.9, 26.2	1.60, 4H, bs	24.8, 24.9	1.60, 4H, bs	24.7	1.60, 4H, bs	24.9	1.60, 4H, bs	24.6, 24.9
4–7, 12–14, 16	1.32, 16H, bs	30.6–31.7, 38.2	1.31, 16H, bs	29.0–29.7, 36.4	1.32, 16H, bs	28.9–29.5, 36.2	1.31, 16H, bs	29.0–29.7, 36.3	1.31, 16H, bs	28.9–29.7, 36.3
8, 11	2.04, 4H, bs	28.6	1.98–2.08, 4H, m	27.1	1.98–2.08, 4H, m	26.9	1.98–2.08, 4H, m	27.0, 27.1	1.98–2.08, 4H, m	27.1
9, 10	5.35, 2H, bs	130.7, 130.8	5.35, 2H, bs	129.7, 129.8	5.35, 2H, bs	129.5, 129.6	5.35, 2H, bs	129.6, 129.8	5.35, 2H, bs	129.7, 129.9
17	3.82–3.87, 1H, m	79.4	3.66–3.73, 1H, m	77.5	3.66–3.73, 1H, bs	77.3	3.66–3.73, 4H, m	77.5	3.66–3.73, 4H, m	77.5
18	1.25, 3H, d, *J*=6.1 Hz	22.4	1.22, 3H, d, *J*=6.1 Hz	21.1	1.22, 3H, d, *J*=6.1 Hz	21.0	1.22, 3H, d, *J*=6.2 Hz	21.1	1.22, 3H, d, *J*=6.1 Hz	21.1
1′	4.45, 1H, d, *J*=7.5 Hz	105.1	4.49, 1H, d, *J*=7.6 Hz	101.0	4.49, 1H, d, *J*=7.5 Hz	100.9	4.49, 1H, d, *J*=7.6 Hz	101.0	4.49, 1H, d, *J*=7.6 Hz	101.1
2′	3.35–3.59, 1H, m	82.3	3.66–3.73, 1H, m	77.8	3.66–3.73, 1H, bs	77.6	3.66–3.73, 4H, m	77.8	3.66–3.73, 4H, m	77.8
3′	3.35–3.59, 1H, m	78.7	5.06–5.17, 1H, m	74.5	5.03–5.21, 1H, m	74.4	5.06–5.17, 3H, m	74.5	5.06–5.17, 3H, m	74.5
4′	3.20–3.31, 1H, m	71.9	5.06–5.17, 1H, m	67.8	5.03–5.21, 1H, m	67.9	5.06–5.17, 3H, m	68.0	5.06–5.17, 3H, m	68.1
5′	3.20–3.31, 1H, m	78.2	3.66–3.73, 1H, m	71.2	3.66–3.73, 1H, bs	71.0	3.66–3.73, 4H, m	71.1	3.66–3.73, 4H, m	71.1
6′	3.64, 2H, s	63.5	4.27, 2H, m	62.1	4.22–4.34, 2H, m	61.9	4.27, 2H, m	62.1	4.27, 2H, m	62.1
1″	4.64, 1H, d, *J*=7.7 Hz	103.2	4.75, 1H, d, *J*=7.6 Hz	100.3	4.74, 1H, d, *J*=7.7 Hz	100.1	4.75, 1H, d, *J*=7.6 Hz	100.2	4.75, 1H, d	100.3
2″	3.20–3.31, 1H, m	76.3	4.91–4.93, 1H, m	71.6	4.87–4.97, 2H, m	71.4	4.91–4.93, 2H, m	71.5	4.91–4.93, 2H, m	71.5
3″	3.35–3.59, 1H, m	78.2	5.06–5.17, 1H, m	72.9	5.03–5.21, 1H, m	72.8	5.06–5.17, 3H, m	72.9	5.06–5.17, 3H, m	72.9
4″	3.20–3.31, 1H, m	72.2	4.91–4.93, 1H, m	68.7	4.87–4.97, 2H, m	69.2	4.91–4.93, 2H, m	68.7	4.91–4.93, 2H, m	68.7
5″	3.64, 1H, s	78.7	3.66–3.73, 1H, m	71.7	3.66–3.73, 1H, bs	71.5	3.66–3.73, 4H, m	71.7	3.66–3.73, 4H, m	71.7
6″	3.82–3.87, 2H, m	63.1	4.08, 2H, d, *J*=10 Hz	61.8	4.03–4.10, 4H, bm	61.6	4.08, 2H, d, *J*=7.5 Hz	61.8	4.08, 2H, d	61.8
$-OCH_3$	3.64, 3H, s	52.00	3.66–3.73, 3H, m	51.4	–	–	–	–	–	–
$-C(O)CH_3$	–	–	–	169.2–170.6	–	169.0–170.3	–	169.1–170.5	–	169.3–170.6
$-C(O)CH_3$	–	–	1.98–2.08, 21H, m	20.4–20.7	1.98–2.08, 21H, m	20.2–21.0	1.98–2.08, 21H, m	20.3–21.1	1.98–2.08, 21H, m	20.4–20.7
$-OCH_2CH_2CH_2CH_3$	–	–	–	–	4.03–4.10, 4H, bm	63.8	–	–	–	–
$-OCH_2CH_2CH_2CH_3$	–	–	–	–	1.32, 2H, bs	30.5	–	–	–	–
$-OCH_2CH_2CH_2CH_3$	–	–	–	–	1.32, 2H, bs	18.8	–	–	–	–
$-OCH_2CH_2CH_2CH_3$	–	–	–	–	0.92, 3H, t, *J*=6.9 Hz	13.4	–	–	–	–
$-OCH_2CH(CH_3)_2$	–	–	–	–	–	–	3.85, 2H, d, *J*=6.7 Hz	70.2	–	–
$-OCH_2CH(CH_3)_2$	–	–	–	–	–	–	1.90–1.95, 1H, m	27.5	–	–
$-OCH_2CH(CH_3)_2$	–	–	–	–	–	–	0.93, 6H, d, *J*=6.7 Hz	18.9	–	–

Table 3. Assignment of ^{1}H and ^{13}C signals of perpropionyl sophorolipid derivatives (**5**, **8**, **9**, and **11**)

Position	**5**		**8**		**9**		**11**	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1	–	174.3	–	173.8	–	173.9	–	179.0
2	2.21–2.39, 2H, bm	34.0	2.21–2.35, 2H, bm	34.2	2.14–2.31, 2H, bm	34.2	2.18–2.36, 2H, bm	33.9
3, 15	1.60–1.63, 4H, bs	24.9, 25.0	1.58–1.64, 4H, bs	24.8	1.53–1.55, 4H, bs	24.9	1.60–1.63, 4H, bs	24.6, 24.9
4–7, 12–14, 16	1.30, 16H, bs	29.0–29.7, 36.4	1.31, 16H, bs	29.3–29.6, 36.3	1.24, 16H, bs	29.0–29.7, 36.3	1.31, 16H, bs	28.9–29.7, 36.4
8, 11	2.03, 4H, bs	27.1, 27.2	2.03, 4H, bs	27.1	1.96, 4H, bs	26.9, 27.1	2.03, 4H, bs	27.0, 27.2
9, 10	5.35, 2H, bs	129.6, 129.9	5.35, 2H, bs	129.5, 129.8	5.28, 2H, bs	127.5–127.8	5.35, 2H, bs	129.6, 129.9
17	3.65–3.71, 1H, m	77.3	3.67–3.74, 1H, m	77.2	3.60–3.67, 1H, m	77.2	3.67–3.74, 1H, m	77.3
18	1.16, 3H, d, J=5.1 Hz	21.1	1.21, 3H, d, J=6.0 Hz	21.1	1.14, 3H, d, J=5.8 Hz	21.1	1.14, 3H, d, J=5.1 Hz	21.1
1′	4.47, 1H, d, J=7.5 Hz	100.3	4.49, 1H, d, J=7.4 Hz	101.0	4.42, 1H, d, J=7.0 Hz	100.2	4.49, 1H, d, J=7.5 Hz	100.3
2′	3.65–3.71, 1H, m	77.8	3.67–3.74, 1H, m	77.7	3.60–3.67, 1H, m	77.8	3.67–3.74, 1H, m	77.8
3′	5.11–5.18, 1H, m	74.5	5.08–5.22, 1H, m	74.4	5.02–5.11, 1H, m	74.4	5.08–5.19, 1H, m	74.5
4′	4.93–4.95, 1H, m	68.6	4.90–4.94, 2H, m	68.6	4.83–4.91, 1H, m	69.0	4.91–4.98, 1H, m	68.6
5′	3.65–3.71, 1H, m	71.9	3.67–3.74, 1H, m	71.8	3.60–3.67, 1H, m	71.8	3.67–3.74, 1H, m	71.9
6′	4.27, 2H, m	62.1	4.21–4.33, 2H, m	62.0	4.14–4.21, 2H, m	62.0	4.27, 2H, m	62.1
1″	4.74, 1H, d, J=7.9 Hz	101.0	4.72, 1H, d, J=7.9 Hz	100.2	4.66, 1H, d, J=7.9 Hz	101.0	4.73, 1H, d, J=7.9 Hz	101.0
2″	4.93–4.95, 1H, m	71.3	4.90–4.94, 1H, m	71.3	4.83–4.91, 1H, m	71.2	4.91–4.98, 1H, m	71.3
3″	5.11–5.18, 1H, m	72.8	5.08–5.22, 1H, m	72.7	5.02–5.11, 1H, m	72.7	5.08–5.19, 1H, m	72.8
4″	5.11–5.18, 1H, m	68.0	5.08–5.22, 1H, m	67.9	5.02–5.11, 1H, m	67.8	5.08–5.19, 1H, m	67.9
5″	3.65–3.71, 1H, m	71.4	3.67–3.74, 1H, m	71.4	3.60–3.67, 1H, m	71.3	3.67–3.74, 1H, m	71.4
6″	4.11, 2H, d	61.8	4.08, 4H, d	61.7	4.02, 2H, d	61.7	4.09, 2H, d, J=12 Hz	61.8
$-OCH_3$	3.65–3.71, 3H, m	51.4	–	–	–	–	–	–
$-OC(O)CH_2CH_3$	–	172.7–174.0	–	172.5–173.4	–	172.5–173.6	–	172.7–174.0
$-OC(O)CH_2CH_3$	2.21–2.39, 14H, bm	27.1	2.21–2.35, 14H, bm	27.1	2.14–2.31, 14H, bm	27.1	2.18–2.36, 14H, bm	27.2
$-OC(O)CH_2CH_3$	1.01–1.17, 21H, brm	8.9	1.02–1.17, 21H, bm	8.74	0.97–1.07, 21H, bm	8.8	0.91–1.17, 21H, bm	8.8
$-OCH_2CH_2CH_2CH_3$	–	–	4.08, 4H, d	63.8	–	–	–	–
$-OCH_2CH_2CH_2CH_3$	–	–	1.31, 2H, bs	30.5	–	–	–	–
$-OCH_2CH_2CH_2CH_3$	–	–	1.31, 2H, bs	18.9	–	–	–	–
$-OCH_2CH_2CH_2CH_3$	–	–	0.94, 3H, t, J=7.2 Hz	13.5	–	–	–	–
$-OCH_2CH(CH_3)_2$	–	–	–	–	3.78, 2H, d, J=6.3 Hz	70.2	–	–
$-OCH_2CH(CH_3)_2$	–	–	–	–	1.86–1.89, 1H, m	27.1	–	–
$-OCH_2CH(CH_3)_2$	–	–	–	–	0.86, 6H, d, J=6.4 Hz	18.9	–	–

spectra of **4** and **6**, the disappearance of the methyl ester carbon (C(O)OCH_3) in product **6** and concomitant new resonance signals appearing at 63.8 (C(O)OCH_2–), 30.5 (C(O)OCH$_2$–CH_2–), 18.8 (C(O)OCH$_2$–CH$_2$–CH_2–) and 13.4 (C(O)OCH$_2$–CH$_2$–CH$_2$–CH_3) ppm suggested formation of the butyl ester upon Novozym-435 catalyzed transesterification in the presence of 1-butanol. Importantly, a downfield shift was observed for the three methylene-group protons in the butyl chain as compared to that of 1-butanol further suggesting formation of the butyl ester. The assignments were supported by a DEPT-135 and a $^1H-^{13}C$ HETCOR spectra (see Supporting Information). For example in the DEPT-135 spectrum of **6**, negative resonance signals at 63.8, 30.5 and 18.8 ppm and a positive resonance signal at 13.4 ppm were assigned to the methylene and the methyl carbons, respectively, of the butyl group. In the $^1H-^{13}C$ HETCOR spectrum a direct correlation was observed between the butyl carbons and corresponding protons allowing us to unambiguously assign the resonance signals of the hydrogens for this independent spin system (Tables 2 and 3). The methylene carbon at 63.8 along the f_2 axis of $^1H-^{13}C$ HETCOR spectrum showed correlation with the resonance signals on the f_1 axis at 4.03–4.10 ppm, which integrated to four hydrogens. The –OCH_2– hydrogens in the butyl ester were thus assigned to the resonance multiplet at 4.03–4.10 ppm. Furthermore, the two different methylene carbons at 30.5 and 18.8 ppm showed correlations to the broad singlet (4H) at 1.32 ppm and the methyl carbon at 13.4 ppm to the triplet (3H) at 0.92 ppm.

Importantly, carbon resonances of the sophorose, the acetates groups, and the fatty acid chain were relatively unchanged (Fig. 2). It was therefore concluded that regioselective transesterification of methyl ester took place and the acetate groups on sophorose sugar did not participate in this reaction. In a separate experiment, when the peracetate **4** was incubated with Novozym-435 in dry THF in presence of 2-methylpropanol under similar transesterification reaction conditions, disappearance of the methyl ester at 52.0 ppm was also observed with concomitant resonance signals appearing at 70.2 (C(O)OCH_2–), 27.5 (C(O)OCH$_2$C$H(CH_3)_2$), and 18.9 (C(O)OCH$_2$C$H(CH_3)_2$) ppm suggesting the formation of the isobutyl ester at the carboxyl end of the octadecenoic acid. Again, the carbon resonances of the sophorose moiety and the acetate groups were relatively unaffected (Fig. 2). It is important to mention that all reactions were carried out using 2:1 and 5:1 molar excess of the alcohol to the substrate and that the resulting product was the same regardless of the quantity of alcohol used. After column chromatographic purification yields were typically above 75%.

The ^{13}C NMR spectrum of the perpropionate **5** was more complex because of additional resonances arising from the propionate groups. Assignments shown in Table 3 were established using DEPT-135, $^1H-^{13}C$ HETCOR, and $^1H-^1H$ COSY spectra. The transesterification reaction catalyzed by Novozym in the presence of 1-butanol and 2-methylpropanol resulted in formation of the corresponding butyl (**8**) and isobutyl (**9**) esters, respectively at the carboxyl end of the octadecenoic acid. To reiterate, carbon resonances of the sophorose moiety and propionate groups were unchanged in products **8** and **9**, compared to the starting perpropionate methyl ester, **5**. It was thus established that Novozym catalyzed regioselective transesterification of the methyl ester in the peracetate **5** and perpropionate **6**.

Hu et al.[12d] have recently reported lipase catalyzed deacetylation of 6′,6″-diacetoxy lactonic SL in anhydrous organic solvents suggesting that the lactonic SL fit well in the binding pocket of several lipases such that 6′-OAc is well accessible to the active site. Therefore, it was somewhat intriguing that the non-lactonic (or acidic) peracylated SLs were not deacylated under the reaction condition described in this report. Bisht et al.[14a] have previously investigated lipase catalyzed acylation of non-lactonic SL-methyl ester (**3**) in anhydrous organic solvents. In absence of an acylating agent, the authors reported formation of a (17-hydroxyoctadec-9-enoic acid)-1′,6″-lactonized sophorolipid. The observation made by Bisht et al.[14a] and those made in this report suggest that presence of the macrolactone is necessary for proper placement of the peracylated sophorose moiety in the binding pocket of a lipase such that it is accessible to the active site of the lipase for deacylation of the sophorose esters in SLs. In this report, we suggest that the macrolactone provides a structural motif to the SLs that allow it to fit in the binding cavity of the lipase such that an acylated sophorose moiety is accessible to the active site. In absence of the lactonic structural motif, the binding of the SLs in the lipase binding pocket takes place such that the carboxyl group of the octadecenoic acid, not the acyl groups on sophorose sugar, is preferentially accessible to the active site.

The formation of 6′-acyloxy-(17-hydroxyoctadec-9-enoic acid)-1′,6″-lactonized sophorolipid in two steps, reported by Bisht et al.,[14a] thus can be rationalized as a two step mechanistic process. In the first step, the SL methyl ester binds such that an enzyme-activated complex (EAC) is formed upon attack of the Ser-105 (in the active triad of the lipase-B from *C. antarctica*) on the carbonyl of the methyl ester group in the 17-hydroxyoctadec-9-enoic acid chain. In absence of another nucleophile (the reaction was done under anhydrous solvents) the 6″-hydroxyl of the sophorose attacks the EAC to yield a macrolactone. In the second step, the macrolactone reenters the binding pocket such that the sophorose unit is now accessible to the active site and the acylation proceeds to give the 6′-acyloxy-(17-hydroxyoctadec-9-enoic acid)-1′,6″-lactonized sophorolipids in presence of an acylating agent. If this sequence of events were actually what was happening in the lipase active site then the presence of another nucleophile, during the first step, would not result in formation of a macrolactone. If the nucleophile were an alcohol, a transesterification of the methyl ester and the alcohol would result.

To explore this hypothesis, we preformed an experiment to investigate formation of the (17-hydroxyoctadec-9-enoic acid)-1′,6″-lactonized sophorolipids as described previously by Bisht et al.[14a] but in presence of a nucleophile, i.e. 1-butanol. The SL-Me ester was incubated with Novozym in THF at 40°C in presence of 3 mol equiv. of 1-butanol for 72 h (Scheme 3). Progress of the reaction was monitored by

thin layer chromatography. The product **12** was characterized from its detailed NMR spectral analysis. The ^{1}H NMR spectrum of **12**, had the methyl ester singlet missing, observed at 3.64 ppm (3H) in the proton NMR spectrum of **3**, with concomitant appearance of a triplet at 3.96 ppm (2H), a broad singlet at 1.21 ppm (4H) and another triplet at 0.84 ppm (3H) suggesting the formation of the butyl ester. The triplets at 3.96 and 0.84 ppm were assigned to the methyleneoxy ($-C(O)OCH_2$) and the methyl ($-CH_3$) groups, respectively, and the broad singlet at 1.21 ppm was assigned to the two methylene groups ($-CH_2CH_2-$) of the butyl ester. These assignments were supported by the ^{13}C NMR spectrum in which the $-OCH_3$ signal at 52.0 ppm, observed in **3**, disappeared with new resonance signals appearing at 65.2, 31.8, 20.2 and 14.1 ppm for the butyl carbons. The carbon resonances of the sophorose moiety were unperturbed, i.e. formation of the macrolactone was not observed. The structure of the resulting product (Scheme 3) was thus determined to be butyl-17-L-[(2β-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)-oxy]-*cis*-9-octadecenoate (**12**), i.e. butyl ester of sophorolipids.

2.4. Lipase-catalyzed hydrolysis

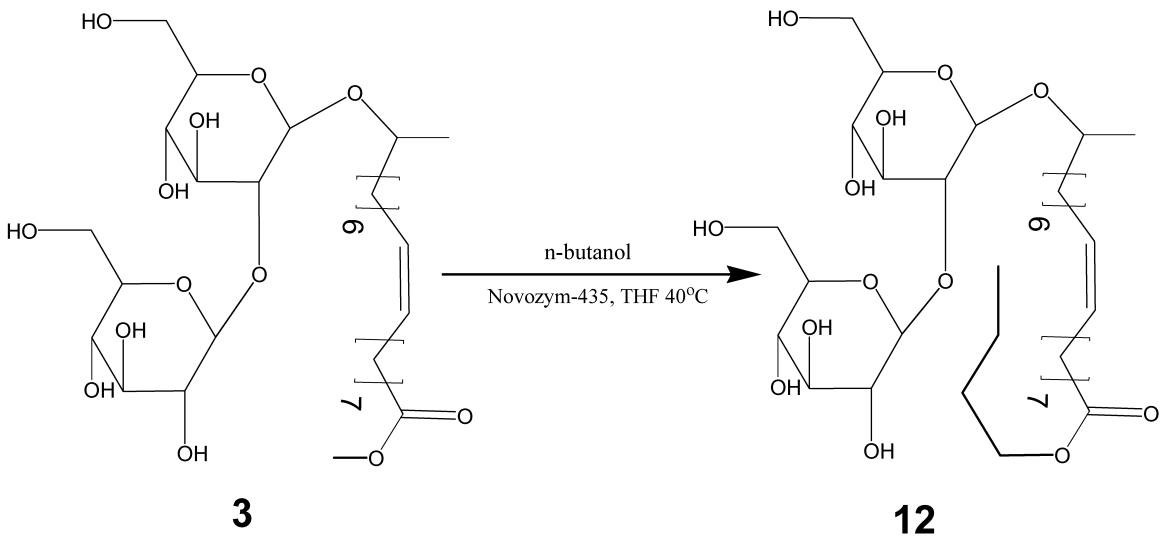
Otto et al.[12a] have described the selective deacetylation of lactonic sophorolipid 6′,6″-diacetate using lipase AYS (from *C. rugosa*) in a phosphate buffer medium (pH=7.4, 0.2 M). The author reported that the 6′,6″-diacetate was converted to the 6′-hydroxy lactonic sophorolipid. Choice of a reaction medium in an enzymatic reaction is very important because organic solvents may alter enzyme conformation or at worst denature the enzyme.[16] Hydrolysis of the peracetate **4** and perpropionate **5** was therefore investigated upon incubation with the lipases Novozym-435 and AYS in phosphate buffer (pH=7.4, 0.2 M) at 25°C.

For the current study, the reaction procedure needed to be modified due to poor solubility of the compounds **4** and **5** in buffer. A common approach for improving the biocatalytic reaction rates of water insoluble substrates is the use of cosolvents.[15c] Compounds **4** or **5** was therefore dissolved in minimum amount of acetone prior to their addition to the buffer solution followed by addition of the lipase. The reaction product was isolated upon its extraction in ethyl acetate followed by rotoevaporation of the solvent. In separate reactions with compound **4** and **5** only one hydrolysis product in each was isolated. In the ^{1}H NMR spectra of the compound **10** and **11**, isolated from reaction of **4** and **5**, respectively, the methyl ester resonance between 3.65 and 3.73 ppm disappeared. The ^{13}C NMR spectra of **10** and **11** corroborated the hydrolysis of the methyl ester. For example, in the ^{13}C NMR spectrum of the product **10**, the resonance for *O*-methyl ester carbon ($C(O)OCH_3$) at 51.4 ppm had disappeared along with an observed downfield shift in the carboxylic acid carbonyl carbon to 178.8 ppm (in **4**). More significantly, the resonances for sophorose carbons and acetate groups remained unchanged confirming that sophorose acetate esters were not hydrolyzed by the lipase under reaction conditions described. Similarly, the ^{13}C NMR spectrum of the product **11** (of the perpropionate **5**) also had the *O*-methyl ester carbon ($C(O)OCH_3$) resonance missing at 51.4 ppm along with a carboxyl carbonyl carbon signal shifted downfield to 179 ppm. The resonances for the sophorose carbons and the perpropionates groups were unchanged in the ^{13}C NMR spectrum of **11** reaffirming that deacylation of the propionate esters was not catalyzed by the lipase. The NMR results thus established that enzymatic hydrolysis of the peracylated SL-Me esters **4** and **5** by Novozym-435 and AYS occurred with high chemo and regioselectivity, i.e. only hydrolysis of the methyl ester took place while the peracyl esters were left untouched. These observations lend additional support to the proposition that the macrolactone ring is necessary for proper binding of the SLs in the lipase binding pocket such that the acyl ester on the sophorose moiety are accessible to the lipase active site.

3. Experimental

3.1. Chemicals and enzymes

All reagents were purchased from commercial sources and used as received. All solvents were purified and dried prior



Scheme 3. Formation of the butyl ester of sophorolipid methyl ester.

to use by known literature procedures. Prior to their use, the sophorolipids were dried over P_2O_5 in a vacuum desiccator (0.1 mm Hg, 12 h, 56°C). Porcine pancreatic lipase (PPL) Type II Crude (activity=61 units/mg protein) and *C. rugosa* lipase (AYS) TypeVII (activity=4570 u/mg protein) were purchased from the Sigma Chemical Co. The lipase PS-30 from *P. cepacia* (20000 units/g) was obtained from Amano Enzymes Co., Ltd. The carrier fixed lipase Novozym 435 (from *C. antarctica*, fraction B; specified activity at pH 7.0 is 10000 units/g) was a gift from Novo Nordisk Inc.

Column chromatographic separations were performed over silica gel 60 (Silicycle Inc.) using ethyl acetate/hexane mixture as the eluent.

3.2. Instrumental methods

^{1}H NMR and ^{13}C NMR spectra were recorded using Bruker ARX-250 spectrometer. Chemical shifts in parts per millions are reported downfield from 0.00 ppm using deuterated chloroform (unless stated otherwise) with trimethylsilane (TMS) as the internal reference. Unambiguous assignments were derived from COSY and HETCOR spectra. The following abbreviations are used to present the spectral data: s=singlet, bs=broad singlet, bm=broad multiplet, d=doublet, t=triplet.

Optical rotations were measured with an AutoPol-IV (Rudolph research analytical) automatic polarimeter. IR spectra were recorded on a Nicolet Avatar Smart Miracle 320 FT-IR spectrometer. High resolution mass analyses (HRESIMS) were obtained using a JEOL JMS-600H spectrometer at the Florida State University's (Tallahassee) Mass Spectral Analysis Facilities.

3.2.1. Synthesis of methyl 17-L-([2β-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl]-oxy)-*cis*-9-octadecenoate (SL-Me, 3).[14a] In a 100 mL round-bottomed flask equipped with a reflux condenser 10 g of dry crude sophorolipid and 10 mL 0.022N freshly prepared sodium methoxide in methanol were added. The reaction assembly was protected from atmospheric moisture by a $CaCl_2$ guard tube. The reaction mixture was refluxed for 3 h, cooled to room temperature (25°C), and neutralized using glacial acetic acid. The reaction mixture was concentrated by rotoevaporation and poured with stirring onto 100 g of crushed ice that resulted in the precipitation of the sophorolipid methyl ester as a white solid. The white precipitate was filtered, washed with ice-water, and dried overnight under pressure in a vacuum oven (8.77 g, yield=95.0%).

3.2.2. Synthesis of methyl 17-L-([2″,3′,3″,4′,4″,6′,6″-heptaacetoxy-2′-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl]-oxy)-*cis*-9-octadecenoate (4). In a 50 mL round-bottomed flask was added 2 g (3.06 mmol) of methyl 17-L-([2′-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl]-oxy)-*cis*-9-octadecenoate (**3**) and dissolved in dry THF (30 mL). Acetic anhydride 8 mL (8.47×10^{-2} mol) and 0.15 g of dimethylamino pyridine (DMAP) was added to the solution and the reaction was allowed to stir at room temperature for 6 h while protected from atmospheric moisture by a $CaCl_2$ guard tube. The reaction mixture was concentrated by rotoevaporation followed by extraction with ethyl acetate and washing with sodium bicarbonate solution. The organic layer was dried over anhydrous sodium sulfate and concentrated by rotoevaporation. The viscous liquid was then dried overnight in a vacuum desiccator at 40°C to yield the peracetylated sophorolipid methyl ester (**4**) (2.79 g, yield=98%) as a clear viscous liquid. $[\alpha]_D^{25}=-7.3°$ ($CHCl_3$, c=0.01); IR ν 2923, 1748, 1430, 1376, 1240, 1037, 888 cm^{-1}; HRESIMS m/z: 930.4466 $[M+Na]^+$ (calcd for $C_{45}H_{70}O_{20}$, 930.4461).

3.2.3. Synthesis of methyl 17-L-([2″,3′,3″,4′,4″,6′,6″-heptapropionyloxy-2′-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl]-oxy)-*cis*-9-octadecenoate (5). A procedure similar to the one above for the peracetylated sophorolipid methyl ester (**4**) was used to prepare the perpropionated sophorolipid methyl ester. Sophorolipid methyl ester **3** (2.0 g, 3.06 mmol), propionic anhydride (10.0 g, 7.68×10^{-2} mol) and DMAP (0.15 g) were dissolved in dry THF (30 mL). The reaction mixture was allowed to stir for 6 h at room temperature. After usual workup the perpropionated sophorolipid methyl ester (**5**) was isolated as a light amber colored viscous liquid (3.0 g, yield=97%). $[\alpha]_D^{25}=-4.5°$ ($CHCl_3$, c=0.01); IR ν 2936, 1740, 1450, 1351, 1165, 1053, 1012, 813 cm^{-1}; HRESIMS m/z: 1028.5571 $[M+Na]^+$ (calcd for $C_{52}H_{84}O_{20}$, 1028.5556).

Screening of lipases and general procedure of lipase catalyzed transesterification reactions. In a 50 mL round-bottomed flask was added the appropriately substituted analogue (**4** or **5**, ~0.8 g) dissolved in dry THF (30 mL). To this solution, the appropriate enzyme (AYS, Novozym-435, PPL, PS-30; ~0.4 g) was added followed by addition of ~6 molar equivalent of *n*-butanol (or *iso*-butanol) and the reaction mixture was stirred at 40°C for 72 h. Novozym-435 was found to be the only enzyme that catalyzed the transesterification reaction and was used in subsequent reactions. The reaction mixture was then filtered through a bed of celite and concentrated by rotoevaporation. The resulting crude product was purified by wet column chromatography using an ethyl acetate–hexane mixture as eluent.

3.2.4. Synthesis of *n*-butyl 17-L-([2″,3′,3″,4′,4″,6′,6″-heptaacetoxy-2′-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl]-oxy)-*cis*-9-octadecenoate (6). Transesterification procedure similar to the one above was used to prepare **6** (0.159 g, yield=83%) as a light amber viscous liquid. $[\alpha]_D^{25}=-5.9°$ ($CHCl_3$, c=0.01); IR ν 2921, 1749, 1376, 1229, 1045, 903 cm^{-1}; HRESIMS m/z: 972.4937 $[M+Na]^+$ (calcd for $C_{48}H_{76}O_{20}$, 972.4930).

3.2.5. Synthesis of *n*-butyl 17-L-([2″,3′,3″,4′,4″,6′,6″-heptapropionyloxy-2′-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl]-oxy)-*cis*-9-octadecenoate (8). Transesterification procedure similar to the one above was used to prepare **8** (0.645 g, yield=77%) as an amber colored viscous liquid. $[\alpha]_D^{25}=-5.2°$ ($CHCl_3$, c=0.01); IR ν 2942, 2871, 1739, 1450, 1352, 1169, 1057, 1031, 804 cm^{-1}; HRESIMS m/z: 1070.6022 $[M+Na]^+$ (calcd for $C_{55}H_{90}O_{20}$, 1070.6026).

3.2.6. Synthesis of isobutyl 17-L-([2″,3′,3″,4′,4″,6′,6″-heptaacetoxy-2′-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl]-oxy)-*cis*-9-octadecenoate (7). Transesterification procedure similar to the one above was used to prepare **7**

(0.245 g, yield=58%) as a light amber colored viscous liquid. $[\alpha]_D^{25}=-6.7°$ ($CHCl_3$, c=0.01); IR ν 2929, 2840, 1757, 1374, 1227, 1036, 906 cm^{-1}; HRESIMS m/z: 972.4931 $[M+Na]^+$ (calcd for $C_{48}H_{76}O_{20}$, 972.4930).

3.2.7. Synthesis of isobutyl 17-L-([2″,3′,3″,4′,4″,6′,6″-heptapropionyloxy-2′-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl]-oxy)-*cis*-9-octadecenoate (9). Transesterification procedure similar to the one above was used to prepare **9** (0.231 g, yield=55%) as a light amber colored viscous liquid. $[\alpha]_D^{25}=-4.2°$ ($CHCl_3$, c=0.01); IR ν 2929, 1735, 1454, 1347, 1174, 1049, 1022, 804 cm^{-1}; HRESIMS m/z: 1070.6038 $[M+Na]^+$ (calcd for $C_{55}H_{90}O_{20}$, 1070.6026).

General procedure of lipase screening for hydrolysis reactions. To a 50 mL Erlenmeyer flask containing 20 mL phosphate buffer solution (pH=7.4, 0.2 M), the appropriately substituted sophorolipid analogue dissolved (~0.8 g) in 5mL acetone was added. To this solution, lipase (AYS or Novozym-435, ~0.4 g) was added and the flask was closed with a rubber stopper (to prevent evaporation of the co-solvent). The reaction mixture was stirred at room temperature for 72 h. The reaction mixture was extracted with ethyl acetate (3×30 mL), and the organic layer was dried over anhydrous sodium sulfate and concentrated by rotoevaporation. Purification was achieved using ethyl acetate–hexane as the eluent in a silica gel chromatographic column.

3.2.8. Synthesis of 17-L-([2″,3′,3″,4′,4″,6′,6″-heptaacetoxy-2′-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl]-oxy)-*cis*-9-octadecenoic acid (10). Hydrolysis procedure similar to the one above was used to prepare **10** (Novozym-435, yield 85%; AYS, yield 91%) as a clear viscous liquid. $[\alpha]_D^{25}=-11.1°$ ($CHCl_3$, c=0.01); IR ν 2925, 2854, 1746, 1366, 1218, 1019 cm^{-1}; HRESIMS m/z: 916.4308 $[M+Na]^+$ (calcd for $C_{55}H_{90}O_{20}$, 916.4304).

3.2.9. Synthesis of 2″,3′,3″,4′,4″,6′,6″-heptapropionyloxy-17-L-([2′-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl]-oxy)-*cis*-9-octadecenoic acid (11). Hydrolysis procedure similar to the one above was used to prepare **11** (Novozym-435, yield 90%; AYS, yield 54%) as a light amber viscous liquid. $[\alpha]_D^{25}=-5.0°$ ($CHCl_3$, c=0.01); IR ν 2929, 2846, 1754, 1362, 1164, 1058, 1021 cm^{-1}; HRESIMS m/z: 1014.5399 $[M+Na]^+$ (calcd for $C_{51}H_{82}O_{20}$, 1014.5400).

3.2.10. Synthesis of butyl 17-L-([2′-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl]-oxy)-*cis*-9-octadecenoate (12). In a 50 mL round bottomed flask was added 0.1 g (0.153 mmol) of **5** and dissolved in dry THF (3 mL). To this solution, 0.1 g of Novozym-435 was added followed by 0.057 g (0.077 mmol) of *n*-butanol and the reaction was stirred at 40°C for 72 h. The reaction mixture was then filtered over a celite bed and concentrated by rotoevaporation. The resulting crude product was purified by silica gel column chromatography using a 35:65 ethyl acetate–hexane mixture as eluent to afford **12** (0.103 g, yield=95%); IR ν 3328, 3248, 2921, 2846, 1749, 1258, 1078, 1011, 813 cm^{-1}; HRESIMS m/z: 678.4195 $[M+Na]^+$ (calcd for $C_{34}H_{62}O_{13}$, 678.4190). ^1H NMR (250 MHz, CD_3OD) δ 0.84 (3H, t, J=7.5 Hz, $-O(CH_2)_3CH_3$), 1.21 (16H, brs, H-4-7,12-14,16), 1.21 (4H, brs, $-OCH_2(CH_2)_2CH_3$), 1.50 (4H, bs, H-3,15), 1.93 (4H, bs, H-8 and -11), 2.20 (2H, t, J=7.5 Hz, H-2), 3.16 (4H, m, H-2″,-4″,-4′, and -5′), 3.34 (3H, m, H-3″,H-3′,H-2′), 3.54 (2H, m, H-6′), 3.76 (3H, m, H-6″ and H-17), 3.96 (2H, t, $-OCH_2(CH_2)_2CH_3$), 4.35 (1H, d, J=7.5 Hz, H-1′), 4.54 (1H, J=7.5 Hz, H1″) and 5.24 (2H, bs, H-9 and -10); ^{13}C NMR (62.5 MHz): 14.1, 20.2, 21.9, 24.8, 26.1, 26.2, 28.1, 28.1, 30.1, 30.2, 30.4, 30.7, 30.8, 30.9, 31.8, 35.1, 37.8, 62.6, 63.0, 65.2, 68.2, 71.3, 71.7, 75.8, 77.7, 78.2, 78.3, 78.9, 81.7, 102.7, 104.6, 130.7, 130.9, 175.6.

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References

1. Cooper, D. G.; Paddock, D. A. *Appl. Environ. Microbiol.* **1984**, *47*, 173.
2. Stüwer, O.; Hommel, R.; Haferburg, D.; Kleber, H. P. *J. Biotechnol.* **1987**, *6*, 259.
3. (a) Tulloch, A. P.; Spencer, J. F. T.; Gorin, P. A. J. *Can. J. Chem.* **1962**, *40*, 1326. (b) Tulloch, A. P.; Hill, A.; Spencer, J. F. T. *Can. J. Chem.* **1968**, *46*, 3337. (c) Asmer, H. J.; Lang, S.; Wagner, F.; Wray, V. *J. Am. Oil Chem. Soc.* **1988**, *65*, 1460.
4. (a) Haferburg, D.; Hommel, R.; Claus, R.; Klebel, H. P. *Adv. Biochem. Engng Biotechnol.* **1986**, *33*, 53. (b) Busscher, H. J.; Neu, T. R.; Van der Mei, H. C. *Appl. Microbiol. Biotechnol.* **1994**, *41*, 4. (c) Willumsen, P. A.; Karlson, U. *Biodegradation* **1997**, *7*, 415.
5. (a) Fiechter, A. *Trends Biotechnol.* **1992**, *10*, 208. (b) Lin, S. C. *J. Chem. Tech. Biotechnol.* **1996**, *66*, 109. (c) Rahman, K. S. M.; Rahman, T. J.; McClean, S.; Marchant, R.; Banat, I. M. *Biotechnol. Prog.* **2002**, *18*, 1277.
6. (a) Koch, A. K.; Kappeli, O.; Fiechter, A.; Reiser, J. *J. Bacteriol.* **1991**, *173*, 4212. (b) Goswami, P.; Singh, D. *Biotechnol. Bioengng* **1991**, *37*, 1. (c) In *Biosurfactants: Production, Properties, Applications*; Kosaric, N., Ed.; Marcel Dekker: New York, 1993.
7. Isoda, H.; Kitamoto, D.; Shinmoto, H.; Matsumura, M.; Nakahara, T. *Biosci. Biotechnol. Biochem.* **1997**, *61*, 609.
8. Piljac, G.; Piljac, V. US Patent 5,514,661, 1996. Piljac, G.; Piljac, V. US Patent 5,455,232, 1995.
9. Zhou, Q. H.; Kosaric, N. *J. Am. Oil Chem. Soc.* **1995**, *72*, 67.
10. Asmer, H. J.; Lang, S.; Wagner, F.; Vray, V. *J. Am. Oil Chem. Soc.* **1988**, *65*, 1460.
11. Rau, U.; Manzke, C.; Wagner, F. *Biotechnol. Lett.* **1996**, *18*, 149.
12. (a) Otto, R. T.; Daniel, H. J.; Pekin, G.; Müller-Decker, K.; Fürstenberger, G.; Reuss, M.; Syldatk, C. *Appl. Microbiol. Biotechnol.* **1999**, *52*, 495. (b) De Koster, C. G.; Heerma, W.; Pepermans, H. A. M.; Groenewegen, A.; Peters, H.; Haverkamp, J. *Anal. Biochem.* **1995**, *230*, 135. (c) Weber, L.; Stach, J.; Haufe, G.; Hommel, R.; Kleber, H. P. *Carbohydr. Res.* **1990**, *206*, 13. (d) Hu, Y.; Ju, L. *Biotechnol. Prog.* **2003**, *19*, 303.

13. Rau, U.; Heckmann, R.; Wray, V.; Lang, S. *Biotechnol. Bioengng* **1999**, *63*, 233.
14. (a) Bisht, K. S.; Gross, R. A.; Kaplan, D. L. *J. Org. Chem.* **1999**, *64*, 780. (b) Bisht, K. S.; Gao, W.; Gross, R. A. *Macromolecules* **2002**, *33*, 6208.
15. (a) Kondaveti, L.; Al-Azemi, T. F.; Bisht, K. S. *Tetrahedron: Asymmetry* **2002**, *13*, 129–135. (b) Al-Azemi, T. F.; Kondaveti, L.; Bisht, K. S. *Macromolecules* **2002**, *35*, 3380–3386. (c) Carr, J. A.; Al-Azemi, T. F.; Long, T. E.; Shim, J.-Y.; Coates, C. A.; Turos, E.; Bisht, K. S. *Tetrahedron*, accepted for publication.
16. *Biotransformations In Organic Chemistry*. 4th ed. Faber, K., Ed.; Springer: Berlin, 200; pp 13–15.
17. Pleiss, J.; Fischer, M.; Schmid, R. D. *Chem. Phys. Lipids* **1998**, *93*, 67.