Effect of Substrate on Sophorolipid Properties

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ABSTRACT: This research demonstrates that the formation of a crystalline sophorolipid product by *Candida bombicola* can be attributed to two overlapping types of discrimination by the enzymes of this yeast. The first of these is a preference for direct incorporation of hydrocarbons having a chain length between 15 and 18 carbon atoms if these are in the medium. The second is a preference for the formation of the diacetylated lactone structure if hydroxycarboxylic acids that are 16 or 17 carbon atoms long are attached to the disaccharide. The combined effect is to produce a product mixture that contains a large amount of a single structure of sophorolipid that can result in a crystalline product if either hexadecane or heptadecane is the sole lipophilic carbon source. Surface tension measurements of the various components isolated from the sophorolipid mixtures showed that the mono- and diacetylated lactones were the most effective surfactants. This is consistent with literature reports that the lactone form of the sophorolipids is more useful for a number of applications than the open acidic structure. The surface tension behavior of some of the sophorolipids demonstrated patterns similar to those usually observed for carboxylic acids and other sparingly soluble amphipathic compounds.

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Candida species (including *C. apicola, C. gropengiesseri,* and *C. bombicola*) are known to produce extracellular glycolipids in high yields. These glycolipids are mixtures of partially acetylated sophorosides and are commonly known as sophorolipids (1). The hydrophilic portion always consists of sophorose glycosidically bound *via* the 1-*O* position to a hydroxyl group on either the penultimate or terminal carbon of a carboxylic acid that is from 14 to 20 carbon atoms long. The acid function can be either free or esterified to the 4-hydroxyl group of the sophorose, forming a macrocyclic lactone (2). Sophorolipids are produced as complex mixtures containing both the free acid and lactone forms. Furthermore, there are many possible variations of these two basic forms, involving differences in the hydroxycarboxylic acid chain length such as degree of unsaturation and the position of the hydroxyl group as well as the degree of acetylation of the sophorose component (3,4).

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Sophorolipids have commercial potential because they can be easily produced in high yields from cheap raw materials (5–7). They have found applications in cosmetics and deodorants (8–10) and are also useful in the manufacture of detergents (11). Other uses for sophorolipids include oil recovery processes and soil decontamination (12,13) and the production of specialty chemicals (14).

It has been shown that the lactone forms are preferable if not essential, for many of these applications (8). For example, lactone-type sophorolipids have higher antimicrobial activity than the acid form (15). The lactones, specifically the acetylated lactones, are patented as stimulating agents for skin dermal fibroblast cell metabolism and for collagen neosynthesis (16). Other patents include the use of lactones for the treatment of dandruff and body odor (10). Fortunately, the lactones usually represent the largest fraction of the crude products (2–4). However, it would be desirable to increase the amount of this form obtained from the fermentation because separation costs are prohibitive (8,17).

Candida bombicola produces sophorolipids that usually are viscous brown oils (1,17). There have been a few reports that it is possible to obtain the sophorolipids in a crystalline form (2,18), and this would be advantageous for separation of the sophorolipids from the broth. Recently, it was shown that the physical nature of the sophorolipid mixture can be controlled by altering the carbon substrate used to grow the yeast. A crystalline product was observed when hexadecane or heptadecane was used as the lipophilic substrate, but only oils were obtained when other substrates, including other pure hydrocarbons, were used (17). More importantly, from the point of view of eventual applications, these crystals consisted of mainly the diacetylated lactone form of the product. Because of the high degree of direct incorporation observed with hexadecane and heptadecane, the carboxylic acid components of these sophorolipids were mainly a single length, which was the same number of carbons as the lipophilic substrates. Thus, it was postulated that direct incorporation was a cause for the unusually large amounts of diacetylated lactone produced in these cases (17). The results of the present work lead to a more realistic evaluation of the significance of direct incorporation.

Surface tension data are readily available for the synthetic surfactants that are extensively used in industrial processes. The surface activities of some biosurfactants have also been investigated. Very few studies have been made on the surface activity of sophorolipids, and most of the reported values are for mixtures of sophorolipids (15,19). The efficacy of a given surfactant is usually determined by the critical micelle concentration

(CMC) and the minimum surface tension (20). For water-insoluble surfactants, where monolayers or surface films are formed, surface pressure–area isotherms are usually used to describe the surfactant's surface activity (20,21). In these cases, owing to the extremely low solubility of the compounds, the change in surface pressure of the monolayer is measured as a function of available molecular area. As described by Adams (22,23), most systems undergo phase changes as compression increases and area decreases until the monolayer collapses.

EXPERIMENTAL PROCEDURES

Growth conditions. The yeast *C. bombicola* (ATCC 22214) was maintained on agar plates supplemented with yeast extract and stored at 4°C. The growth medium used throughout these studies consisted of 10 g/L glucose, 1 g/L yeast extract, 0.1 g/L urea, and 5 g/L lipophilic substrate with a culture volume of 100 mL. The lipophilic substrates used were the hydrocarbons dodecane, tetradecane, pentadecane, hexadecane, heptadecane, octadecane, eicosane, as well as octadecanoic acid. To prevent caramelization, the glucose was sterilized separately and, on cooling, was aseptically transferred to the rest of the medium. After inoculation, shake flasks were incubated for 11 d at room temperature in a New Brunswick Scientific Shaker, at 200 rpm. Another set of growth studies used media containing 2,2,4,4,6,8,8-heptamethylnonane (3:1, vol/vol to lipophilic substrate) to dissolve the two solid lipophilic substrates octadecane and eicosane. Measurements showed no loss of the heptamethylnonane, and control experiments showed zero growth on this hydrocarbon by *C. bombicola*.

Quantification of sophorolipids. When the product was a viscous oil, the entire contents of the flasks were extracted at the completion of growth using two 50-mL volumes of ethyl acetate. The fractions were combined and evaporated under reduced pressure. The amorphous product was washed with hexane to remove any residual lipophilic substrate and redissolved in ethyl acetate. The ethyl acetate was then transferred to a preweighed aluminum dish and allowed to evaporate at atmospheric pressure and room temperature. The weight of sophorolipid was determined by weighing the aluminum dish a second time. Crystalline product was collected after being allowed to settle. Distilled water at 4°C was used to wash the crystals, and the product was weighed after drying at ambient temperature. Extraction of the remaining aqueous phase as described above resulted in only trace amounts of sophorolipids.

Characterization of sophorolipids. The structural classes present in the sophorolipid products were evaluated using TLC. The components were identified by comparison of the calculated R_f values with those reported elsewhere (3) using the same TLC developing solvent system (chloroform/methanol/water = 65:15:2, by vol).

Analysis of hydroxycarboxylic acid components. The hydroxycarboxylic acid components of the sophorolipids were isolated and identified according to the method described by Cavalero and Cooper (17). A methanolysis reaction was used to convert the hydroxycarboxylic acids to hydroxy acid methyl esters, which were then analyzed using GC (Varian CP-3800 with a WCOT Fused-Silica, CP-Sil 5 CB capillary column with an FID). Any unknown compounds were identified by considering their mass spectra, which were obtained using GC–MS (Varian Thermo Quest model TRACE GC, 2000/Finnigan POLARIS containing an RTX-5 MS column) and interpreted as described by Cavalero and Cooper (17).

Calculation of percent direct incorporation. The percent direct incorporation of the hydrocarbon substrates into the sophorolipids was estimated by comparing the pattern of hydroxycarboxylic acids of the sophorolipids produced using each substrate to the pattern obtained for the sophorolipids when dodecane was the substrate. The sophorolipids produced using dodecane showed no indication of direct incorporation (zero percent dodecanoic acid) and were thus taken to represent sophorolipids that were produced completely through *de novo* synthesis. This is where FA in the sophorolipids are synthesized from acetate units when the hydrocarbon or other substrates are broken down completely, usually resulting in large amounts of 18- and 16-carbon acids (24). For example, the percent direct incorporation for tetradecane was estimated to be 18.8% since the tetradecane products contained 18.8% 14-carbon acids and dodecane products contained zero percent 14-carbon acids.

Measurement of surface tension. A modified evaporating drop technique (23) was used to examine the surface properties of sophorolipids. Surface tension measurements were made with a Fisher Autotensiomat that operates on the principles of the du Nouy ring method. Distilled water (25 mL) was placed in each of four identical glass dishes. A stock solution of between 7 and 9 g/L sophorolipid in ethyl acetate was prepared. With a 1-mL gas-tight syringe, 1, 2, 3, or 5 drops of solution were deposited on the water surfaces of the four identical dishes. The solvent was allowed to evaporate, and the surface tensions of the remaining water were measured. Four more drops were added to each dish to increase the concentration of sophorolipids. Once the solvent evaporated, the surface tensions were measured. This pattern of addition and measurement was repeated until after the CMC had been exceeded and constant surface tension measurements were obtained.

Fractionation of sophorolipids. Fractionation of the sophorolipid products was done using column chromatography. The sophorolipids were chromatographed on a short column of silica gel using a three-stage solvent system based on the early work of Tulloch *et al.* (2). New silica gel columns were prepared for each experiment using the slurry method with chloroform as the packing solvent. Sophorolipid (0.10–0.15 g) was dissolved in ethyl acetate and mixed with a small amount of silica gel. The solvent was evaporated under reduced pressure until the silica gel moved freely and then added to the column. Column fractions were collected in glass test tubes using a fraction collector (Spectra/Chrom CF-1). A chloroform/methanol (98:2, vol/vol) solution was used to elute the diacetylated lactone from the column. A second chloroform/methanol (96:4, vol/vol) solvent solution was used to elute first the monoacetylated lactone and then to slowly elute

TABLE 1
Sophorolipid Yields and Morphologies When Grown On Various Lipophilic Substrates

Lipophilic substrate	Yield (g/L)	Morphology
Dodecane	1.82	Oil
Tetradecane	2.19	Oil
Pentadecane	2.51	Oil
Hexadecane	5.67	Crystalline
Heptadecane	5.87	Crystalline
Octadecane	0.56	Oil
Octadecane–heptamethylnonane	1.03	Oil
Eicosane	0.49	Oil
Eicosane–heptamethylnonane	0.95	Oil
Octadecanoic Acid	2.1	Oil

the nonacetylated lactone. A final solvent solution containing 3.3% acetic acid in chloroform/methanol (96:4, vol/vol) was used to elute any remaining lactone structures from the column. The column packing was then transferred to a beaker where ethyl acetate was used to extract the acid sophorolipids that were strongly bound to the column packing.

RESULTS AND DISCUSSION

Sophorolipid yields. The variations in product yields and morphologies with the different lipophilic substrates are shown in Table 1. Higher sophorolipid yields were obtained when the products were crystals rather than oils. When the solid alkanes octadecane and eicosane were the lipophilic substrates, the yields were very low. Higher yields were obtained when a sufficient amount of heptamethylnonane was added to dissolve these solids.

Composition of sophorolipid mixtures. As was previously reported, the crystalline sophorolipids were only obtained when hexadecane or heptadecane was used as substrate (Table 1). TLC showed that the crystals contained mainly the diacetylated lactone with only trace amounts of the other sophorolipid structures (Fig. 1a). TLC of the oily products obtained when using any of the other lipophilic substrates showed a complex pattern with a mixture of different structural forms (Fig. 1b). The sophorolipid yields were at a maximum when the product was crystalline.

Hydroxycarboxylic acid constituents of sophorolipids. Earlier work concluded that obtaining a product that was mainly a diacetylated lactone was due to a high degree of direct incorporation of certain hydrophobic substrates into the lipids (17). The present work demonstrates that the explanation is not this simple. Tables 2 and 3 contain data for the hydroxycarboxylic acid compositions of the total sophorolipid products as well as the diacetylated lactones isolated from each of these. As expected, the distribution of acids is very dependent on the chain length of the substrate. In every case, the distribution found for the lactone was different from that found for the mixture, showing that there is a relationship between the type of sophorolipid and the type of hydroxycarboxylic acid likely to be observed. It can be seen that there is a wider distribution of acids in the oily mixtures (Table 2) than those from the crystalline products (Table 3). The carboxylic acids were all found to be mixtures of terminally hydroxylated or subterminally hydroxylated acids. For example, C14 sophorolipids, recovered as an oil, consisted of approximately 19% tetradecanoic acids, 26% hexadecanoic acids, and 39% octadecanoic acids, whereas heptadecane sophorolipids, recovered as crystals, had an acid distribution of approximately 92% heptadecanoic acids and 6% octadecanoic acids. These trends can be seen more clearly in Table 4, which contains the total amounts based on only the number of carbon atoms of the most prevalent acids observed for the products obtained with hydrocarbon substrates between 15 and 18 carbon atoms long. This includes the two crystalline products as well as two oily products produced from the hydrocarbons that were one carbon atom shorter or longer than those that produced the crystals.

The percent direct incorporation of hydrocarbon substrates into sophorolipids according to substrate chain length is shown in Figure 2. An observation from the earlier study was that the solid hydrocarbons octadecane and eicosane showed only very small amounts of direct incorporation (17). The results in this paper show that this is not an intrinsic property of growth on

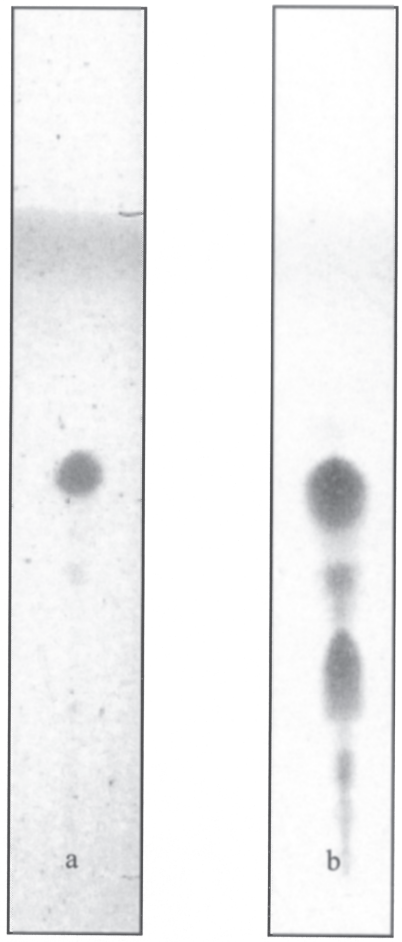

FIG. 1. TLC plates of sophorolipids from *Candida bombicola* when grown on (a) hexadecane and recovered as crystals or (b) octadecane and recovered as an oil.

TABLE 2
Hydroxycarboxylic Acid Compositions of Oily Sophorolipids from Various Hydrocarbon Substrates

		Sophorolipid structures (%[c]) as function of hydrocarbon substrate											
Hydroxy acid	Position of	C12		C14		C15		C18		C20		C18:1	
chain length[a]	hydroxyl group[b]	Mix[d]	Di-ace. lac.[e]	Mix[d]	Di-ace. lac.[e]	Mix[d]	Di-ace. lac.[e]	Mix[d]	Di-ace. lac.[e]	Mix[d]	Di-ace. lac.[e]	Mix[d]	Di-ace. lac.[e]
14:0	ω-1			13.4	3								
	ω			5.4	3.1								
15:0	ω-1					20	13.5						
	ω					17	4.9						
16:0	ω-1	7	8.2	8.4	25	1.1	1.8	2.4	15	8.1	7.2	1.2	1.1
	ω	5.6	6.1	8	21.2	1		1.8	3.5	5.8	4.7	1	1
16:1	ω-1	1.5	1.2	2.4					5	1.1	1.14	0.7	0.5
	ω	4.5	2.4	7	3.7	0.3				1.4	0.4		
17:0	ω-1					16.1	32.8						
	ω												
17:1	ω-1					15.9	20.2						
	ω												
18:0	ω-1	19.2	22	9.7	14.8	6.5	1.9	77.6	35.2	43.4	24.8	5.3	4.4
	ω							2.4		1.2	0.6	0.6	0.4
18:1	ω-1	53.3	50.7	25.3	27.1	13.5	13.6	12.3	34.7	18.7	53.4	75.3	84.4
	ω	6.1	3.9	3.5		1.4	11.2	1.6		1.4	2.1	11.9	6.7
20:0	ω-1									7.6			
	ω									1.3			

[a](XX:0) denotes saturated acid chain; (XX:1) denotes unsaturated acid chain.
[b](ω-1) denotes hydroxyl group on penultimate carbon; (ω) denotes hydroxyl group on terminal carbon.
[c]Some columns do not add up to 100% because various minor acids were not included.
[d]Denotes crude sophorolipid mixture.
[e]Denotes the diacetylated lactone sophorolipid isolated using column chromatography.

TABLE 3
Hydroxycarboxylic Acid Compositions of Crystalline Sophorolipids from Hexadecane and Heptadecane

		Sophorolipid structures (%[c]) as function hydrocarbon substrate			
Hydroxy acid	Position of hydroxyl	C16		C17	
chain length[a]	group[b]	Mixture[d]	Di-ace. lac.[e]	Mixture[d]	Di-ace. lac.[e]
12:0	ω-1				
	ω				
14:0	ω-1				
	ω				
15:0	ω-1				
	ω				
16:0	ω-1	39	44		
	ω	37	44		
16:1	ω-1				
	ω				
17:0	ω-1			78.6	82.7
	ω			4.5	5.1
17:1	ω-1			8.4	9.1
	ω				
18:0	ω-1	4	3	1.8	0.9
	ω				
18:1	ω-1	13	6	3.5	1.7
	ω	1		0.6	0.15

[a–e]For footnotes see Table 2.

hydrocarbons of this length because an appreciable amount of direct incorporation was observed when these solid substrates were made more bioavailable by the addition of the nondegradable hydrophobic liquid heptamethylnonane. When the octadecane was readily available to the yeast, the result was an appreciable amount of direct incorporation; and with eicosane, at least a small degree of direct incorporation was observed. The new data for octadecane and eicosane lead to a more regular pattern and a smooth curve for the effect of substrate chain length on the percentage of direct incorporation. Also, a clear optimum for direct incorporation was observed for heptadecane with appreciable amounts for both hexadecane- and octadecane-grown cultures.

The fact that two data points are shown for the pentadecane product in Figure 2 reflects the observation of appreciable amounts of 15- and 17-carbon length acids in these sophorolipids. This is unusual, as it is known that *C. bombicola* cannot directly synthesize carboxylic acids with odd chain lengths (24). The pentadecanoic acids are a result of the yeast's ability to incorporate the pentadecane substrate directly. In this case, the heptadecanoic acids are a result of chain lengthening of the pentadecanoic acids by the yeast (25). The higher data point for this substrate in Figure 2 represents the total of both the pentadecanoic and heptadecanoic acids found in the sophorolipids, whereas the lower percentage point, which actually falls on the curve, is only the pentadecanoic acids.

It can now be argued that obtaining a product with a high concentration of the lactone is not simply a consequence of direct incorporation of the hydrocarbon substrate into the sophorolipid. Both pentadecane and octadecane show high degrees of direct incorporation, comparable to hexadecane, but their products are oily mixtures of different types of sophorolipid structures.

A more reasonable explanation can be seen by considering just the distribution of hydroxycarboxylic acids found in the sophorolipids. Neither the position of the hydroxyl function nor the presence or absence of a double bond appears to be important (see Tables 2 and 3) so the effect is most obvious in Table 4, which combines the data of the first two tables to consider only the number of carbon atoms in each acid. It can be seen that the sophorolipids are preferentially converted to the lactone when the carboxylic acid component is either 16 or 17 carbon atoms long. This is related to direct incorporation because the carboxylic acids formed from the oxidation of either hexadecane or heptadecane are easily incorporated into the sophorolipids, and the resulting lipids are the correct length for conversion to the lactone form. Although pentadecane results in a high degree of direct incorporation of odd chain-length carboxylic acids in the sophorolipids, a significant portion of these are only 15 carbon atoms long, and this is less favorable for the lactone form. The large amount of 18-carbon carboxylic acids containing sophorolipids obtained from direct incorporation during growth on octadecane are also less favored for lactone formation.

The discrimination in the formation of the lactones was also apparent in all of the products obtained from the other hydrocarbons if the appropriate analyses were carried out. When the diacetylated lactones were separated from the oily product mixtures from the cells grown on hydrocarbons other than hexadecane or heptadecane, they consistently contained larger amounts of 16- and 17-carbon hydroxycarboxylic acids than was observed for each of the original mixtures. For example, the

TABLE 4
Total Amounts of Hydroxycarboxylic Acids for Mixtures of Sophorolipids and Their Major Components

Number of carbon atoms[a]	Sophorolipid structures (%[b]) as function of hydrocarbon substrate							
	C15		C16		C17		C18	
	Mixture[c]	Di-ace. lac.[d]	Mixture[c]	Di-ace. lac.[d]	Mixture[c]	Di-ace. lac.[d]	Mixture[c]	Di-ace. lac.[d]
15	37	18.4						
16	2.4	1.8	76	88			4.2	23.5
17	32	53			91.5	96.9		
18	21.4	26.7	18	9	5.9	2.8	93.9	69.9

[a]Includes all the various isomers of the acids with these carbon numbers listed in Tables 1 and 2.
[b]Some columns do not add up to 100% because various minor acids were not included.
[c]Denotes crude sophorolipid mixture.
[d]Denotes the diacetylated lactone sophorolipid isolated using column chromatography.

diacetylated lactones isolated from the products from both tetradecane and octadecane contained more hexadecanoic acids than the parent mixtures. Similarly, higher amounts of heptadecanoic acids were observed in the diacetylated lactones isolated from the pentadecane-grown products as compared with the total product composition.

This discrimination was particularly dramatic with the products from the cells grown on octadecenoic acid and octadecane liquefied with the addition of the heptamethylnonane. Both sophorolipids contained very large amounts of various 18-carbon acids, but the total amounts of diacetylated lactone in the product mixtures were smaller than that observed for the product from heptadecane. This demonstrates that even one extra carbon atom noticeably reduces the amount of lactone produced. There is a marked preference for hydroxycarboxylic acids that are either 16 or 17 carbon atoms long. The hexadecane-grown products, which were largely of the diacetylated lactone structure, consisted of only 88% 16-carbon acids, whereas with even larger percentages of 18 carbon-length acids, such as in the octadecanoic acid (93%)- and liquefied octadecane (94%)-grown products, mixtures of both lactone and acid structures were observed

Although a high yield of the diacetylated lactone is desirable (8,17), it is also beneficial to have a crystalline product. It can be seen that the condition for a crystalline product is not just direct incorporation. Uniformity of both the sophorolipid structural type and its carboxylic acid lengths are important. However, if direct incorporation results in increased amounts of a diacetylated lactone, the concentration of this particular product can be high enough to allow crystallization. Significant amounts of the other forms of the sophorolipids as impurities inhibit the formation of crystals. The two types of crystals recovered are examples of this purity requirement. It is interesting that only the uniformity of the length of the acid seems to be important for crystal formation (*cf.* Tables 2 and 3 with Table 4). The crystalline products were actually mixtures of

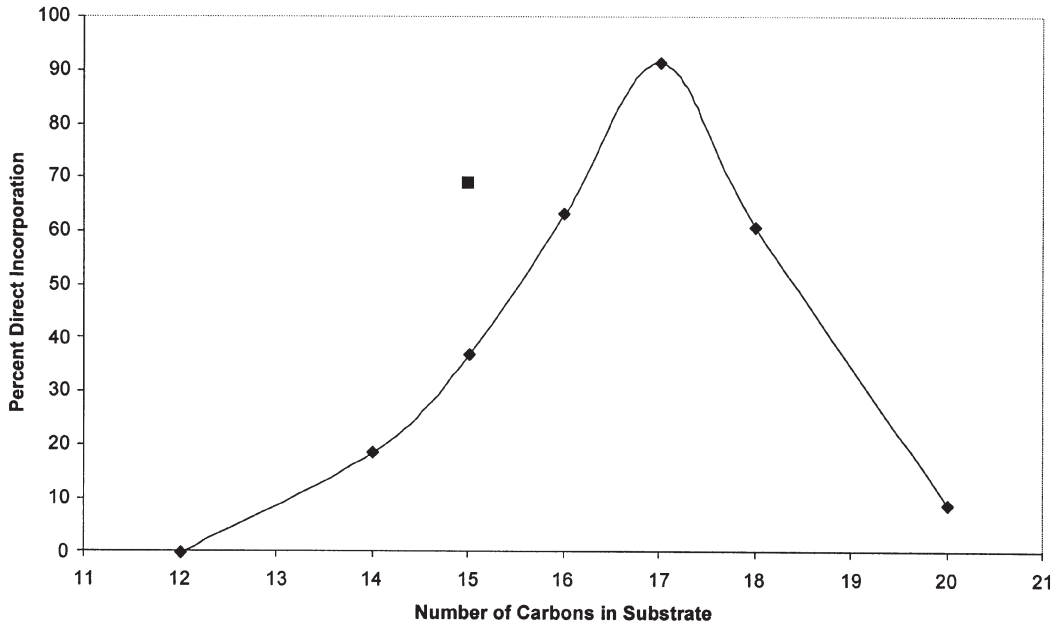

FIG. 2. Direct incorporation of hydrocarbon substrates (◆) into sophorolipids. Direct incorporation of pentadecane when the total of both pentadecanoic and heptadecanoic acids are considered (■).

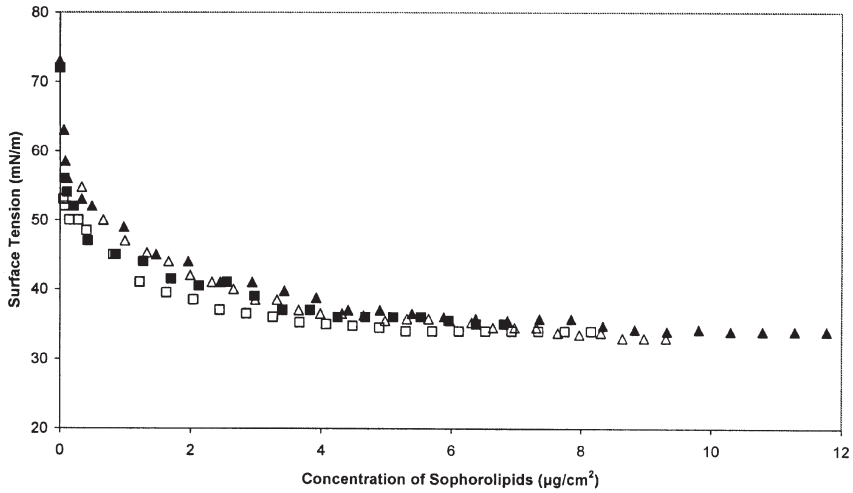

FIG. 3. Surface tension curves for diacetylated lactones isolated from sophorolipid mixtures obtained with various substrates: (△) dodecane, (■) hexadecane, (□) octadecane, and (▲) eicosane.

isomers, and there was no apparent preference for the hydroxyl function being on the terminal carbon or on the penultimate carbon. Similarly, it did not seem to matter whether the acids were saturated or unsaturated.

Surface tension of sophorolipids. There have been a few reports of surface tension measurements of sophorolipid mixtures, but a more precise examination of this property was possible with the various individual isomers isolated in this study. Sophorolipids have extremely low water solubility and form monolayers on the water surface. Thus data in Figures 3, 4, and 5 are in $\mu g/cm^2$. Well-behaved curves were obtained in each case.

A wide range of carboxylic chain lengths is not possible for the sophorolipids, but within the small range found there does not seem to be much effect on the surface tension as can be seen in Figure 3. It is possible to say that the diacetylated lactone containing mainly 18-carbon acids was the most effective of these biosurfactants, but this was a very small difference.

The most significant differences were observed between the open-chain form of the sophorolipids and the lactones. This effect is shown for the components isolated from a product mixture obtained when dodecane was the substrate (Fig. 4), but the results in the other cases were similar. The lactones were more effective surfactants, and it was tempting to relate this to the observations that the lactones were more effective for a number of applications (8). The degree of acetylation of the lactones did not seem to be very important. All of these structures

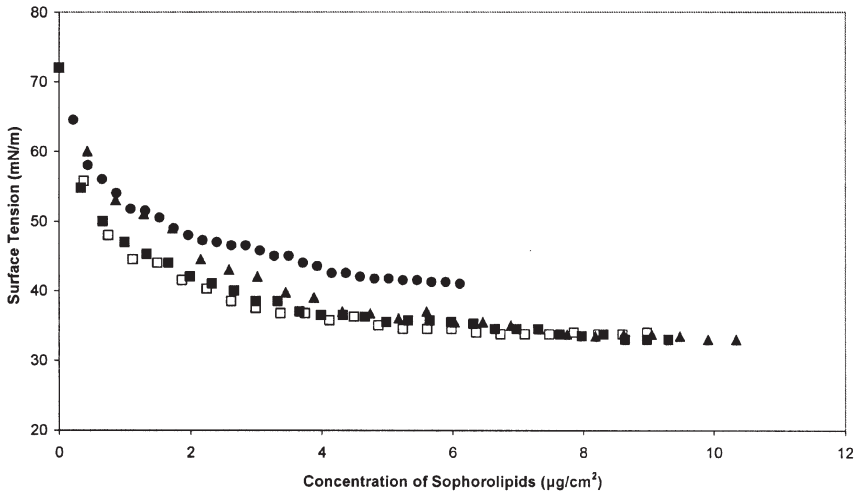

FIG. 4. Surface tension curves of different sophorolipid structures isolated from a sophorolipid mixture produced using dodecane as the lipophilic substrate. These include (■) diacetylated lactone, (□) monoacetylated lactone, (▲) nonacetylated lactone, and (●) acid sophorolipids.

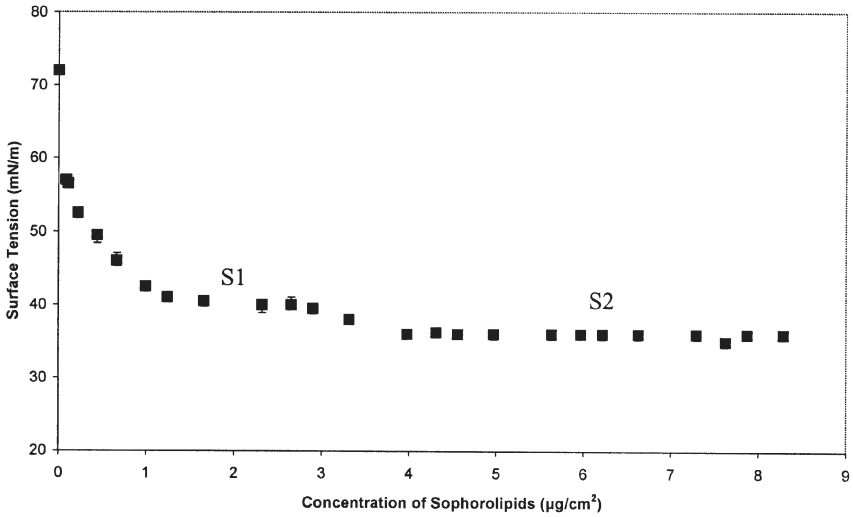

FIG. 5. Surface tension profile of crystalline sophorolipids obtained when heptadecane was the lipophilic substrate. There are two distinct "steps": S1 and S2. Error bars, where visible, represent SD.

were more effective surfactants than the acid form. The two acetylated lactones were slightly more effective at reducing surface tension than the nonacetylated lactones. Bulky head groups on the polar part of an amphipathic molecule are known to restrict molecular movement and increase packing density (26,27), and this could explain the effect of acetylation on the surface tension.

The surface tension pattern obtained with the crystalline sophorolipids produced using heptadecane is shown in Figure 5. Two "steps" or regions of constant surface tension are observed in the graph. The first step occurs at approximately 40 mN/m and 1.2 μg/cm^2, and the second step occurs at approximately 35 mN/m and 4.0 μg/cm^2. The minimum surface tension of 35 mN/m was consistent with that observed for the surface tension profiles obtained for the other diacetylated lactones. This unusual two-step plot was also obtained for the crystalline sophorolipids produced using hexadecane, although the steps were less pronounced. These "step" patterns are attributed to the high degree of uniformity of these crystalline products, revealing "fine structure" in the usual surface tension vs. concentration plots. The two "steps" observed in Figure 5 have not been reported for any other biosurfactant, but this is similar to the pattern observed by Adams for pure carboxylic acids and attributed to phase transitions (22,23). These phase transitions are analogous to the pressure/volume relationships for gas/liquid/solid systems (20,21). With increasing surface compression, the molecular area available on the water surface decreases. This results in a rearrangement of the molecules to increasingly ordered states.

If a comparison with the work done by Adams can be made here, then it is possible to suggest reasons for the existence of the two "steps" or regions of constant surface tension. The first plateau at the lower concentration of sophorolipid S1 would be comparable to the liquid expanded (LE) region. The next plateau (S2) would be the liquid condensed (LC) region. It has been proposed that in the LE region, surface rearrangement is beginning to occur. The molecules are moving freely yet do not have sufficient space to allow randomly tilted orientations flat on the water surface. At the higher concentrations in the LC region, the molecules are closely packed and nearly vertically oriented.

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