Microbial Production, Structure Elucidation and Bioconversion of Sophorose Lipids

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Cultivation of *Torulopsis bombicola* ATCC 22214 on a mixture of glucose and oleic acid (*A*) or oleic acid alone (*B*) produced large amounts of sophorose lipids. In the case of *A*, 38 g/l of crude product were finally isolated; fermentation *B* led to 77 g/l. After separation by MPLC and TLC, six glycolipids were obtained and identified by NMR and fast atom bombardment-mass spectrometry (FAB-MS). In general, a 17-hydroxyoctadecanoic acid at the C-1′-position and acetate groups at the C-6′-and C-6″-positions of sophorose were found as substituents in the lactone and acidic forms of these lipids.

The composition of product from *A* was as follows: 62% of sophorolipid 1′,4″-lactone 6′,6″-diacetate (SL-1), 4% of sophorolipid 1′,4″-lactone 6″-monoacetate (SL-2), 4% of sophorolipid 1′,4″-lactone (SL-3), 4% of sophorolipid 1′,6′-and 1′,6″-lactones (SL-4a,b), 4% of sophorolipid 6′-monoacetate acid (SL-5), 4% of sophorolipid acid (SL-6) and finally 17% of other lipids.

In *B*, the principal lactone (40%) had a double bond in the fatty acid moiety; the other components were identical with the above products. Yields of 13% SL-2 and of 35% lipids containing no carbohydrate were significant. SL-1 was deacetylated to SL-3 (yield: 25–30%) using acetyl-esterase in a two-phase system (cyclohexane/water).

Since 1961, it has been known that the yeast *Torulopsis bombicola* produces a mixture of sophorose lipids during growth on glucose, yeast extract and urea (1). Studies on the improvement of biosurfactant yield showed that the step-wise addition of long chain fatty acid esters or n-alkanes promoted the biosynthesis (2). In this paper, the influence of different lipophilic second carbon sources on the composition of the total hydroxyfatty acids within the crudesophorolipids was investigated.

In 1968 after use of n-octadecane as second carbon source, three glycolipids were isolated and their structures were elucidated (3). The principal lactone component was 17-L-([2′-O-β-D-glucopyranosyl-β-D-glucopyranosyl]-oxy)-octadecanoic acid 1′,4″-lactone 6′,6″ diacetate (= SL-1) while 17-L-([(2′-O-β-D-glucopyranosyl-β-D-glucopyranosyl]-oxy)-octadecanoic acid 1′,4″-lactone 6″-monoacetate (= SL-2) was present as a minor component. The principal acidic compound was 17-L-([(2′-O-β-D-glucopyranosyl-β-D-glucopyranosyl]-oxy)-octadecanoic acid 6′,6″-diacetate (= SL-A). Using 100 g/l glycose, 10 g/l yeast extract, 1 g/l urea and 36 g/l *n*-octadecane as nutrient, the yield of the crude sophorolipid mixture was 40 g/l; the approximate composition was 41% SL-1, 8% SL-2, 31% SL-A, 14% of other possible isomeric lactones, and 6% of monoacetylsophorosyl hydroxyacid (3).

Fermentation of oleyl alcohol produced the oleyl alcohol ester of the sophoroside of 17-hydroxyoleic acid (40%) together with lactone and acidic hydroxy-acid sophorosides (4).

Other authors have not investigated the distribution and structure elucidation of the sophorolipid components. Thus, Inoue and Ito reported a yield of 32 g/l of crude product after addition of 100 g/l of safflower oil to the basic medium (5), while Cooper and Paddock (6) obtained 67 g/l crude product using 10% triacylglycerols (among them sunflower oil) as the second carbon source. Although six glycolipids were observed by TLC, no structure elucidation was described.

Our own studies demonstrated that resting cells of the same yeast always synthesize the same sophorose lipid with octadecenoic acid, independent on the carbon source (carbohydrates, fatty acids or triglycerols), in a yield of 90% (7).

The target of further investigations was to find optimized cultivation conditions for *Torulopsis bombicola*, to identify all glycolipids and to convert the principal sophorose lipid 6′,6″-diacetate lactone (SL-1) into more hydrophilic compounds by biochemical methods.

EXPERIMENTAL

Materials. The yeast *Torulopsis bombicola* ATCC 22214 was procured from the American Type Culture Collection (Rockville, Maryland). The enzymes acetylesterase, acetylcholine esterase and pig liver esterase were obtained from Sigma Chemical Co. (München, FRG). Glucose and oleic acid were purchased from Merck (Darmstadt, FRG).

Growth conditions. The yeast was maintained on YM agar slants and transferred at monthly intervals. The basic medium used throughout these studies consisted of 100 g/l glucose, 10 g/l yeast extract and 1 g/l

TABLE 1

The influence of Carbon Sources on the Sophorolipid Production by *Torulopsis bombicola* in a 30-l Bioreactor at 30 C[a]

First carbon source	Second carbon source	Crude product (g/l)	Main component (%)
Glucose	Glucose	15	60[b]
Glucose	Soybean oil	33	51[b]
Glucose	Stearic acid	20	56[b]
Glucose	Stearic acid methylester	32	60[b]
Glucose	Oleic acid	38	62 (SL-1)
Oleic acid	Oleic acid	77	40 (SL-7)

[a]Conditions: medium: 10% of first carbon source, 1% YE, 0.1% urea. Addition of second carbon source (3.6%): from t = 24 hr to t = 84 hr (step-wise or continuously). Total incubation time: 120–144 hr.
[b]Sophorolipid 1′4″-lactone 6′,6″-diacetate, mainly containing 17-OH-octadecanoic acid.

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urea, which were sterilized together at 121 C for 20 min. Medium (100 ml in 500 ml Erlenmeyer flask) was inoculated with one platinum loop of the strain and incubated for 48 hr at 30 C with shaking (100 rpm, shaker RS 206, Infors, München, FRG). Then 10 ml was added to 500 ml of the same medium in a 2 l shake flask; altogether six of these second precultures were incubated. The conditions were as above except that after 24 hr, the whole broth (3 l) of all flasks were combined and transferred into 27 l of fresh medium in a 30 l bioreactor (30D, Braun, Melsungen, FRG) equipped with a flat-blade stirrer.

The conditions in the bioreactor were 550 rpm; aeration rate: 0.6 (v/v/m), T=30 C; pH not adjusted (starting pH=); continuous addition of 36 g/l oleic acid for 60 hr beginning at t=24 hr. The total incubation time was 120 hr.

Alternatively, 3 l of the second preculture were inoculated into 27 l of a similar medium, containing 100 g/l oleic acid instead of glucose. The same conditions as above were employed except that the incubation time was 168 hr.

Estimation of biomass and glucose. For determination of biomass, 10 ml of the whole culture broth were mixed vigorously with 10 ml of EtOH/n-butanol/$CHCl_3$ = 10/10/1 (v/v/v) and centrifuged for 30 min at 13,500 rpm (Labofuge 15.000, Heraeus Christ, Osterode, FRG). The solvent mixture facilitated the separation of oleic acid and glycolipids. The residual cell mass was washed with water, dried at 105 C for 48 hr, and weighed. The concentration of glucose in the supernatant was determined enzymatically (glucose oxydase) with a glucose analyzer (model 27, Yellow Springs Instruments, OH).

Estimation of the total sophorolipid contend. Whole broth (200 ml) was extracted twice with 200 ml ethyl acetate and the extract was used for determination of sophorolipids by: a) thin layer chromatography (TLC) (qualitative analysis): stationary phase: TLC plates with Kieselgel 60_{F254} (Nr. 5554, Merck, Darmstadt, FRG). Developing system: $CHCL_3/CH_3OH/H_2O$ = 65/15/2 (v/v/v). Detecting reagent: anisaldehyde/sulfuric acid/acetic acid = 0.5/1/50 (v/v/v); 150 C. Green spots were observed. b) Gravimetry (quantitative analysis): The crude product was dissolved in 100 ml $CHCl_3$ and bound to 100 g silica gel (Nr. 7734, Merck, Darmstadt, FRG) in a glass column. The elution was started with 100 ml of $CHCl_3$, to separate the remaining oleic acid. The more hydrophilic sophorolipids were eluted with 400 ml of $CHCl_3/CH_3OH$ = 2/1 (v/v) and weighed after evaporation of the solvent mixture. c) Anthrone method (8): quantitative analysis, modified as described (9).

Isolation of the sophorose lipids. At the end of the cultivation, the whole broth of the 30 l bioreactor was stored at 4 C for 20 hr and centrifugated continuously at 17,000 rpm at a flow rate of 50 l/hr (Cepa Z61, Padberg, Lahr, FRG). The wet residue, containing biomass and the glycolipids, was extracted twice with 10 l of ethylacetate. Alternatively, the solution was evaporated to dryness or concentrated to a smaller volume followed by crystallization at 4 C. In the latter case, the sophorolipid 1′,4″-lactone 6′,6″-diacetate (SL-l) was enriched in the precipitate.

Purification and identification of the sophorose lipids. The crude products of the two fermentations were initially separated using medium pressure liquid chromatography (MPLC): Pump: 681 (Büchi, Eislingen, FRG), maximum pressure, 12 bar; Column, 460 × 49 mm (Büchi); stationary phase, Kieselgel 60, 70-230 mesh ASTM, 250 g (Merck, Darmstadt, FRG); loading, 15 g crude sophorolipids; developing system, $CHCl_3$/CH_3OH mixtures (98/2 (v/v) to 60/40 (v/v), 2% steps; 900 ml per step); detection of sophorolipids, usually TLC of 300 ml fractions.

After this, the individual sophorolipid fractions were purified by thick layer chromatography using 100 × 20 cm plates: Stationary phase, Kieselgel 60_{F254} (Nr.

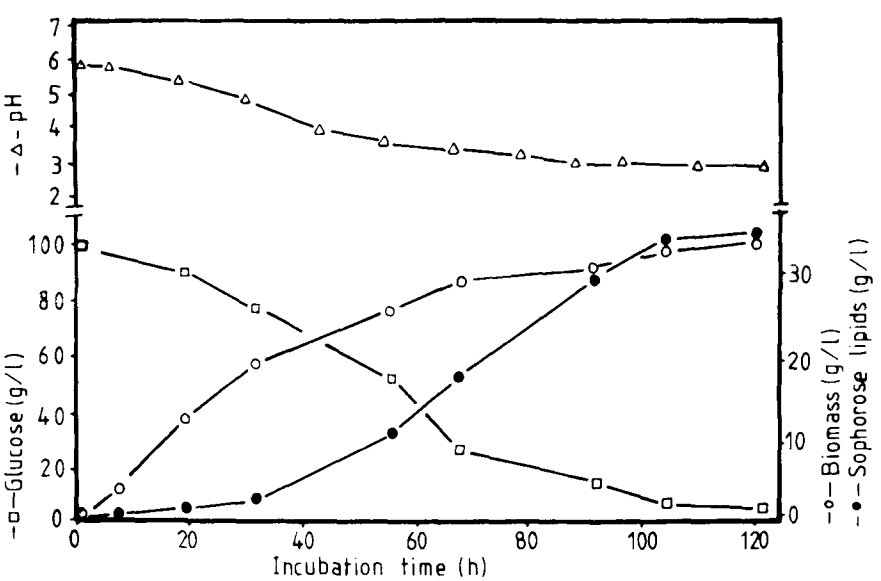

FIG. 1. Growth and sophorolipid production of *Torulopsis bombicola* on Glucose/oleic acid (A). Conditions: 30-l bioreactor. Basic medium: 100 g/l glucose, 10 g/l yeast extract, 1 g/l urea. Second carbon source: 36 g/l oleic acid (continuous addition from t = 24 hr to t = 84 hr).

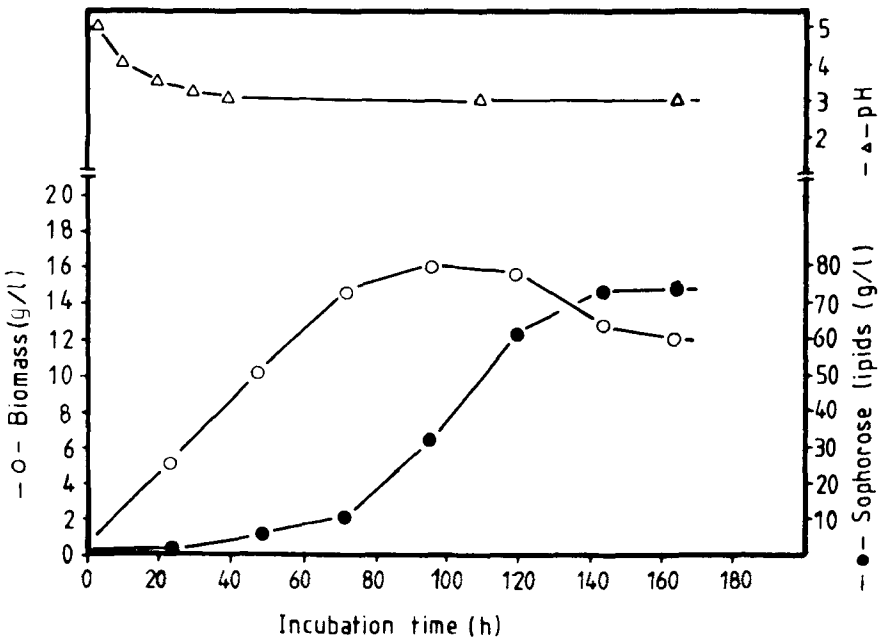

FIG. 2. Growth and sophorolipid production of *Torulopsis bombicola* on oleic acid (B). Conditions: 30-l bioreactor. Basic medium: 100 g/l oleic acid, 10 g/l yeast extract, 1 g/l urea. Second carbon source: 36 g/l oleic acid (continuous addition from t = 24 hr to t = 84 hr).

7734, Merck, Darmstadt, FRG); layer thickness: 2 mm; developing system 1, $CHCl_3/CH_3OH$ = 90/10 (v/v); developing system 2, $CHCl_3/CH_3OH/H_2O$ = 65/15/2 and 60/30/4 (v/v/v); detection, UV (254 nm) or anisaldehyde-reagent. Elution of the sophorolipids was performed by using $CHCl_3/CH_3OH$ in a ratio of 2/1 (v/v).

The pure glycolipids were analyzed by the following spectroscopic methods: a) ^{1}H-and ^{13}C-NMR: Bruker WM-400 and Bruker AM-300 spectrometers were used. All 1D and 2D spectra were recorded using the standard Bruker software. The internal standard was tetramethylsilane and 3-(trimethylsilyl)-propanesulfonic acid sodium salt (for D_2O). The solvent system was $CDCl_3/CD_3OD$ = 1/1 (v/v) and D_2O. b) GC/MS: Finnigan MAT 4515 (Finnigan, Bremen, FRG). c) FAB-MS: Finnigan MAT 8430 (Finnigan, Bremen, FRG). The sophorolipids were hydrolyzed under acidic conditions in methanol, and the 17-hydroxyl groups of the resulting fatty acid methylesters were silylated with N-methyl-N-(trimethylsilyl)-trifluoroacetamide (MTSFA). Gas chromatography-mass spectrometry (GC-MS) data of these derivatives were recorded.

Enzymatic deacetylation of sophorolipid SL-1. A 1.35 mL $(NH_4)_2SO_4$-suspension of acetylesterase (acetic ester hydrolase EC 3.1.1.6), corresponding to 50 U, were added to a mixture of 10 ml distilled water and a solution of 500 mg sophorolipid SL-1 in 40 ml cyclohexane. The reaction was carried out under pH-static conditions with permanent stirring (30 ml vessel, Metrohm, Herisau, Swizerland). The pH was adjusted to 7.0 with 0.1 N NaOH. Samples were analyzed quantitatively every 24 hr with a thin layer chromatography/flame ionization detector (TLC/FID)-scanner. TLC/FID measurement employed the following: stationary phase, Chromarods SII (SES GmbH, Niederolm, FRG); developing system, $CHCl_3/CH_3OH$ = 90/4 (v/v); quantitative measurement, coupling of TLC with FID using an Iatroscan TH 10 (SES GmbH, Niederolm, FRG).

RESULTS AND DISCUSSION

Growth and sophorose lipid production. Initial studies on the sophorose lipid production by the yeast *Torulopsis bombicola* using both 100 ml and 30-l cultures showed the following results: 1) The basic medium of

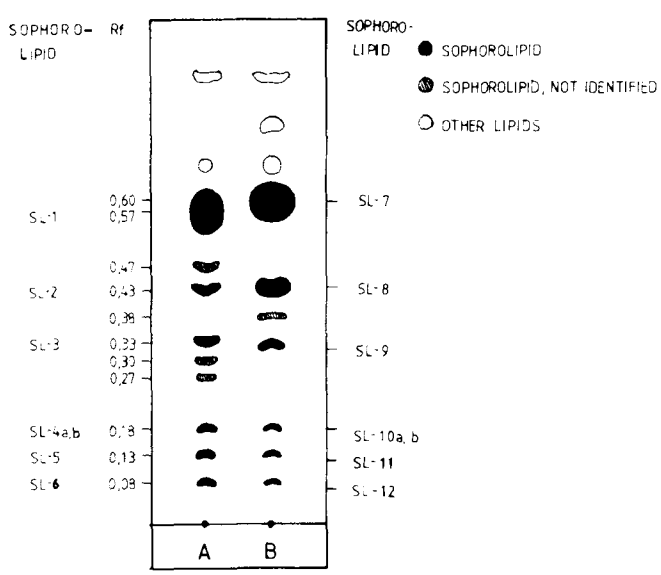

FIG. 3. Comparison of the TLC of crude sophorolipids from the glucose/oleic acid cultivation (A) and oleic acid cultivation (B). Detection: anisaldehyde reagent.

TABLE 2

Lipid Concentrations of the Glucose/Oleic Acid Fermentation (A) and Oleic Acid Fermentation (B), after MPLC

Lipids (A)	Content (%)	Lipids (B)	Content (%)
SL-1[b]	62	SL-7	40
SL-2	4	SL-8	13
SL-3	4	SL-9	4
SL-4	4	SL-10	2
SL-5	4	SL-11	2
SL-6	4	SL-12	2
Other SL[c]	1	Other SL[c]	2
Other lipids[c]	17	Other lipids[c]	35

[a]Total lipids: A: 38 g/l; B: 77 g/l. SL, sophorolipid.
[b]Including about 10% of SL-7.
[c]Not identified.

Tulloch et al. (3), containing 10% glucose, 1% yeast extract and 0.1% urea, was the best. All components had to be sterilized together. 2) The pH was adjusted to 6.0 before sterilization. During cultivation, pH was not adjusted. 3) Incubation temperatures between 23 C and 30 C promoted growth and glycolipid production. 4) Step-wise or continuous addition of the second carbon source of triglycerols, fatty acids, esters, alcohols and n-alkanes, after 24 hr to the basic medium enhanced the sophorolipid production. Lipophilic compounds containing a C-18 chain length (with or without a double bond) were particularly suitable. 5) A ratio of glucose/2nd carbon source of about 3/1 in the medium favored the overproduction of a single sophorolipid (SL-1). Lower ratios led to more hydrophilic sophorolipids.

For the determination of the optimum conditions for a high overproduction of the sophorolipid SL-1 in a 30-l or 50-l bioreactor, soybean oil, stearic acid, stearic acid methylester and oleic acid were used as second carbon sources. At a total concentration of 136 g/l of carbon sources (100 g/l glucose + 36 g/l of second carbon source), the yields of crude product were in the range 20 to 40 g/l (see Table 1); only glucose as substrate gave lower values. The best result was found when glucose was replaced by oleic acid also as first carbon source. In this case, 77 g/l of crude product were isolated. Corresponding cultivations with 136 g/l of the other lipophilic substrates led to less than 25 g/l of crude product. The concentration of the principal sophorolipid 1′,4″-lactone 6′-6″-diacetate with either 17-hydroxy-octadecanoic or -octadecenoic acid within the crude product amounted to 40%–60%. By precipitation of the organic extracts at 4 C, these compounds could be enriched to 80%–90% purity.

It could be concluded that in the case of glucose/soybean oil, intensive studies showed that the intensor type is to be preferred to the blade-stirrer. In all other cases, the latter gave satisfactory results. The stirrer speed of 550 rpm for the blade-stirrer and 1,500 rpm for the intensor system with an aeration rate of 0.6 (v/v/m) prevented pO_2-limitation within the culture broth.

The whole course of both glucose/oleic acid and oleic acid cultivations in a 30 l bioractor are shown in Figures 1 and 2, respectively. Biomass and sophorolipid curves indicate that overproduction began in the stationary phase of growth and maximum yields of total glycolipids were reached after 120 hr and 144 hr, respectively. The pH decreased to 3–4 in both cases.

Analysis of crude sophorolipids. The analysis of the ethyl acetate extract (Fig. 3) shows that the R_f-values of sugarpositive spots were similar in both batches. Six glycolipids were distinguishable in both cases. The predominant spots belonged to SL-1 and SL-7, respectively. The size of all other spots were 10% or less.

A quantitative analysis of the crude products was performed using MPLC where 15 g portions of raw material were separated by gradient elution. The results in Table 2 are extrapolated to the final product values of 38 g/l and 77 g/l (see Table 1), respectively. The percentage content of glycolipid SL-1 (62%) was more dominant in the case of the glucose/oleic acid fermentation than that of SL-7 (40%) in the oleic acid fermentation. The percentage of other lipids was higher in the oleic acid cultivation.

Structure elucidation studies. After a second purification step by thick layer chromatography on silica gel for each compound, the structures of the sophorolipids SL-1 to SL-12 were elucidated by application of ^1H, ^{13}C-NMR-spectroscopy and fast atom bombardment-mass spectroscopy (FAB-MS) of the intact substances, as well as by GC/MS analysis of the fatty acid moiety

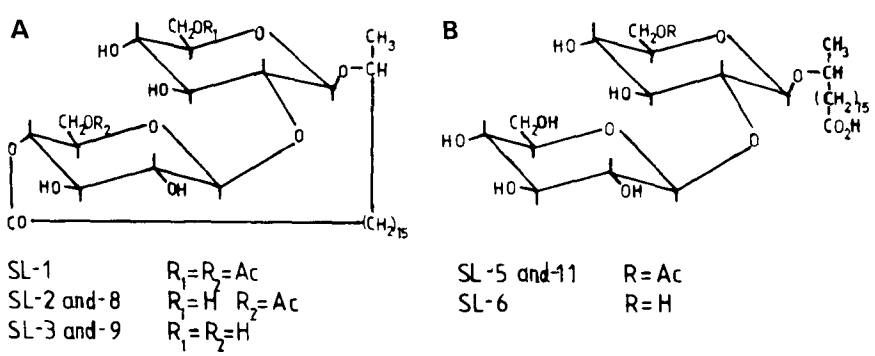

FIG. 4. Molecular structures of sophorolipids. a) lactone forms (left), b) acidic forms (right).

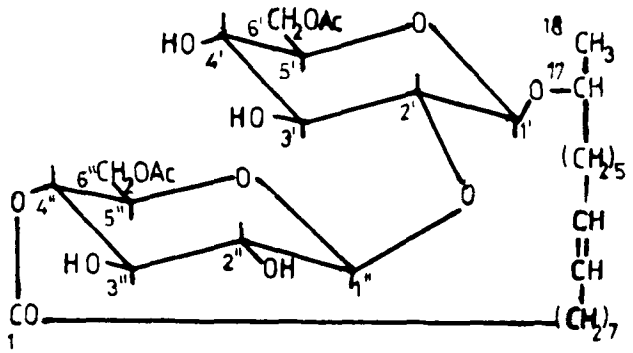

FIG. 5. Molecular structure of SL-7.

after acidic hydrolysis. The results are presented in Figure 4-6. In general, the structures are independent of the fermentation substrates, and only the double bond in SL-7 indicates a difference between the fermentations.

The lactones SL-1, SL-7 and SL-2(-8) have been described by Tulloch et al. in the case of a glucose/octadecane cultivation, while lactones SL-3(-9) and SL-4a,b (-10a,b) and the open acid form SL-5 (-11) are reported for the first time. The mixture of two similar sophorose lipids SL-4a,b (-10a,b), in which the lactone ring closure is at C-6′ and C-6″, could not be separated. In Sl-5(-11), the acetategroup is connected to C-6′ and not to C-6″, in contrast with the lactone SL-2. An acidic compound with C-6′,6″-diacetate groups (= SL-A, see Introduction) observed by Tulloch et al. (3) was not detected.

Important structural information was derived from both the ^{1}H and ^{13}C-NMR spectra (C-atom numbering is given in Fig. 5), where characteristic shift and coupling constant data allowed unambiguous identification and positional assignments of the various substituents in the molecules. In the case of the 1′,4″-lactones (SL-1 to -3, -7 to -9) there was always a triplet at ca. 4.95 ppm (^{1}H-NMR), showing the couplings of 9.5 Hz, for H-4″ and a signal at 174 ppm (^{13}C NMR) for C-1. In the corresponding spectra of the open acid forms (SL-5, -6 and -11), the signal for H-4″ had moved upfield and the signal for C-1 was at ca. 178 ppm. Acetate groups gave singlets between 2.05 and 2.00 ppm (^{1}H-NMR) and at ca. 172 and 21 ppm (^{13}C-NMR), the number of such groups being determined by the number of signals. The presence of an acetate group of C-6′ or C-6″ was indicated by shifts of the corresponding H-6′ or H-6″ signals from 3.6-3.8 to 4.2-4.0 ppm. The double bond of the fatty acid moiety was indicated by ^{1}H-NMR signals at 5.4-5.2 (-C**H**=C**H**-) and 2.0-1.9 ppm (-C**H**$_2$-CH=CH-C**H**$_2$-), respectively, and by ^{13}C-NMR signals at about 130 ppm.

The identity of the sugar moiety as sophorose in all the glycolipids isolated followed from the 1D and 2D ^{1}H-NMR data. All the protons in a particular pyranose moiety were identified from cross peaks in the 2D COSY ^{1}H-NMR spectrum starting from the H-1 signal of the sugar (as an example the 2D COSY ^{1}H-NMR spectrum of SL-1 is shown in Fig. 7). This data, together with the coupling constant from the 1D spectra indicated the presence of two β-glucopyranose systems, which from the correlation of the ^{1}H and ^{13}C were shown to be connected via C-2 of the first system to give a sophorose residue.

The position of the acetate group in SL-5(-11) was carefully established as this contrasted with that found for SL-2(-8). The shifts of the protons of the two sugar moieties were identified from a 2D COSY ^{1}H spectrum. This data, together with selective proton decoupled ^{13}C-NMR spectra, indicated the sugar moiety with substitution at C-2 also had an acetate group at C-6.

The structure of compounds SL-4 a,b(-10 a,b) were established from the fact that there were no acetate signals in either the ^{1}H or ^{13}C-NMR spectra. One set of shifts for C-6′ or C-6″ were to low field (^{1}H-NMR) indicative of an ester bond, the C-1 signal in the ^{13}C-NMR was at 175 ppm indicative of lactone and there was no triplet at low field for H-4″ (^{1}H-NMR).

Support for the structures derived fron NMR was provided by the negative and positive FAB-MS data shown in Table 3, from which the molecular weights could be calculated.

To supplement the studies on biosynthetic pathways in sophorolipid formation summarized by Spencer et al. (10), we propose a pathway (Fig. 8) to the principal compound SL-1 in the glucose/oleic acid fermentation. The presence of the various compounds in the fermentations and the fact that there is no change during cultivation of the ratios from t = 0 to t = 120 hr (TLC-analysis of crude products) suggests that the first sophorolipid synthesized by glycosylation between sophorose and 17-hydroxyoctadecanoic acid should be the acidic form SL-6. After an esterase-catalysed lactonization SL-4a and -4b should be in equilibrium, fol-

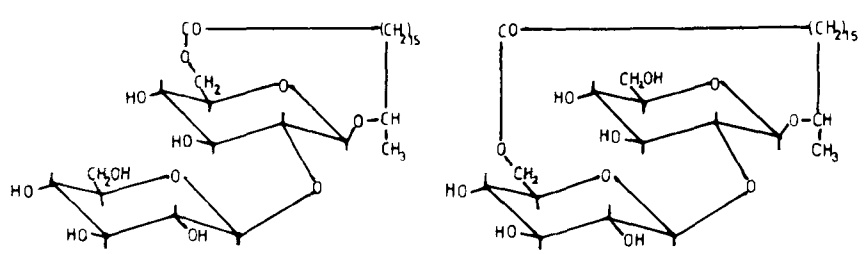

FIG. 6. Molecular structures of the sophorolipid mixtures SL-4a (SL-10a) (left) and SL-4b (SL-10b) (right).

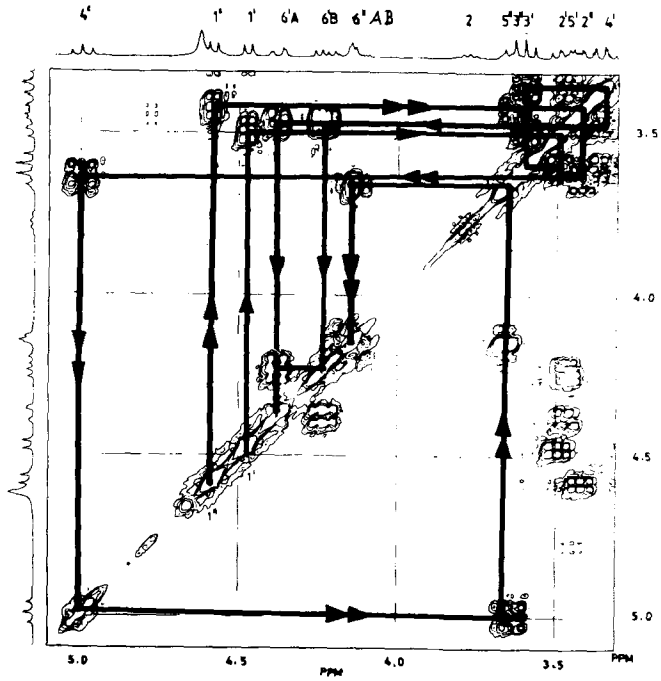

FIG. 7. 2D COSY ^{1}H-NMR spectrum of sophorolipid SL-1.

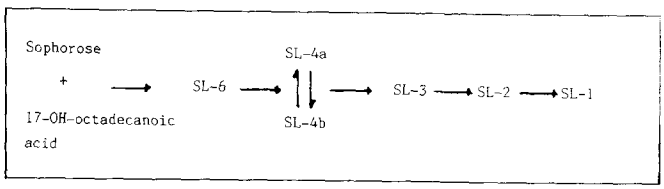

FIG. 8. A proposal for the last steps in the biosynthesis of SL-1.

lowed by formation of SL-3 and acetylation leading to SL-1.

The alternative pathway, starting with SL-1 followed by degradation steps to eventually give SL-6, seems improbable.

Biochemical conversion of sophorolipid SL-1. For testing in various fields of technical application, such as oil pollution abatement at sea and in coastal waters, larger quantities of the most hydrophilic sophorolipid lactone SL-3 were necessary. Preliminary studies on alkaline hydrolysis of both acetate groups at C-6′ and C-6″ were unsuccessful as the lactone also was cleaved. Hence, hydrolyzing enzymes such as acetylcholine esterase (ACE), pig liver esterase (PLE) and acetylesterase (AE) were tested.

PLE was suitable for deacetylation of *cis*-1,4-diacetoxy-cyclopentene (11), ACE for deacetylation of D,L-acetylcarnitine (12), and AE in conversion of cephalosporin C to the deacetyl compound (13,14). Initial experiments with only AE were positive, and the conditions for the deacetylation reaction were optimized by using this enzyme. Because SL-1 is poorly soluble in water, a two-phase system was necessary. Among 20 organic solvents usually employed in enzymatic conversions of lipophilic compounds, cyclohexane seemed to be the most suitable, particularly in the ratio of 4/1 (v/v) cyclohexane/H_2O. Optimum values of pH and temperature were found at 7.0 and 40 C, respectively.

Figure 9 shows the conversion of SL-1 to SL-3, identified by TLC and NMR, and quantified by TLC/FID. It is conspicuous that the reaction time was longer than 10 days. Unfortunately only 25%–30% of the theoretical yield of SL-3 was found, and this could not be increased because further degradation prevented the accumulation of the desired product. Chemical or

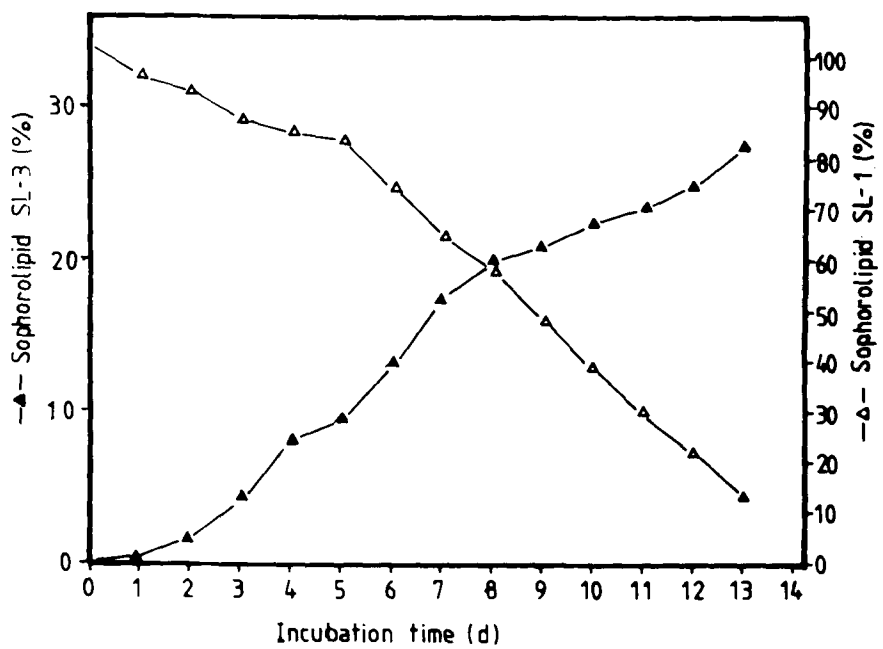

FIG. 9. Bioconversion of SL-1 to SL-3 by acetylesterase.

TABLE 3

The Various Molecular Ions of Sophorolipids by FAB-MS with Glycerol as Matrix

Sophorolipid	$[M+Na]^+$	$[M+H]^+$	$[M-H]^-$	$M_{calc.}$	Molecular formula
SL-1	713	691	689	690	$C_{34}H_{58}O_{14}$
SL-2	-	649	647	648	$C_{32}H_{56}O_{13}$
SL-3	-	607	605	606	$C_{30}H_{54}O_{12}$
SL-4	-	-	605	606	$C_{30}H_{54}O_{12}$
SL-5	703	-	-	680	$C_{33}H_{60}O_{14}$
SL-7	711	-	687	688	$C_{34}H_{56}O_{14}$
SL-8	-	-	647	648	$C_{32}H_{56}O_{13}$
SL-9	629	-	-	606	$C_{30}H_{54}O_{12}$
SL-11	703	-	-	680	$C_{33}H_{60}O_{14}$

enzymatic cleavage of the lactone ring and glycosidic bonds seem to be responsible for the large decrease to less than 20% of SL-1.

In recent studies using immobilized acetylesterase, further degradation of the sophorolipid SL-3 was not observed. Hence, this may afford a method of accumulating the desired deacetylated sophorolipid.

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