

Analysis of Intact Hopanoids and Other Lipids from the Bacterium *Zymomonas mobilis* by High-Performance Liquid Chromatography¹

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Hopanoids and other lipids were extracted from *Zymomonas mobilis* and quantitatively analyzed by high-performance liquid chromatography. Previous methods for hopanoid analysis required derivatization of the hopanoids via periodate oxidation or acetylation. The current method employs a normal-phase silica gel column, a ternary gradient of hexane-isopropanol-water-triethylamine, and detection with a flame ionization detector. Three major hopanoid classes were separated and quantified by this new method, and together they comprised 30 to 40% of the total lipid in these cells. Mass spectrometry confirmed that chemical structures of these hopanoids were consistent with those previously proposed. In addition, several other common lipid classes (free fatty acids and six classes of common phospholipids), comprising the remaining 60 to 70% of the total lipids, were also separated and quantified. This new chromatographic method represents the first technique for the analysis and purification of intact hopanoids. This method should be useful for the analysis and purification of hopanoids from other bacterial species. © 1995 Academic Press, Inc.

Hopanoids are a class of pentacyclic triterpene lipids found in many species of bacteria and cyanobacteria (1), a few species of fungi (2,3), a lichen (4), and a few species of higher plants (5). It has been proposed that hopanoids stabilize prokaryotic biological membranes similar to the way that cholesterol and other sterols stabilize eukaryotic membranes (6). Several methods have been de-

veloped for the analysis of hopanoids. The most popular method involves periodate oxidation of the entire lipid extract and analysis of the resulting hopane fragment, bacteriohopane-32-ol, by gas-liquid chromatography (GLC)³ (1). Unfortunately, there are two problems with this technique: (i) each of the hopanoid classes is derivatized to the same fragment so it is impossible to ascertain which types of intact hopanoids were present in the original sample, and (ii) the oxidation reaction may not be quantitative (7). Two previous analytical approaches employed HPLC methods, but each of these also required derivatization of the samples prior to HPLC analysis. In the first, the lipid extract was acetylated and five hopanoids were separated via a reversed-phase HPLC system with uv detection (7). In the second system, the lipid extract was first subjected to periodate oxidation, and the resulting bacteriohopane-32-ol was derivatized with common uv chromophores (8). The derivatized material was then separated by reversed-phase HPLC and the hopanoid derivatives were monitored with a uv detector. Because of the high extinction coefficient of the attached chromophore, the sensitivity of the latter method was very high, but like the earlier GLC method, all of the original hopanoid classes were converted to the same derivative. Thus, it was only possible to quantify the total amount of hopanoid present and not the individual hopanoid classes.

A HPLC method recently reported by our laboratory was recently used to identify high levels of one hopanoid, bacteriohopanetetrol, in *Frankia*, a nitrogen-fixing actinomycete (9). We subsequently used a variation of the

¹ Reference to a brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

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³ Abbreviations used: GLC, gas-liquid chromatography; FID, flame ionization detector; RP, reverse phase; NP, normal phase; EIMS, electron impact mass spectrometry; SIMS, secondary ion mass spectrometry; PE, phosphatidylethanolamine; FAB, fast atom bombardment; THBH, tetrahydroxybacteriohopane.

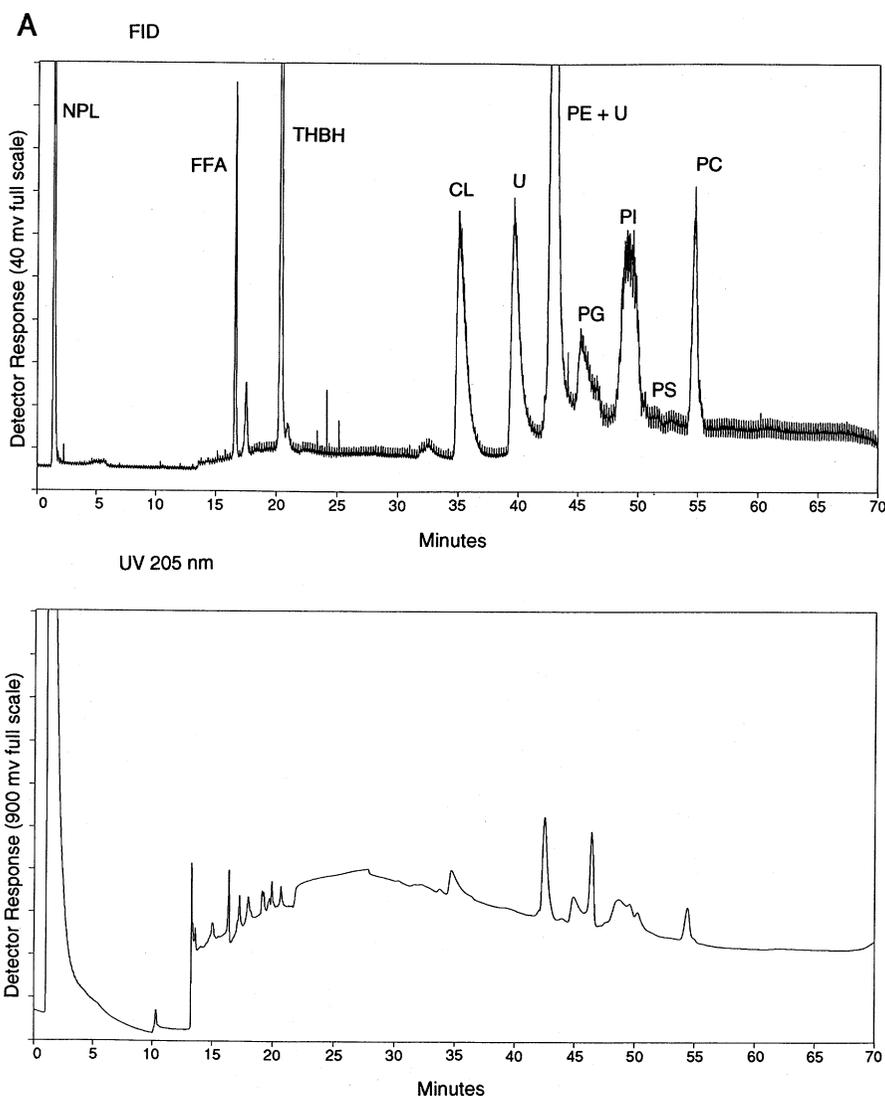


FIG. 1. NP-HPLC gradient analysis of lipid classes in *Z. mobilis*. (A) Chromatogram obtained using the hexane-isopropanol-water gradient as described previously (9). (B) Chromatogram obtained after adding 0.04% triethylamine to the C solvent of hexane-isopropanol-water gradient (9). For each sample the top chromatogram was obtained with the flame ionization detector (FID) and the bottom chromatogram was obtained with the uv detector at 205 nm (uv 205 nm). The lipid sample was from *Z. mobilis* cells grown anaerobically with 10% glucose, and 250 μg of total lipid was injected in 50 μl of chloroform-methanol (85/15, v/v). See Table 2 for abbreviations used. The unknown peak at 40.1 min was labeled U.

same method to detect a second hopanoid (bacteriohopanetetrol phenylacetate monoester) in *Frankia* vesicles, the specialized nitrogen-fixing structures of the root nodule-forming bacterium (10). This was the first time that an esterified hopanoid had been reported. In the current study we have modified our previous HPLC-FID system to separate and quantify the hopanoids in *Zymomonas mobilis*, an ethanol-producing bacterium previously reported to contain five hopanoids (7).

MATERIALS AND METHODS

Growth of Microbes

Strains of *Z. mobilis* (ATCC 29191 and ATCC 31821) were maintained on a solid medium [2% glucose, 1%

yeast extract (Difco), and 1.5% agar (Difco)] at 28°C and subcultured weekly (all media compositions are reported as w/v). For the isolation of hopanoids, the bacterium was grown in liquid medium consisting of 2% glucose, 1% yeast extract, 0.2% KH_2PO_4 , pH 6.0 (1 liter media, in 2.8 liter Fernbach flasks). Starter cultures (20 ml of liquid medium in a 100-ml flask) were inoculated with a turbid cell suspension (approximately 1 ml) prepared from cells grown overnight on the above solid medium at 28°C. Starter culture flasks were incubated overnight at 28°C with shaking (250 rpm) and one 20-ml starter culture was used to inoculate each 1-liter flask. The 1-liter cultures were incubated at 28°C with shaking (250 rpm) for 1 to 3 days. Anaerobic cultures were grown in a 70-

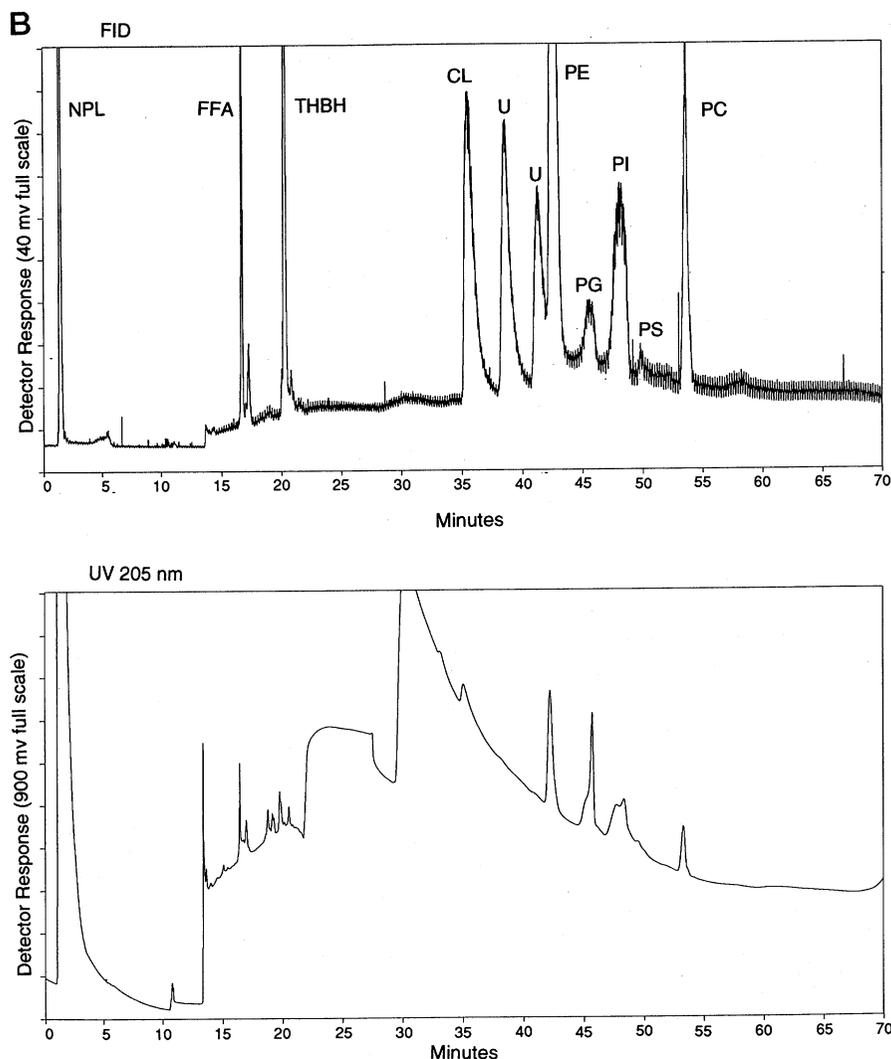


FIG. 1—Continued

liter pilot plant fermentor (ABEC, Inc., Allentown, PA) at 28°C under nitrogen sparging. The medium (40 liter total volume) consisted of 10% glucose, 0.5% yeast extract, 0.1% $(\text{NH}_4)_2\text{SO}_4$, 0.1% KH_2PO_4 , and 0.05% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$; pH was controlled at approximately 5.0 by periodic additions of 0.5 M KOH. Glucose concentrations were measured with a Yellow Springs Instruments (Yellow Springs, OH) Model 2000 glucose analyzer. Cells were harvested by centrifugation in a Sharpels (Philadelphia, PA) solid-bowl centrifuge.

Lipid Extraction

When the cells (aerobically or anaerobically grown) had reached stationary phase (based on stable optical density measurements), they were harvested by centrifugation at $5000g \times 30$ min. Cell pellets were washed once with distilled water and re-centrifuged ($16,000g \times 20$ min). The washed cells were lyophilized and stored at

-20°C until used. Lyophilized cells (200 mg) were homogenized in chloroform/methanol/water (8/16/4.8 ml) with a Polytron Homogenizer (Brinkman), and lipids were extracted according to the method of Bligh and Dyer (11). The yield of total lipid extract was 9–11 mg from 200 mg dry wt of cells.

Normal-Phase High-Performance Liquid Chromatography (NP-HPLC)

The hopanoids and other lipids were separated and quantified on a stationary phase which consisted of LiChrosorb 5 Si 60 (3×100 mm; Chrompack, Raritan, NJ) and a mobile-phase gradient described in Table 1. This method is a modification of a method we have used for hopanoid analyses in other species (9,10). The HPLC system consisted of an ISCO (Lincoln, NE) Model 2350 pump, an ISCO Model 2360 gradient programmer, an ISCO Model V4 uv detector operated at 205 nm, and a

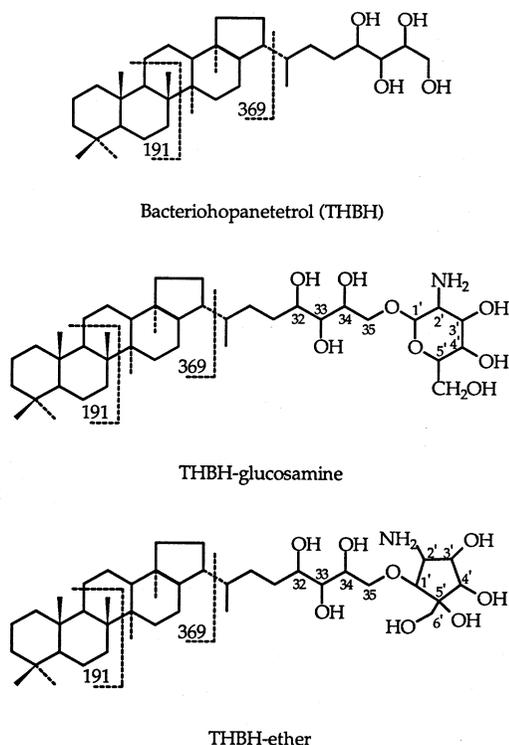


FIG. 2. Proposed structures of the three major hopanoids in *Z. mobilis*. The common fragments obtained by mass spectroscopy are indicated by the dotted lines.

Tremetrics (Austin, TX) Model 945 FID operated at 120°C. The FID was capable of evaporating all of the solvents at this low flow rate (0.5 ml/min) and detecting hopanoids and other lipids with very low background noise.

Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC)

Hopanoids were acetylated (1 mg sample in 1 ml pyridine/acetic anhydride, 1:1, (v/v), for 18 h at 25°C) and separated by a modified version of the method of Schulenberg-Schell *et al.* (7). The column was a LiChrosorb 7 RP18 (3 × 100 mm, Chrompack) and a mobile phase consisting of the gradient program as previously described (7). The HPLC system and detectors were as described above.

Mass Spectrometry

Electron impact (EIMS), chemical ionization, and secondary ion mass spectrometry (SIMS) were performed on a Hewlett-Packard 5989A spectrometer. For electron impact and chemical ionization, samples were introduced into the ionization chamber via a Hewlett-Packard 59980B particle beam separator interfaced with a Hewlett-Packard 1050 HPLC. The HPLC conditions described above were used for the HPLC analysis of

crude samples, whereas purified samples (collected from previous HPLC analyses) were analyzed directly without the use of a HPLC column. The chemical ionization reagent gas was ammonia. A Phasor CES5F cesium gun was used to obtain SIMS spectra from the sample in a glycerol matrix. Fast atom bombardment MS was performed on a VG Analytical ZAB 2-SE high-field mass spectrometer. A cesium gun was used to generate ions and the instrument was calibrated with oligomers of PEG 1000 as molecular weight standards.

Chemical Identification of HPLC Fractions

Fractions (0.5 ml) were collected and subjected to the following analyses: Lipid phosphorus was quantified by the method of Dittmer and Wells (12), glycolipids were estimated according to the phenol-sulfuric acid method of Roughn and Batt (13), and free amino groups were estimated by a spectrophotometric ninhydrin assay (14).

Hydrolysis and Identification of Sugar and Aglycone Moieties

Various methods were tested to hydrolyze the sugar moiety from the hopanoid conjugates. These included refluxing at 110°C for 2 to 20 h in 2.0 or 2.5 N trifluoroacetic acid, respectively, refluxing at 100°C for 18 h in 2 N aqueous HCl, and refluxing at 75°C for 2 or 40 h in 2 N methanolic HCl. Of these methods the most effective was hydrolysis of 0.5 mg of Hop 2 (unknown at R_t 42.3 min) in 1.0 ml of 2 N aqueous HCl at 100°C for 18 h in a 7-ml screw-cap tube sealed after flushing with N₂. After refluxing, the sample was extracted with 2 ml of chloroform to remove most of the aglycone and nonhydrolyzed hopanoid conjugate. The chloroform phase was dried under N₂, and the aglycones were redissolved in chloroform/methanol 85:15 (v/v) and analyzed using the normal-phase HPLC system described above. The aqueous HCl phase was evaporated under a stream of N₂ at 40°C. The dried residue was dissolved in 1 ml of ethanol-water, 1:4, filtered (0.45- μ m Teflon membrane), and dried

TABLE 1
Linear Gradient Program Used for the Normal-Phase Separation of Hopanoids and Other Lipids

Time (min)	% A	% B	% C
0	100	0	0
5	95	5	0
10	85	15	0
15	40	60	0
53	40	51	9
68	40	51	9
73	40	60	0
78	100	0	0
100	100	0	0

Note. Column was LiChrosorb 5 Si 60 (3 × 100 mm) and a flow rate of 0.5 ml/min. A, hexane; B, isopropanol; and C, 0.04% triethylamine in water (C was prepared fresh daily).

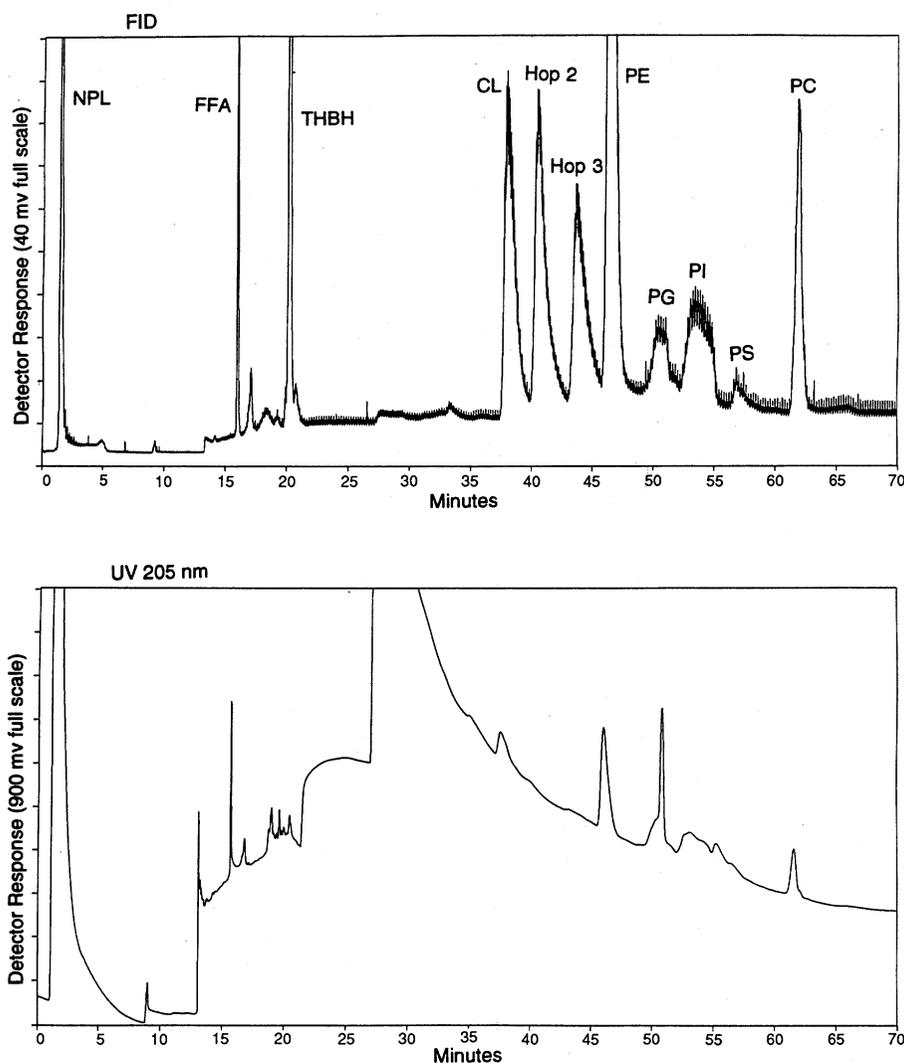


FIG. 3. NP-HPLC gradient analysis of lipid classes in *Z. mobilis*, using the hexane-isopropanol-water-triethylamine gradient described in Table 1. The unknown hopanoid at 42.3 min was labeled Hop 2 and the unknown hopanoid at 44.5 min was labeled Hop 3.

under a stream of N_2 . Monosaccharides in the residue were derivatized to alditol acetates by the method of Blakenly *et al.* (15). The alditol acetates were dissolved in $30 \mu\text{l}$ of ethyl acetate prior to analysis by capillary GLC and GLC-MS.

GLC of the alditol acetates of Hop 2- and Hop 3-derived sugars and known standards was performed on a $15\text{-m} \times 0.25\text{-mm-i.d.}$ fused silica SP-2330 capillary column (Supelco, Inc., Bellefonte, PA) inserted in a Perkin-Elmer 8320 gas chromatograph. The column oven temperature was programmed as follows: held at 200°C for 2 min, heated to 230°C at $2^\circ\text{C}/\text{min}$, and then held at 230°C for 28 min. Injector and FID temperatures were 260 and 300°C , respectively, with a carrier gas (He) head pressure of 10 psi (69 kPa). Analysis of alditol acetates by GC-MS was performed using a Hewlett-Packard 5890 GC interfaced with a HP5970A mass selective detector as described by Gross and Acosta (16).

RESULTS AND DISCUSSION

Lyophilized cells of *Z. mobilis* typically yielded about 50 mg of total lipid per gram of dry cells when extracted with chloroform-methanol-water (11). When the total lipid extract was chromatographed using our hexane-isopropanol-water HPLC system (9), a complex chromatogram was obtained (Fig. 1A). Preliminary MS studies indicated that the largest peak, which cochromatographed with phosphatidylethanolamine (PE) standards, also contained hopanoids (as evidenced by a major 191 m/z fragment indicated in Fig. 2). We succeeded in separating the "PE peak" into two separate peaks by adding a small amount of triethylamine to the gradient (Fig. 1B) and by programming the middle part of the gradient more gradually, as indicated in Table 1. After incorporating these two changes to the HPLC gradient system we also obtained a complex chromatogram (Fig.

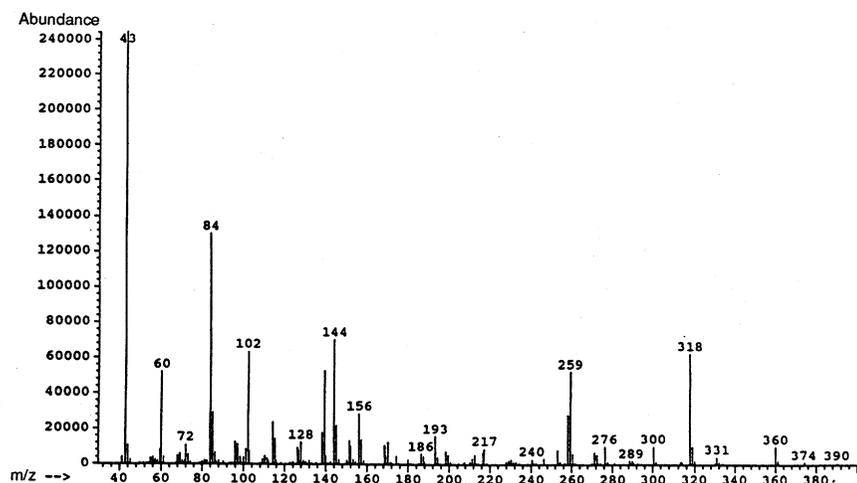


FIG. 4. GC-MS fragments from alditol acetate derivatives of water-soluble components of the acid-hydrolyzed 42.3-min peak fraction. The retention time and fragmentation pattern are identical to those obtained with the alditol acetate derivative of glucosamine.

3), but further investigations revealed that the hopanoids and phospholipid classes were successfully separated. By using two HPLC detectors in series (first, a uv detector at 205 nm, followed by the FID) we could compare the mass of the lipid peaks (as detected with the FID), with the relative degree of unsaturation and perhaps conjugation, of the lipid peaks (as detected with the uv detector). Our previous studies of HPLC analyses of hopanoids revealed that bacteriohopanetetrol was detected with the FID, but was not detected by the uv detector at 205 nm (9,10,17). Using this criterion with the *Z. mobilis* lipids, we detected a large FID peak at 20.1 min, which was not detectable (essentially invisible) with the uv detector. Later MS analysis of this peak confirmed that it was bacteriohopanetetrol (m/z 546, for the intact hopanoid, and m/z 714, for the acetylated derivative). Several other lipid peaks (free fatty acids, cardiolipin, phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol, and phosphatidylserine) cochromatographed with authentic standards and did not contain any MS fragments (i.e., 191 m/z) which would indicate that they contained hopanoids.

Multiple injections of the total lipid extract were made and individual peaks were collected and analyzed. Perchloric acid digestion and phosphate analysis (12) revealed that the putative phospholipid peaks contained phosphate.

The only major peaks not chemically identified were at retention times of 42.3 and 44.5 min. Analyses of lipid phosphorous and carbohydrate were negative, indicating that phospholipids and glycolipids were not present. The fraction from the 42.3-min peak reacted slightly with ninhydrin, but the fraction from the 44.5-min peak did not react with ninhydrin. Because these peak fractions gave m/z 191 fragments, we suspected that they were hopanoids and tentatively referred to them as Hop 2 and Hop 3 (Fig. 3).

Hydrolysis of the two unknown peak fractions with base (1 N NaOH, 18 h at 100°C) produced no identifiable lipid or nonlipid fragments. Hydrolysis of Hop 2 with 2 N HCl for 18 h at 100°C resulted in the recovery of a small amount of bacteriohopanetetrol (equivalent to about 10 mol% of starting material) and a comparable amount of glucosamine, as confirmed by GC-MS of the alditol acetate derivative (Fig. 4).

High-resolution fast atom bombardment mass spectroscopy (FAB-MS) of the two unknown peak fractions revealed that each had essentially identical molecular weights, $m/z = 708.5461$ ($M + H^+$) for the earlier peak fraction and $m/z = 708.5444$ ($M + H^+$) for the second peak fraction, which corresponded to a chemical formula of $C_{41}H_{74}O_8N_1$. High-resolution FAB-MS of the acetylated derivative of the two unknowns again yielded identical molecular weights of 1002.888, which corresponded to seven degrees of acetylation. EIMS of the two components yielded fragments of m/z 191, 369, and 493, which have been reported to be characteristic for hopanoids (18,19). Because Hop 2 was acid-hydrolyzed to bacteriohopanetetrol and glucosamine, we identified it as bacteriohopanetetrol-glucosamine (Fig. 2), previously reported to occur in *Z. mobilis* (7,20). Similarly, because Hop 3 had a molecular weight identical to Hop 2, and was not acid-hydrolyzed, it appears to be bacteriohopanetetrol-ether (Fig. 2), also previously reported for this species (7,18). We do not know why Hop 2 (bacteriohopanetetrol-glucosamine) did not test positive for glycolipids with the phenol sulfuric acid assay and why Hop 3 (bacteriohopanetetrol-ether) did not react with ninhydrin, but we believe that the mass spectral data are more definitive than colorimetric assays. The uv chromatogram (Fig. 3) revealed little or no uv absorption for the 42.3- and 44.5-min peak fractions, consistent with the proposed chemical structures (Fig. 2), since they are saturated.

TABLE 2

Analysis of the Lipid Classes in Total Lipid Extracts of *Z. mobilis* (ATCC 29191) Using the Method Described in Table 1

Lipid class	RT (min)	2% glucose ^a		10% glucose ^b	
		Area%	mg/gdw	area%	mg/gdw
Nonpolar lipids (NPL)	1.3	24.0 ± 3.4		10.6 ± 1.9	
Free fatty acids (FFA)	16.4	5.8 ± 0.7		4.4 ± 0.2	
Bacteriohopanetetrol (THBH)	20.1	13.2 ± 1.1	5.2 ± 0.3	8.7 ± 0.8	3.4 ± 0.3
Cardiolipin (CL)	37.6	9.2 ± 0.2		9.9 ± 0.4	
THBH-glucosamine (THBH-GA)	42.3	6.3 ± 0.5	8.1 ± 0.6	10.9 ± 1.0	14.1 ± 1.2
THBH-ether (THBH-Et)	44.5	5.6 ± 0.3	7.3 ± 0.4	8.6 ± 0.2	12.7 ± 0.3
Phosphatidylethanolamine (PE)	46.2	19.4 ± 1.4		28.1 ± 1.7	
Phosphatidylglycerol (PG)	51.3	1.2 ± 0.3		2.0 ± 0.1	
Phosphatidylinositol (PI)	55.7	6.7 ± 0.7		9.1 ± 0.3	
Phosphatidylserine (PS)	57.4	0.5 ± 0.1		1.1 ± 0.1	
Phosphatidylcholine (PC)	62.3	8.0 ± 0.3		6.8 ± 0.4	

Note. Area% values were calculated from the areas under each peak in the chromatograms. The values of mg hopanoid component/mg dry weight of bacterial cells (mg/gdw) were calculated for each component using area% values and the standard curves in Fig. 5. Results are the mean of three injections of each sample SD.

^a Cells were grown aerobically in the presence of 2% (w/v) glucose.

^b Cells were grown anaerobically in the presence of 10% (w/v) glucose.

To use this new HPLC method quantitatively, one must convert peak area to units of mass. Several milligrams of each of the three major hopanoids in *Z. mobilis* were purified using this new gradient system by detaching the FID and collecting the effluent from the uv detector at appropriate retention times. Several injections were then made with each compound, and standard curves of mass vs peak area were constructed (Fig. 5). The first hopanoid eluted by the gradient, bacteriohopanetetrol, exhibited a linear correlation in the range of 1–50 μg per injection. The latter two hopanoids exhibited a linear correlation in the range of 10 to 50 μg per injection. The decreased sensitivity exhibited by the latter

two hopanoids may result from the rather broad peak shapes observed with these compounds. Although attempts were made to sharpen these peaks (make them less broad), not one was successful. The standard curves were then used to quantify the levels of hopanoids in *Z. mobilis* in units of mg hopanoid/gram dry weight of cells (Table 2). These levels compare well with those reported previously (7,20) using other analytical methods. The construction of standard curves for the phospholipids and other lipid components was not considered necessary for the current study.

It has been hypothesized that in *Z. mobilis*, an ethanol-producing bacterium, the hopanoids may serve to protect the organism from ethanol toxicity (6,21–23). Although there have been two reports that the levels of hopanoids increased when the cells of *Z. mobilis* were grown in the presence of ethanol (21,22), the most recent report did not confirm this hypothesis (23). With the two growth regimes used in this study (2% glucose under aerobic conditions and 10% glucose under anaerobic conditions, where ethanol levels rose to 5.0% and the levels of glucose became undetectable), there were measurable differences in the levels of the three major hopanoid lipid classes (Table 2). In the low-glucose aerobic cells, the three hopanoids constituted about 25 mol% of the total lipids, and bacteriohopanetetrol was the most abundant hopanoid. In contrast, in the high-glucose anaerobic cells, hopanoids constituted 37 mol% of the total lipids, and the predominant hopanoids were bacteriohopanetetrol-glucosamine and bacteriohopanetetrol-ether. This preliminary evidence indicates that cultural conditions can dramatically influence the hopanoid composition of *Z. mobilis*. In addition to the above studies, which indicate that hopanoids may function to protect the mem-

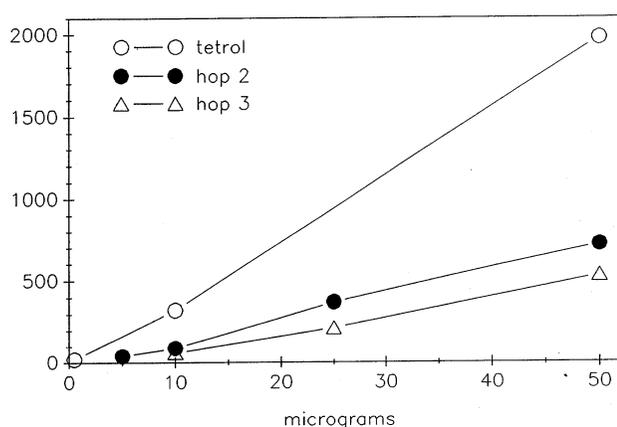


FIG. 5. Mass vs HPLC-FID peak area for the three hopanoids in *Z. mobilis*. Individual hopanoids were purified using the NP-gradient system and redissolved at 1.0 mg/ml in chloroform-methanol (85/15, v/v), and samples of each (1–50 μg) were injected. Because each of these three hopanoids are saturated they were only detected by the FID and were undetected by the uv detector at 205 nm.

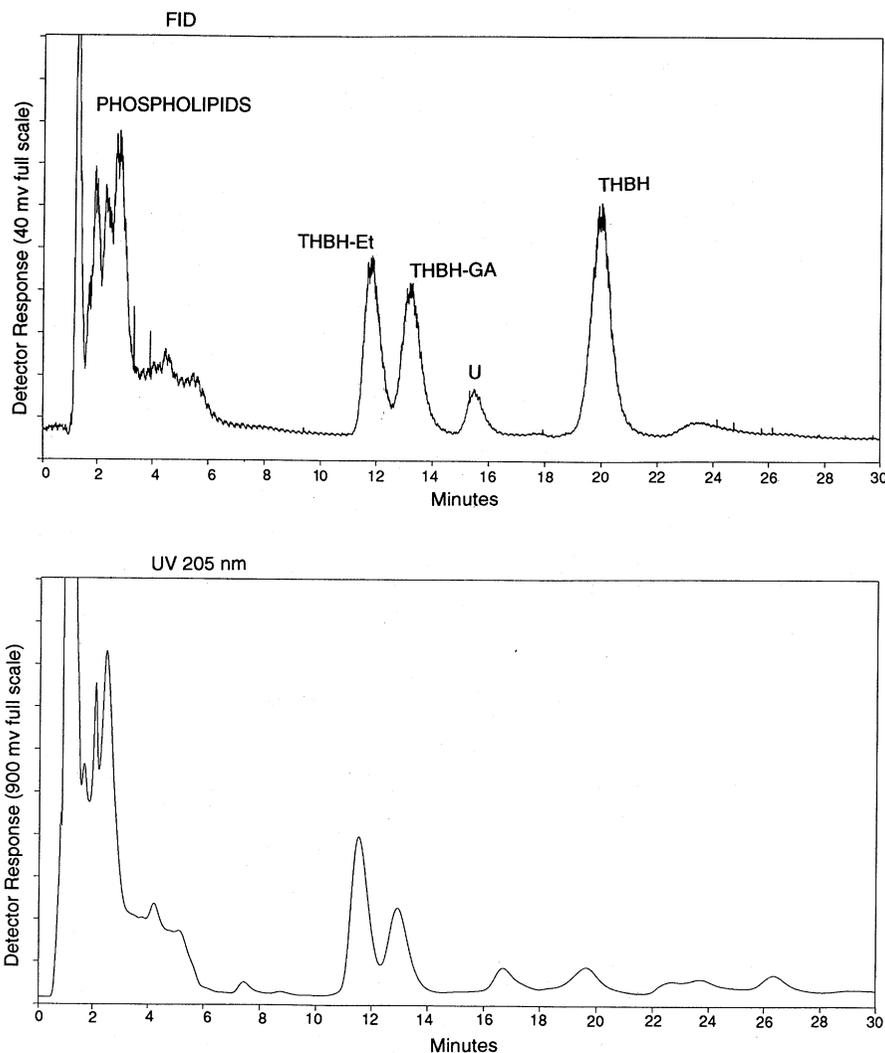


FIG. 6. RP-chromatogram of hopanoids in an acetylated total lipid extract of *Z. mobilis* performed as previously described (7) except that detection was with a flame ionization detector. The three individual hopanoids were also purified by NP-HPLC, acetylated, and chromatographed in this RP-HPLC system to verify the identity of each peak.

branes from ethanol toxicity (or perturbation), others have suggested that the high levels of vaccenic acid (cis-11-octadecenoic acid) in phospholipids of *Z. mobilis* are responsible for protection from ethanol toxicity (24). Further studies are required to determine whether hopanoids or vaccenic acid, or both, functions in protecting *Z. mobilis* from ethanol toxicity.

In the future we plan to conduct experiments to determine whether increasing the level of ethanol (or perhaps an increase in glucose or some other compound) in the medium induces an increase in the levels of certain classes of hopanoids. Although all of the experiments described in this study were performed with *Z. mobilis*, strain ATCC 29191, a second strain, ATCC 31821, was found to contain nearly identical levels of hopanoids and other lipids (data not shown) when grown under identical conditions.

In the last set of experiments we compared the levels of hopanoids as measured with our normal-phase HPLC-FID system with analyses performed using a reverse-phase HPLC method recently reported for the analysis of acetylated hopanoids (7). The RP-chromatogram (Fig. 6) revealed the same three major hopanoid lipids which we had separated in our NP system (Fig. 3), but the order of elution of these three components in the RP system was reversed, as previously reported (7). Comparison of the FID and uv chromatograms revealed that acetylated derivatives of the two hopanoid conjugates (THBH-Et and THBH-GA) were detected with approximately equal sensitivity with the two types of detectors. However, the third hopanoid, THBH, was detected more sensitively with the FID than with the uv detector. The identity of the peaks of these acetylated components was confirmed by mass spectroscopy. Ho-

panoids were also purified by the NP-HPLC method (Fig. 3), acetylated, and chromatographed in the RP-HPLC system to confirm the identity of these compounds. The FID could also be used with this system and when we compared the analysis of hopanoids by the NP-HPLC-FID system (Fig. 3) and the RP-HPLC-FID system (Fig. 6), the proportions of the three hopanoid peaks appeared to be similar (data not shown). Although the RP-HPLC-FID system could potentially be used for quantitative analysis of the hopanoids, it has two disadvantages when compared to the NP system: (i) it requires derivatization of the hopanoids, and (ii) it separates and quantifies only the hopanoids, and all of the other lipids are not separated. We also found that the three abundant hopanoids in *Z. mobilis* were separated quite adequately when the reverse-phase gradient system (7) was simplified to an isocratic system of methanol-water (96:4) as previously reported by Renoux and Rohmer (18) (data not shown). However, this isocratic system may not be useful if there is interest in measuring hopanoids other than the most abundant three (i.e., diploptene and diplopterol).

Although diplopterol and diplotene were not detected in this study, previous studies revealed that they are minor components in *Z. mobilis*, representing about 1 and 0.2% of the total lipid, respectively (7). It should be possible to use our system as is, or with slight modifications, to separate diplopterol, diplotene, or other minor hopanoid components from the three major hopanoids found in this study.

Using a normal-phase HPLC-FID system, we have developed a method for the analysis of intact hopanoids in *Frankia* (9,10) and *Z. mobilis* (this study). This method should prove useful for the analysis of hopanoids in other bacterial species. Because numerous types of hopanoids have been reported to occur in bacteria (25), it may be necessary to modify the mobile-phase gradient in order to separate and quantify other hopanoids. Although flame ionization detectors for HPLC are uncommon and rather expensive, they have proven to be very useful for the detection of many types of lipids (17), especially saturated lipids such as hopanoids. In addition to the FID, we have also employed a second type of "mass" detector for the analysis of hopanoids and other lipids, the evaporative light-scattering detector. In previous studies with the hopanoids in *Frankia*, we reported that similar results were obtained with either the flame ionization detector or the evaporative light-scattering detector (10).

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