Characterization and synthesis of mono- and diphytanyl ethers of glycerol

C. N. JOO,* T. SHIER,‡ and M. KATES§

Division of Biosciences, National Research Council, Ottawa, Canada

ABSTRACT The methanolyzed lipids of the extreme halophile, *Halobacterium cutirubrum*, were separated into glycerol diether and glycerol monoether fractions. The diether was shown by synthesis to be 2,3-di-*O*-(3′*R*,7′*R*,11′*R*,15′-tetramethylhexadecyl)-*sn*-glycerol. The monoether fraction was separated by thin-layer chromatography on boric acid-impregnated silicic acid into about equal amounts of α- and β-isomers. The α-isomer was found to be identical with the synthetic 3-*O*-(3′*R*,7′*R*,11′*R*,15′-tetramethylhexadecyl)-*sn*-glycerol, and the β-isomer was identical with the synthetic 2-*O*-(3′*R*,7′*R*,11′*R*,15′-tetramethylhexadecyl) glycerol.

SUPPLEMENTARY KEY WORDS bacterial lipids halophilic bacteria

THE CELLULAR LIPIDS of extremely halophilic bacteria are most unusual in that they consist almost entirely of derivatives of a di-*O*-alkyl-glycerol ether identified as 2,3-di-*O*-(3′,7′,11′,15′-tetramethylhexadecyl)-*sn*-glycerol (di-*O*-phytanyl glycerol) (1–3). The structure and the glycerol configuration of the diether was established by synthesis of the 1,2-diphytanyl- and the 2,3-diphytanyl-*sn*-glycerol isomers and comparison of their rotations with that of the natural isomer (2). The phytanyl groups in the natural glyceryl diether contain three asymmetric centers, namely carbon atoms 3, 7, and 11, and the absolute configuration of these has been established as 3-*R*, 7-*R*, and 11-*R* (4). Although the phytanyl groups used in the synthesis had the 3-*RS*,7-*R*,11-*R* configuration, the asymmetric centers in the phytanyl groups are sufficiently far removed from the asymmetric center in the glycerol moiety not to influence the optical rotation of the molecule significantly; the glycerol configuration could thus be established without ambiguity (2). Nevertheless, we considered it necessary to repeat the synthesis with phytanyl groups that have the natural 3-*R*,7-*R*,11-*R* configuration, and to compare the properties of the synthetic and natural diethers.

In addition to the diphytanyl glycerol ether, small amounts of glycerol monoethers were also detected in hydrolysates of lipids of extremely halophilic bacteria (3). These monoethers have now been isolated and were found to be a mixture of the α- and β-isomers of monophytanyl glycerol ether.[1] To confirm the structure of these monoethers and to establish the glycerol configuration of the α-isomer, we synthesized the isomeric monoethers and compared their properties with those of the natural isomers.

METHODS AND MATERIALS

Physical Methods

IR spectra were measured on thin films of oils or on their solutions in carbon tetrachloride, in a Perkin–Elmer model 237B double-beam spectrometer with sodium chloride optics. Optical rotations were measured at 22°C at 589 (sodium D line), 578, 546, and 436 mμ, by means of a Perkin–Elmer polarimeter, model 141, with digital readout.

This paper is National Research Council No. 10,369.

Abbreviations: TLC, thin-layer chromatography; GLC, gas–liquid chromatography.

* N.R.C. postdoctorate fellow, 1965–67.

‡ N.R.C. summer assistant, 1966.

§ Present address: Department of Chemistry, Ottawa University, Ottawa, Canada.

[1] Although the β-isomer (substituent ether grouping on the secondary hydroxyl of glycerol) can be conveniently referred to as the 2-isomer in the *sn* system, the α-isomer (as separated by TLC; ether group on a primary hydroxyl) can be given only the cumbersome designation "1(or 3)-isomer" until identification has been completed. For convenience we continue to use the older terms "α- and β-isomer" for the majority of this paper.

Downloaded from www.jlr.org by guest, on June 20, 2012

ASBMB JOURNAL OF LIPID RESEARCH JLR

Chromatography

Rapid thin-layer chromatographic analyses for purity determinations and monitoring of columns took place on 7.5 × 2.5 cm microscope slides coated with plain silica gel (Research Specialties Co., Richmond, Calif.). The plates were developed in wide-mouthed, screw-capped jars lined with filter paper, and the spots were made visible by spraying the plates with 40% H_2SO_4 and charring them on an open flame. The R_f values quoted are mean values of at least three independent determinations on microplates with an error of ±0.05. For preparative separations of up to 100 mg of material, 20 × 20 cm glass plates with a 0.6 mm-thick layer of plain silica gel (previously washed with chloroform–methanol 1:1) were used. They were developed in ordinary rectangular jars lined with filter paper and bands were detected under UV light or by staining with I_2 vapor; components were eluted from the silica gel with ethyl ether or mixtures of ethyl ether and methanol.

α- and β-isomers of the monoethers were separated by TLC on boric-acid impregnated silicic acid (5, 6) prepared as follows. A mixture of 20 g of plain silica gel and 2 g of boric acid was slurried in 50 ml of 0.01% sodium carbonate solution and spread on five 20 × 20 cm glass plates at a thickness of 0.5 mm.

Column chromatography was performed on Bio-Rad or Unisil silicic acid (200–325 mesh); the weight ratio of silicic acid to the substance applied was 40:1. All solvents were reagent grade and were distilled before use. The boiling range of the petroleum ether used was 30–60°C.

Gas–liquid chromatography (GLC) was carried out on 120-cm glass columns (4 mm I.D.) of 10% butanediol-succinate polyester on Gas-Chrom A at 180°C and 28 psi, in a Pye Argon chromatograph equipped with a ^{90}Sr ionization detector.

Culture of Organism and Extraction of Lipids

Cells of *H. cutirubrum* were grown in a 110 liter fermentor in the standard medium for extreme halophiles (7) at 37°C for 3 days. They were harvested by centrifugation in a Sharples centrifuge and suspended in 4 M NaCl to a volume of 900 ml. To the suspension were added 2 liters of methanol and 1 liter of chloroform, and the mixture was shaken and left overnight at room temperature. The supernatant red extract was decanted carefully through a bed of glass wool, and the residue was suspended in a mixture of 500 ml of methanol, 250 ml of chloroform, and 200 ml of water and left for 1–2 hr. The mixture was filtered through glass wool; the combined filtrates were diluted with 1250 ml each of chloroform and water and left to settle in a separatory funnel. The deep red chloroform phase was withdrawn, diluted with $^1/_{10}$ volume of benzene, and concentrated to dryness in a rotary evaporator. To remove carotenoid pigments and neutral lipids, we dissolved the residue (5.1 g; 46 mg/-liter of culture) in chloroform (15–16 ml), diluted the solution with 10 volumes of acetone, and kept it at 0°C overnight. The tan-colored precipitate was recovered by centrifugation, washed several times with 10-ml portions of cold acetone and dried in vacuo; yield, 4.9 g.

EXPERIMENTAL RESULTS

Preparation of 2,3-Di-O-Phytanyl-sn-Glycerol from H. cutirubrum

The acetone-insoluble lipids (4.9 g) were methanolyzed (1) in 100 ml of 2.5% anhydrous methanolic hydrogen chloride under reflux for 4–5 hr; the mixture was diluted with 10 ml of water, and the crude glycerol diether, together with small amounts of glycerol monoethers (3) was extracted with several 50-ml portions of petroleum ether. The extracts were concentrated in a rotary evaporator and the residue was dried in vacuo; yield, 3.22 g (65% of acetone-insoluble lipids).

The crude diether (referred to as "unsaponifiable material") was fractionated on a column of silicic acid, prepared in petroleum ether, by elution with the solvents given in Table 1. Small amounts of residual pigments and hydrocarbons were eluted with petroleum ether (fraction 1), followed by 1-chloro-2,3-diphytanyl glycerol which emerged with petroleum ether–benzene 1:1 as eluent (fraction 2). The chloro compound was identified by comparison of its R_f values on TLC and relative retentions on GLC with those of the authentic synthetic substance; it was probably formed from the diether during the refluxing with methanolic HCl. The desired diphytanyl glycerol ether appeared in the benzene and benzene–ether eluates (fractions 4 and 5), which were combined and brought to dryness in vacuo, yielding 2.7 g of colorless oil (85% of unsaponifiable material). The diether obtained was chromatographically pure (being free of glycerol monoether and chlorinated derivatives) and had an IR spectrum identical with that previously reported (1, 3) [major bands: OH, 3450 cm^{-1}; CH_2 and CH_3, 2960, 2930, 2860, 1465, and 730 cm^{-1}; $C(CH_3)_2$, 1370–1380 cm^{-1}; C—O—C, 1110 cm^{-1}; C—O—, 1045 cm^{-1}]. The purified diether had $[\alpha]_D$ + 8.4° (3.8g/dl in chloroform).

Analysis: $C_{43}H_{88}O_3$ (653.1);

calculated: C, 79.07; H, 13.58
found: C, 79.43; H, 13.87

Preparation of Natural Phytanyl(3R,7R,11R,15-tetramethylhexadecyl) Bromide

Natural phytanol (320 mg), prepared as described elsewhere (4), was converted to the bromide by reaction with a mixture of 47% HBr (9 ml) and concentrated

Downloaded from www.jlr.org by guest, on June 20, 2012

H_2SO_4 (1 ml) heated under reflux for 2 hr. The reaction mixture was diluted with water and extracted with ethyl ether; the extract was washed with water, dried over sodium sulfate, filtered, and evaporated to dryness in vacuo. The residual crude bromide (370 mg) was purified by preparative TLC in chloroform–ether 9:1 (R_f of bromide, 0.88, and of phytanol, 0.40); yield of chromatographically pure natural phytanyl bromide, 266 mg (70%). GLC retention of the natural bromide relative to octadecyl bromide on butanediolsuccinate polyester at 180°C was 0.77.

Synthesis of 2,3-Di-O-(3'R,7'R,11'R,15'-tetramethylhexadecyl)-sn-glycerol

Naturally-occurring diether was synthesized by the procedure described previously (2). Natural phytanyl bromide (170 mg, 0.47 mmoles) and 1-benzyl-*sn*-glycerol [100 mg, 0.54 mmoles; prepared according to the method of Lands and Zschocke (8)] were allowed to react in boiling benzene (10 ml) in the presence of powdered potassium hydroxide (0.5 g) for 18 hr. The product obtained, a mixture of the desired benzyl glycerol diphytanyl diether and phytene-1, was fractionated by preparative TLC in petroleum ether–benzene 1:1, yielding 60 mg of 1-benzyl-2,3-diphytanyl-*sn*-glycerol (32% yield from phytanyl bromide). This triether gave one spot, R_f 0.23, on TLC in benzene–petroleum ether 1:1, and had the same IR spectrum as reported previously (2). The blocking benzyl group was then removed by hydrogenolysis of the triether (60 mg) in anhydrous ethyl acetate (7 ml) with freshly prepared palladium catalyst on charcoal (9) for 1 hr. The reaction product was isolated as described previously (2), and purified by preparative TLC in chloroform–ethyl ether 20:1 (R_f 0.40); yield 33.5 mg (20% over-all yield) of pure 2,3-di-(3'*R*,7'*R*,11'*R*,15-tetramethylhexadecyl)-*sn*-glycerol. The synthetic diether obtained had the same R_f values on TLC in several solvents and IR spectrum as the natural diether; its optical rotation, $[\alpha]_D$ + 8.5° (3.35 g/dl in chloroform) was also identical with that of the natural compound (Table 2).

Isolation of Monophytanyl Glycerol Ethers from H. cutirubrum

The crude monoether fraction (0.33 g; fractions 6–9, Table 1), obtained during silicic acid column chromatography of the crude diether, was refractionated on a column of silicic acid (28 g) with the following solvents: benzene (150 ml, subfraction 1); benzene–ethyl ether 10:1 (100 ml, subfraction 2); benzene–ether 10:3 (250 ml, subfraction 3); benzene–ether 2:1 (125 ml, subfraction 4); benzene–ether 1:1 (325 ml, subfraction 5); and ethyl ether (200 ml, subfraction 6). The desired monoether (R_f 0.18 in chloroform–ether 3:1) appeared in subfractions 4 and 5, together with traces of faster- and slower-moving material. These subfractions were combined (weight 74 mg) and purified by preparative TLC in ethyl ether, yielding 39.5 mg (about 1% of unsaponifiable material) of chromatographically pure monoether material (R_f 0.30 in ethyl ether) with $[\alpha]_D$ + 1.5° ± 0.5° (2.0 g/dl in chloroform).

For analysis by GLC, the monoether preparation was converted to dimethyl ether derivative(s) as follows. A solution of the monoether (10–30 mg) in methyl iodide (5 ml) was heated under reflux in the presence of silver oxide (100 mg) for 3 hr. The cooled mixture was diluted with an equal volume of ethyl ether and centrifuged to remove silver salts. The insoluble residue was washed

TABLE 1 Chromatographic Isolation of Di- and Monophytanyl Glycerol Ethers from Lipids of *H. cutirubrum**

Fraction No.	Solvent	Solvent Volume	Fraction Weight		Components	R_f Value†
		ml	*g*	*%*		
1	Petroleum ether (bp 30–60°)	500	0.05	1.6	Pigments + hydrocarbons	0.80
2	Petroleum ether–benzene 1:1	500	0.10	3.1	Glycerol diether chloride	0.60
3	Benzene	1000				
4	Benzene	1000	2.72	84.5	Diphytanyl glycerol ether	0.40
5	Benzene–ethyl ether 10:1	500				
6	" " " "	500	0.33	10.2	Glycerol monoether chloride	0.14
7	Ethyl ether	250			+ monophytanyl glycerol	0.05
8	Ethyl ether–methanol 1:1	200				
9	Methanol	200			+ unidentified	0.00
	Recovery		3.20	99.4		

* 3.22 g of unsaponifiable material were chromatographed on a column of 140 g of silicic acid.
† On silica gel microslide plates; solvent, chloroform–ethyl ether 20:1.

Downloaded from www.jlr.org by guest, on June 20, 2012

TABLE 2 PROPERTIES OF NATURAL AND SYNTHETIC MONO- AND DIPHYTANYL GLYCEROLS

Compound	$[\alpha]_D$*	$[M]_D$	TLC R_f Values†			GLC of Dimethyl Ethers Relative Retention‡
			1	2	3	
Natural di-O-phytanyl glycerol	+8.4°	+55	0.56	0.90	—	—
Synthetic 2,3-di-O-phytanyl-sn-glycerol	+8.5°	+56	0.55	0.90	—	—
Natural α-mono-O-phytanyl glycerol	−0.95°	−3.5	0.24	0.33	0.10	1.45
Synthetic 3-O-phytanyl-sn-glycerol	−0.94°	−3.5	0.25	0.32	0.10	1.45
Natural β-mono-O-phytanyl glycerol	+2.4°	+9.0	0.24	0.31	0.18	1.24
Synthetic 2-O-phytanyl glycerol	+3.0°	+11	0.25	0.32	0.18	1.25

* In chloroform.

† In the following solvents: 1, chloroform–ether 9:1; 2, ethyl ether; and 3, chloroform–methanol 49:1 on boric acid-impregnated plates developed three times (6).

‡ Relative to dimethyl ether of 1-hexadecyl glycerol (chimyl alcohol) on butanediol succinate polyester at 180°C.

twice with ethyl ether, and the combined supernates were brought to dryness in vacuo. The residual dimethyl ether, obtained in almost quantitative yield with a purity greater than 90%, was finally purified by preparative TLC in chloroform–ether 9:1 (R_f 0.75); the pure di-O-methyl-O-monophytanyl glycerol had $[\alpha]_D$ + 4.7 ± 0.4° (2.3 g/dl in chloroform).

Analysis: $C_{25}H_{52}O_3$ (400.67);
calculated: C, 74.93; H, 13.02
found: C, 74.60; H, 12.93

Analysis of the natural monophytanylglycerol dimethyl ether preparation by GLC on butanediol succinate polyester (Fig. 1) showed that it contained two components (with retention 1.24 and 1.45 relative to chimyl alcohol dimethyl ether) in about equal amounts (47 and 53%, respectively). The same peaks were obtained after catalytic hydrogenation, which shows that the two components were saturated. However, periodate oxidation of the unmethylated monoether preparation followed by methylation did not affect the fast-moving component but resulted in the disappearance of the slow-moving component. It may thus be concluded that the slow-moving component is the α-monoether, and the fast-moving component is the β-isomer (Fig. 1).

Separation of Natural α- and β-Mono-O-phytanyl Glycerols

The two isomers were separated by preparative TLC on boric acid-impregnated silica gel (5, 6) as follows. The mixture of isomers (21 mg) was applied to a 20 × 20 cm plate that was developed three times in chloroform–methanol 49:1. The faster-moving β-isomer (R_f 0.18) was well separated from the slower-moving α-isomer (R_f 0.10); the band corresponding to each isomer was eluted with chloroform–methanol 1:1, yielding 7.6 mg of the α-isomer and 8.9 mg of the β-isomer. Each isomer gave only a single spot on TLC on boric acid-impregnated plates, and on GLC showed only one peak which corresponded to the respective peak in the chromatogram of the natural mixture of monoethers (Fig. 1). The α-isomer had $[\alpha]_D$ − 0.95° (0.7 g/dl in chloroform) and the β-isomer had $[\alpha]_D$ + 2.4° (0.9 g/dl in chloroform).

The IR spectra of the two isomers (Fig. 2) were quite similar, containing strong absorption bands for OH (3400 cm^{-1}), CH_2 and CH_3 (2960, 2920, 2870, 1465, and 730 cm^{-1}), $C(CH_3)_2$ (1370–1380 cm^{-1}, doublet), and C—O—C ether (1115 cm^{-1}). The only significant difference was in the alcoholic C—O band, which was at 1050 cm^{-1} in the spectrum of the α-isomer, but was a doublet at 1050 and 1075 cm^{-1} in that of the β-isomer.

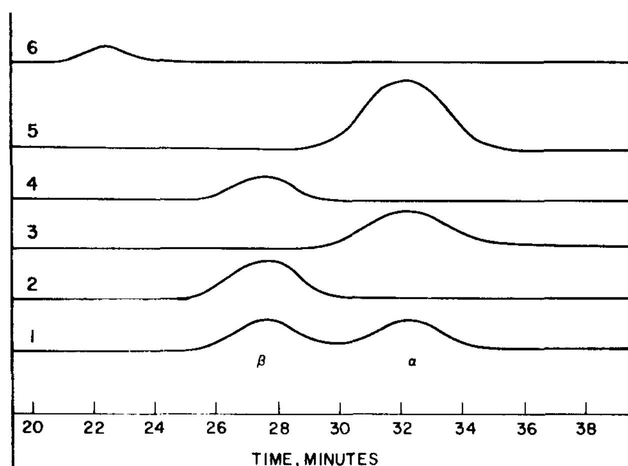


FIG. 1. GLC of di-O-methyl derivatives of: *1*, mono-O-phytanyl glycerol preparation (α- and β-isomers) isolated from *H. cutirubrum; 2*, TLC-separated natural β-O-phytanyl glycerol; *3*, TLC-separated natural α-O-phytanyl glycerol; *4*, synthetic 3-O-phytanyl-*sn*-glycerol; *5*, synthetic 2-O-phytanyl glycerol; *6*, chimyl alcohol (1-O-hexadecyl-*sn*-glycerol). GLC was carried out on butanediol succinate polyester at 180°C and 28 psi inlet pressure.

Downloaded from www.jlr.org by guest, on June 20, 2012

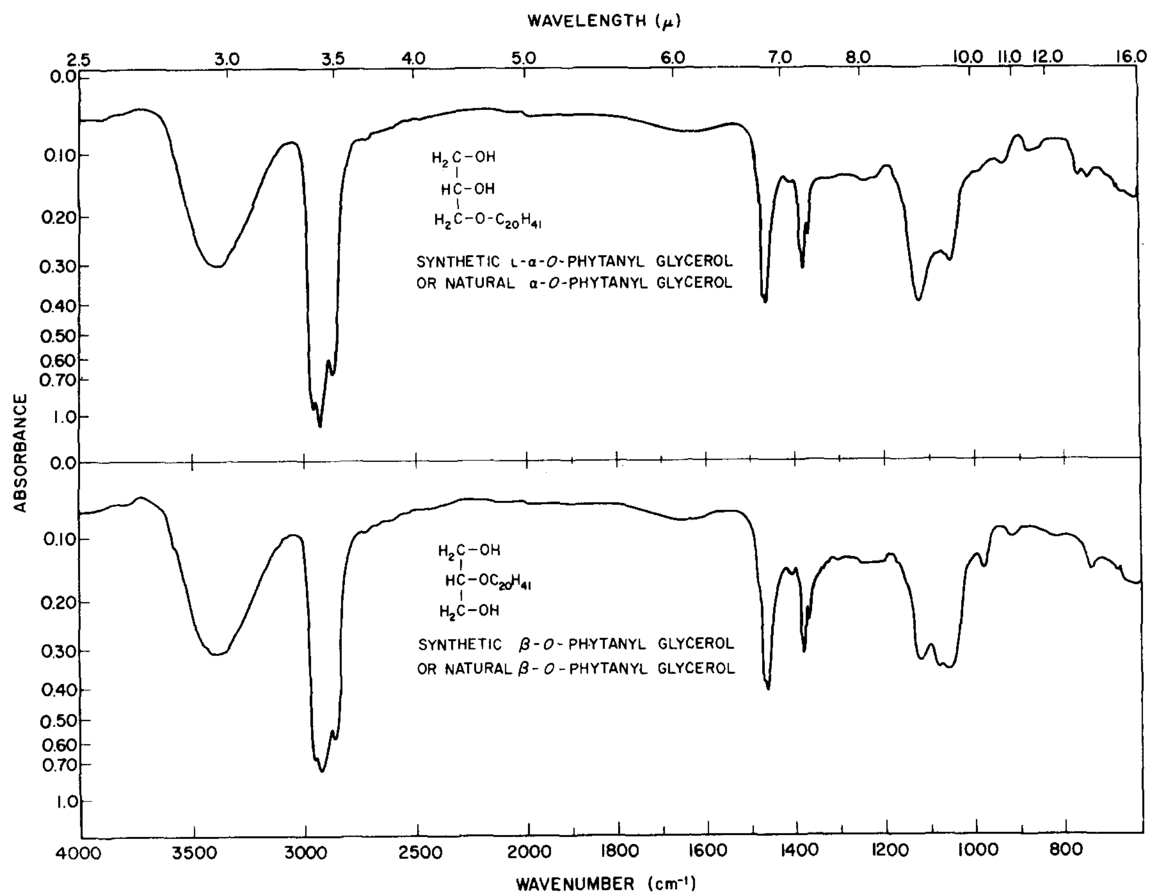


FIG. 2. IR spectra of natural α-*O*-phytanyl and β-*O*-phytanyl glycerol, and of synthetic 3-*O*-phytanyl-*sn*-glycerol and 2-*O*-phytanyl glycerol taken in carbon tetrachloride solution.

To verify the structure of these isomers and establish the configuration of the α-isomer, we undertook their synthesis (see Scheme 1).

Synthesis of 3-O-3'R,7'R,11'R,15'-Tetramethylhexadecyl-sn-glycerol

A solution of phytanyl bromide [90 mg, 0.25 mmoles; prepared from the natural diphytanyl glycerol (2, 4)] and 1,2-isopropylidene-*sn*-glycerol (I) [66 mg, 0.5 mmole; prepared according to Baer (10)] in anhydrous benzene (20 ml) was heated under reflux with stirring in the presence of finely powdered KOH (0.5 g) for 36 hr; the water formed during the reaction was removed by means of a phase-separating head. The cooled reaction mixture was diluted with an equal volume of benzene and the insoluble material was removed by centrifugation and washed twice with ethyl ether. The combined solutions were concentrated under reduced pressure and the residue was dissolved in 20 ml of ethyl ether and washed successively with water, 0.25 N HCl, 2.5% $NaHCO_3$, and finally water. The product obtained on evaporation of the organic solvent was a mixture of the isopropylidene monoether (R_f 0.25 in chloroform) and phytene (R_f 0.80 in chloroform), which were separated by preparative TLC in chloroform; yield of pure isopropylidene 3-*O*-phytanyl-*sn*-glycerol (II), 43.6 mg (42.3%).

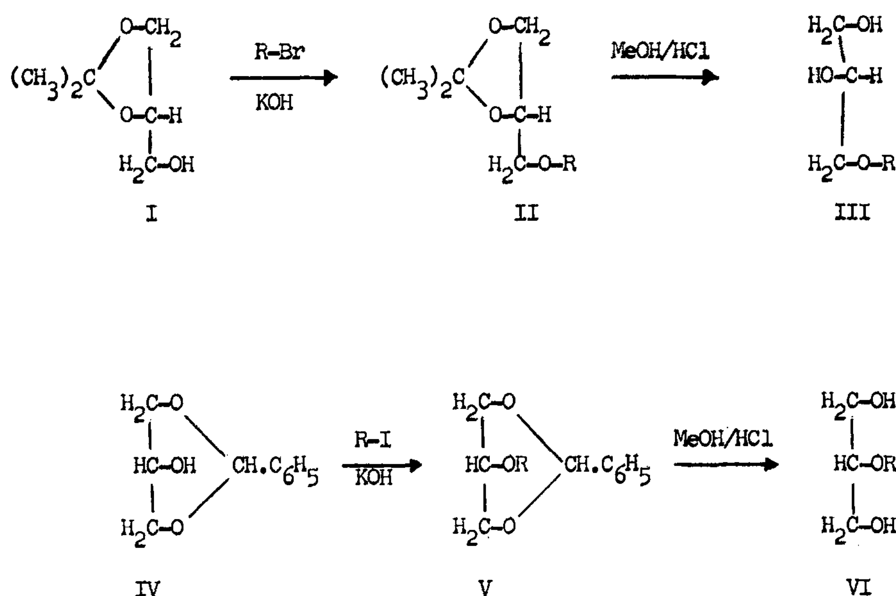
The purified isopropylidene monoether (II) (43.6 mg) was freed of the isopropylidene group by hydrolysis in methanol–water–concentrated HCl 18:1:1 (10 ml) under reflux for 2 hr. The cooled reaction mixture was extracted with several 10-ml portions of petroleum ether; the combined extracts were concentrated in vacuo and the residual crude monoether (III) was purified by preparative TLC with ethyl ether as solvent (R_f 0.33); yield of pure 3-*O*-phytanyl-*sn*-glycerol (III), 20.4 mg (22% over-all yield). The product (III) had a relative retention on GLC (Fig. 1), an IR spectrum (Fig. 2), TLC R_f values, and a specific rotation ($[\alpha]_D$ − 0.94°, 2.0 g/dl in chloroform) identical with those of the natural α-isomer (Table 2).

Analysis: $C_{23}H_{48}O_3$ (372.6);
calculated: C, 74.13; H, 12.99
found: C, 74.70; H, 12.91

Synthesis of 2-O-3'R,7'R,11'R,15'-Tetramethylhexadecyl Glycerol

1,3-Benzylidene glycerol (IV) was prepared by the pro-

Downloaded from www.jlr.org by guest, on June 20, 2012



Scheme 1. Synthesis of 3-*O*-phytanyl-*sn*-glycerol and 2-*O*-phytanyl glycerol. R, $C_{10}H_{41}$ (phytanyl).

cedure of Verkade and van Roon (11); it was recrystallized from benzene–petroleum ether (bp 60–90°C) 1:1 yielding largely the *cis* isomer with mp 71–73°C (6). A mixture of IV (55 mg, 0.32 mmole) and the bacterial phytanyl iodide (4) (112 mg, 0.27 mmole) in benzene (20 ml) was refluxed in the presence of powdered KOH (0.5 g) for 20 hr. The cooled reaction mixture was diluted with an equal volume of ethyl ether, neutralized with dilute HCl, and washed successively with saturated $NaHCO_3$ and water. The organic phase was concentrated in vacuo and the residual crude benzylidene monoether (V) was then hydrolyzed in a mixture of 5 ml of methanol and 0.3 ml of concentrated HCl under reflux for 1 hr. The cooled reaction mixture was diluted with 1 ml of water and extracted repeatedly with petroleum ether. The solvent was evaporated under reduced pressure and the residual crude monoether (VI) was purified by preparative TLC in ethyl ether (R_f 0.25); yield of pure 2-*O*-phytanyl glycerol (VI), 18.2 mg (19% over-all yield). The product (VI) had a relative retention on GLC (Fig. 1), IR spectrum (Fig. 2), TLC R_f values, and specific rotation ($[\alpha]_D + 3.0°$; 3.6 g/dl in chloroform) identical with those of the natural β-isomer (Table 2). The di-*O*-methyl derivative of VI had $[\alpha]_D + 2.8°$ (3.4 g/dl in chloroform).

Analysis: $C_{23}H_{48}O_3$ (372.6);

calculated: C, 74.13; H, 12.99
found: C, 74.47; H, 12.97

DISCUSSION

The results reported here show unambiguously that the diphytanyl glycerol ether component of the lipids in *H. cutirubrum* has the structure and configuration, 2,3-di-*O*-(3′*R*,7′*R*,11′*R*,15′-tetramethylhexadecyl)-*sn*-glycerol. The highly purified diether obtained in the present study had a somewhat higher specific rotation (+8.4°) compared to that (+7.8°) reported for earlier preparations (1, 2). The latter probably still contained traces of monoethers and chlorinated derivatives.

The finding of small amounts of α- and β-isomers of monophytanyl glycerol ethers is of considerable interest, especially since the α-isomer has the 3-*sn*-glycerol (or L) configuration, which is opposite that of the α-alkyl glycerol monoethers found in elasmobranches and other higher organisms (12). It should be noted that the glycerol configuration of the α-monophytanyl ether is the same as that of the diphytanyl ether, which suggests that the monoether may be the biogenetic precursor of the diether. If this is so, then it must be concluded that the introduction of the second phytanyl group into the 2-position of the monoether cannot involve any inversion of configuration.

As to the biosynthetic pathway for the monoether itself, one possibility can be eliminated, namely the alkylation of *sn*-glycerol-3-phosphate, since this would give rise to the 1-*sn*-glycerol ether. It may be argued, then, that the precursor would be the *sn*-glycerol-1-phosphate. However, since it has been shown recently[2] that the glycerophosphate made by cells of *H. cutirubrum* has the usual 3-*sn*-glycerol configuration, this possibility is eliminated. Another possible precursor that could be alkylated is dihydroxyacetone phosphate. Stepwise alkylation coupled with stereospecific reduction of the keto group might well account for the formation of both the 2- and the 3-isomers of the monoether.

[2] Wassef, M. K., J. Sarner, and M. Kates. In preparation.

Downloaded from www.jlr.org by guest, on June 20, 2012

ASBMB JOURNAL OF LIPID RESEARCH

Another point that remains to be cleared up is the original form of the monoethers in the acetone-insoluble lipids. It is quite clear that the monoethers are present in a bound form since they are found only after methanolysis of the lipids. It seems reasonable to suppose that they originate from the unidentified phospholipid observed on chromatograms of halophile lipids (spot *4* in Fig. 5 of reference 4), since this is the only phospholipid whose structure has not been elucidated. As a working hypothesis, it may be assumed that this unknown component might be the monoether analogue of phosphatidyl glycerophosphate. Further studies along these lines are in progress.

Manuscript received 4 June 1968; accepted 29 July 1968.

REFERENCES

1. Kates, M., L. S. Yengoyan, and P. S. Sastry. 1965. *Biochim. Biophys. Acta.* **98:** 252.
2. Kates, M., B. Palameta, and L. S. Yegoyan. 1965. *Biochemistry.* **4:** 1595.
3. Kates, M., B. Palameta, C. N. Joo, D. J. Kushner, and N. E. Gibbons. 1966. *Biochemistry.* **5:** 4092.
4. Kates, M., C. N. Joo, B. Palameta, and T. Shier. 1967. *Biochemistry.* **6:** 3329.
5. Serdarevich, B., and K. K. Carroll. 1966. *J. Lipid Res.* **7:** 277.
6. Serdarevich, B., and K. K. Carroll. 1966. *Can. J. Biochem.* **44:** 743.
7. Sehgal, S. N., and N. E. Gibbons. 1960. *Can. J. Microbiol.* **6:** 165.
8. Lands, W. E. M., and A. Zschocke. 1965. *J. Lipid Res.* **6:** 324.
9. Hessel, L. W., I. D. Morton, A. R. Todd, and P. E. Verkade. 1954. *Rec. Trav. Chim.* **73:** 150.
10. Baer, E. 1952. *Biochemical Prepn.* **2:** 31.
11. Verkade, P. E., and J. D. van Roon. 1942. *Rev. Trav. Chim.* **61:** 831.
12. Baer, E., L. J. Rubin, and H. O. L. Fischer. 1944. *J. Biol. Chem.* **155:** 447.

ASBMB JOURNAL OF LIPID RESEARCH JLR
Downloaded from www.jlr.org by guest, on June 20, 2012