PHOSPHOLIPID OF AN EXTREMELY HALOPHILIC BACTERIUM, *SARCINA MORRHUAE**

By G. C. WOODROW,† H. T. CHEUNG,‡ and K. Y. CHO†

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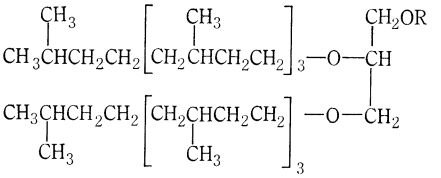
Abstract

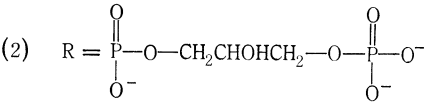
The major phospholipid of the extremely halophilic coccus, *S. morrhuae*, was purified and compared with the major phospholipid of the extremely halophilic rod, *Halobacterium cutirubrum*. Analysis was by infrared and nuclear magnetic resonance spectroscopy, mass spectrometry, and thin-layer chromatography. The last two procedures established structural identity of the coccal lipid with that of the rod, which had been shown to be the 1-phosphatidylglycerophosphate derivative (2) of 2,3-di-*O*-dihydrophytylglycerol (1).

I. INTRODUCTION

The lipids of the extremely halophilic rod, *Halobacterium cutirubrum*, have been extensively studied, and are unusual in that they consist almost entirely of derivatives of a glycerol diether, L-2,3-di-*O*-(3R,7R,11R)-dihydrophytylglycerol (1). The major phospholipid of this bacterium was shown to be (2), the diether analogue of phosphatidylglycerophosphate (Kates *et al.* 1965*a*, 1965*b*, 1967).



(1) R = H



(2)

Chromatographic analysis indicated that the phosphatide (2) was the major lipid in all species of *Halobacterium* studied, but was absent from the moderately halophilic or non-halophilic bacteria so far examined (Kates *et al.* 1966).§

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† Department of Microbiology, University of Sydney, Sydney, N.S.W. 2006.

‡ Department of Pharmacy, University of Sydney, Sydney, N.S.W. 2006.

§ *Note added in proof.*—The presence of the phosphatide (2) as the major lipid of a bacterium isolated from Dead Sea sediment was recently reported (M. F. Mullakhanbhai and G. W. Francis, *Acta chem. scand.*, 1972, **26**, 1399–1410).

The detailed lipid composition of the extremely halophilic cocci is less well known. In three strains of halophilic cocci, a phospholipid composition very similar to that of *H. cutirubrum* was observed (Kates *et al.* 1966). In this paper, we present a more detailed analysis of the major lipid component of the red-pigmented extremely halophilic coccus *Sarcina morrhuae*, and a direct comparison of this lipid with the phosphatidylglycerophosphate of *H. cutirubrum*.

II. Materials and Methods

(*a*) *Organisms and Growth Conditions*

The species used, *S. morrhuae* and *H. cutirubrum*, were grown in the extreme-halophile medium described by Sehgal and Gibbons (1960) with the modification that 40 g Oxoid peptone replaced 7·5 g casamino acids (Difco). Cultures (500 ml medium per 2-litre flask) were grown with shaking on a New Brunswick gyrotary shaker at 37°C for 4–6 days.

(*b*) *Lipid Extraction and Hydrolysis*

The method of Kates (Kates *et al.* 1965*b*) and the following procedure were used. Cells were lyophilized and then homogenized in a Servall Omnimixer for 4 × 3 min with 25 ml chloroform–methanol (2:1 v/v). The solution was filtered and evaporated under reduced pressure. The resulting residue was redissolved in 2 ml of chloroform and stored at −20°C.

Total lipids were differentiated into acetone-soluble and acetone-insoluble fractions by the addition of 10 vol. of cold acetone. The resulting acetone-insoluble precipitate comprised largely polar lipids. This precipitate was removed by centrifugation at 20,000 *g* for 20 min. After decanting, the precipitate was washed in 10 ml of cold acetone. The polar fraction was evaporated, redissolved in 3 ml of chloroform, and stored at −20°C.

Hydrolysis of the phosphate group was carried out using 10% hydrogen chloride in methanol–water (3:1 v/v) under reflux for 2 hr.

(*c*) *Chromatographic Separation*

Thin-layer plates consisted of silica gel G spread to a thickness of 0·25 mm (analytical) and 0·6 mm (preparative) on 20 by 20 cm glass plates. Two solvent systems were used, the first consisting of chloroform–methanol–water (65:25:4 v/v) and the second being di-isobutyl ketone–acetic acid–water (8:5:1 v/v). Lipids were detected by spraying with 0·005% Rhodamine 6G and viewing under ultraviolet light while still wet. Phospholipids were identified by spraying with an acid–molybdate reagent (Vaskovsky and Kostetsky 1968).

The preparative plates were sprayed with water and the bands observed against a dark background. The major band was removed and extracted with chloroform–methanol (2:1 v/v). The silica gel was removed by centrifugation and filtration. The purity of the isolated sample was confirmed by thin-layer chromatography before further analysis.

(*d*) *Infrared Spectroscopy*

Infrared spectra of samples in liquid film form were determined on a Perkin-Elmer 21 spectrometer and calibrated.

(*e*) *Nuclear Magnetic Resonance Spectroscopy*

Nuclear magnetic resonance spectra were measured in deuterochloroform solutions with a Perkin-Elmer R-12 60 MHz spectrometer, using tetramethylsilane as internal standard.

(*f*) *Mass Spectrometry*

Mass spectra were measured at 70 and 12 eV on an A.E.I. MS-982 instrument, using an all-glass, heated inlet system at about 130°C.

III. Results and Discussion

(*a*) *Thin-layer Chromatography*

The total polar phospholipid fractions from *S. morrhuae* and *H. cutirubrum* were run side-by-side in two different systems (Fig. 1). Of particular note are the spots corresponding to the major phospholipid (as gauged from the intensity of the colour reaction with Rhodamine 6G and acid–molybdate sprays). In either solvent system the major phospholipids from both bacteria had the same R_F values.

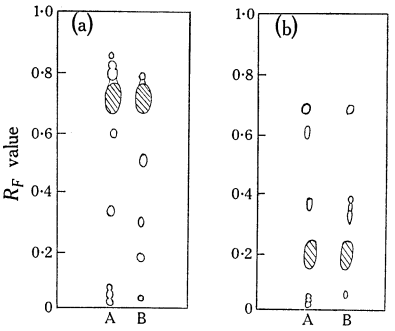


Fig. 1.—Chromatograms of lipid fractions from *S. morrhuae* (*A*) and *H. cutirubrum* (*B*) on silica gel G. The shaded spots represent major components. (*a*) Chromatographed in chloroform–methanol–water (65:25:4 v/v); (*b*) chromatographed in di-isobutyl ketone–acetic acid–water (8:5:1 v/v).

(*b*) *Infrared Spectroscopy*

The spectrum of the purified major phospholipid of *S. morrhuae* showed C–H stretching (2900 cm^{-1}) and bending (1470 and 1370 cm^{-1}) absorption bands. More significant was the absence of a strong C=O absorption band near 1740 cm^{-1}, indicating that the phospholipid is not a glyceride.

(*c*) *Nuclear Magnetic Resonance Spectroscopy*

The n.m.r. spectrum of the major phospholipid of *S. morrhuae* showed signals typical of highly branched hydrocarbon chain(s): δ 0·9–1·0 (CH_3), 1·3 and 1·6–1·7 (CH_2 and CH). A small broad signal near δ 3·6 (CH–O–) was consistent wtih the presence of ether grouping(s). Identical features were present in the n.m.r. spectrum of the major phospholipid (2) isolated from *H. cutirubrum*.

(*d*) *Mass Spectrometry*

The major phospholipids from *S. morrhuae* and from *H. cutirubrum* gave rise to identical fragmentation patterns (Fig. 2). Both samples underwent cleavage of the phosphate group in the heated inlet system and the resulting spectra were identical to those of samples which had been subjected to acid hydrolysis. The fragmentation pattern is in full agreement with a 2,3-di-*O*-dihydrophytylglycerol structure (1). There are three distinct regions in the 70 eV spectrum (Fig. 2). Firstly, there are peaks due to C_3–C_{19} homologous ions typical of hydrocarbon fragmentation. The other two groups of peaks, both also observed at low ionizing voltage (12 eV), consist of (A) high-mass ions of about 1% abundance, *viz.* the molecular ion (*m/e* 652), and ions resulting from simple loss of CH_3, OH, H_2O, CH_2O, CH_2OH, and CH_3OH respectively; and (B) highly characteristic fragments in the region *m/e* 278–383 (abundance 2–20%) which are discussed below.

The ions at *m/e* 341 (5%) and 340 (9%) obviously result from α-cleavage of ethers (Djerassi and Fenselau 1965; Carpenter *et al.* 1967), accompanied in the latter case by hydrogen transfer (cf. glycols; Kossanyi *et al.* 1968).

The molecular ion (M) and the M−CH_2O ion (*m/e* 622) undergo parallel series of fragmentations. Firstly, double hydrogen transfer (Carpenter *et al.* 1967; Spiteller-

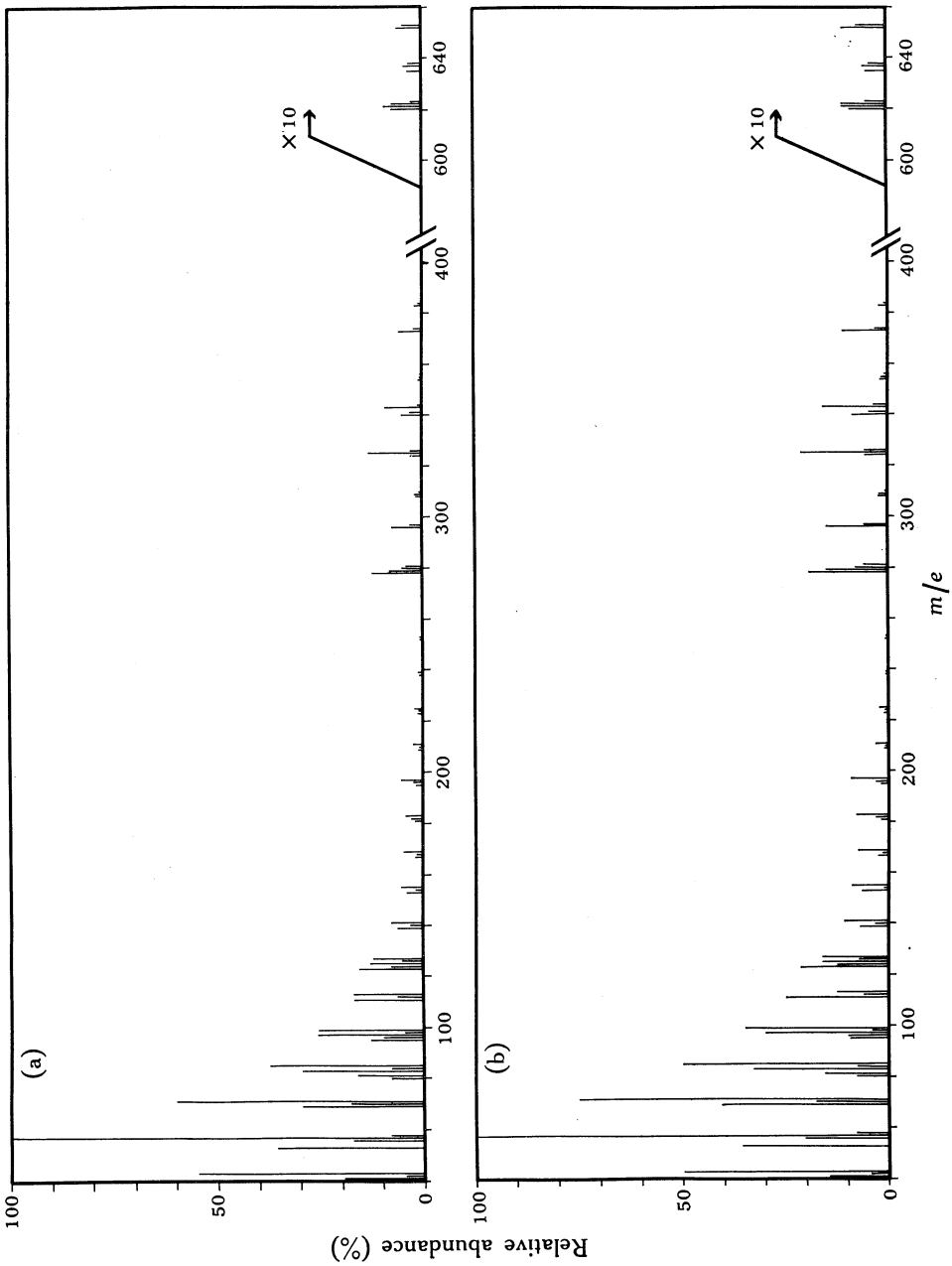
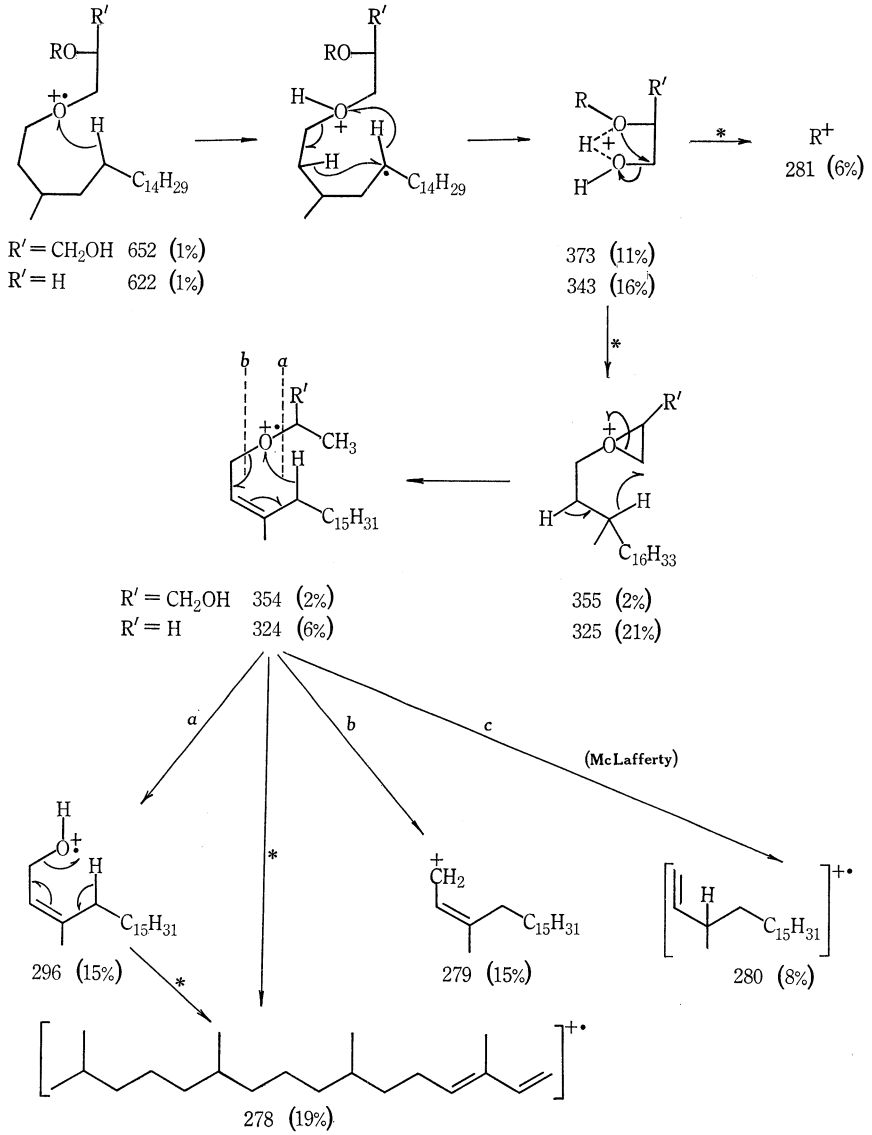
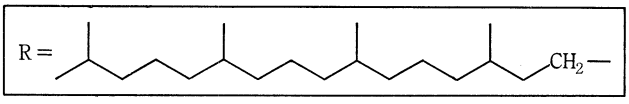


Fig. 2.—Mass spectra of the major phospholipid from *H. cutirubrum* (*a*) and *S. morrhuae* (*b*).

Friedmann and Spiteller 1967) yields respectively ions *m/e* 373 and 343, both of which further decay* to the dihydrophytyl ion *m/e* 281 (Scheme 1). Alternatively, ions at *m/e* 373 and 343 lose water and hydrogen successively to yield two pairs of



Scheme 1



* Fragmentations shown with an asterisk in Scheme 1 are supported by the detection of metastable transitions.

ions *m/e* 355 and 325; 354 and 324 respectively. That the process is probably accompanied by hydrogen transfer (as proposed in Scheme 1) is suggested by the formation from the latter pair of a fragment (*m/e* 278) which is formally an eicosadiene ($C_{20}H_{38}$). Other fragmentation modes of ions *m/e* 354 and 324 (Scheme 1, paths *a*, *b*, and *c*) support our formulation of these ions as ionized allylic ethers (Meyerson and McCollum 1963).

IV. General Discussion

Mass spectral comparison of the major phospholipid components from *S. morrhuae* and *H. cutirubrum* establishes that both phospholipids are derivatives of 2,3-di-*O*-dihydrophytylglycerol (1). Furthermore, chromatographic analysis shows that the major phospholipids from both bacteria are structurally (but not necessarily stereochemically) identical. The major phospholipid of *S. morrhuae* is therefore a 1-phosphatidylglycerophosphate derivative (2) of 2,3-di-*O*-dihydrophytylglycerol.

The presence of diether phospholipids in all species of extreme halophiles examined further strengthens the view (Kushner 1968) that these unusual lipids are of taxonomic and evolutionary importance.

V. References

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