stirred (2 min) with activated charcoal (40 g), filtered and toxin extracted from the charcoal as in [2]. Charcoal extracts from 2 batches, combined in 50 ml H_2O with the pH adjusted to 8.5 (Na_2CO_3), were extracted with EtOAc (4 × 50 ml). The aq. soln was adjusted to pH 2.5 (2M H_2SO_4) and again extracted with EtOAc (4 × 50 ml). This second EtOAc fraction, which contained organic acids and biological activity, was dried (Na_2SO_4) and evaptd; the residue in 10 ml MCA was chromatographed on Sephadex LH 20, the active component cluting in 36 ml, commencing after 215 ml eluent was collected. The combined active fractions, when subjected to PLC as the free acids, separated into 3 components: the major (R_f 0.65) was not biologically active (discarded), the second yielded pure toxin (TLC R_f 0.40), the third (minor, R_f 0.25), also biologically active, was collected for further study. The toxin MS had *m/e* 319 (M^+), 301 ($M^+ - H_2O$), 259, 191, 163 and 145, all of similar intensities in the MS recorded for authentic coronatine [4]; mass measurements: 319.1797, calcd. for $C_{18}H_{25}NO_4$: 319.1784; 191.1054, calcd. for $C_{12}H_{15}O_2$: 191.1072. The toxin was methylated with CH_2N_2, affording a single TLC homogeneous product (R_f 0.50) after elution with EtOAc–$CHCl_3$ (1:4), MS *m/e* 333 (M^+), 301 (M^+ – MeOH), 191, 163, 145 and 142; mass measurements: 333.1934, calcd. for $C_{19}H_{27}NO_4$: 333.1940; 191.1078, calcd. for $C_{12}H_{15}O_2$: 191.1072; 142.0855, calcd. for $C_7H_{12}NO_2$: 142.0868; consistent for methylcoronatine.

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2,3-DI-*O*-PHYTANYL-*sn*-GLYCEROL AND PRENOLS FROM EXTREMELY HALOPHILIC BACTERIA

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Key Word Index—Halophilic bacteria; lipids; ether lipids; prenols; di-*O*-phytanyl glycerol.

Table 1. Contents of 2,3-di-*O*-phytanyl-*sn*-glycerol, geranylgeraniol and phytanol in extremely halophilic bacteria

Species and Strain No.*	% Content of† 2,3-di-*O*-phytanyl-*sn*-glycerol	Geranylgeraniol	Phytanol‡
Halobacterium cutirubrum, 54001	8.5	11.3	0.30
H. halobium, 34020	7	13	0.40
H. halobium M, 34014	10	9	0.20
H. salinarium, 34002	9	13	0.35
Amoebobacter morrhuae, 51001	20	3	0.06
Sarcina litoralis, 16006	12	10	0.25

* Details of the microorganism used and their numbers from the National Research Council of Canada culture collection are given in our previous communication [1].
† % Content is given as % by weight of the total neutral lipids.
‡ Determined by GLC using methyl palmitate as internal standard.

Several strains of extremely halophilic bacteria have been shown to contain carotenoids, MK-8, retinal and squalenes [1]. This paper reports on the remaining neutral lipid components which have been isolated and characterized.

From each of the strains listed in Table 1, three colour-

less compounds, phytanyl-glycerol diether, geranylgeraniol and phytanol, have now been isolated and identified. The phytanyl-glycerol diether was obtained as a colourless viscous oil with optical rotations ranging between $[\alpha]_D$ + 8.5–9.0 ($CHCl_3$; c, 2.6). The dextrorotatory nature of the compound indicated that the diether has *sn*-2,3-structure and configuration because synthetic *sn*-1,2-dialkyl glycerol ethers are laevorotatory [2]. The IR and NMR spectra were identical to those of a synthetic sample [2–4]. Geranylgeraniol was also obtained as a colourless oil and its IR, NMR spectra and GLC retention time (10% SP-2300) were identical with an authentic sample [3]. The identity and the amount of phytanol were determined by GLC.

The wide occurrence of large amounts of free phytanylglycerol diether in several strains of extreme halophilic bacteria is highly significant, since it is the common backbone of all the phospho- and glycolipids in these organisms. It would thus appear that phytanylglycerol diether plays a key role in the biosynthesis of phospho- and glycolipids, although the biosynthetic pathway of the phytanylglycerol diether moiety and diether lipids is still a mystery. Presumably, geranylgeraniol and phytanol play a key role in the biosynthesis of the diether moiety, probably via their pyrophosphates. Work on the biosynthesis of the diether lipids is in progress.

EXPERIMENTAL

Culture methods, harvesting of cells, lipid extraction procedures and separation of total lipids into polar and neutral lipids fractions are described elsewhere [3]. Neutral lipids were fractionated on a 3% H_2O (v/w) deactivated column of Al_2O_3 (18 × 3 cm) with C_6H_6 (600 ml, Fr. I), 1% Me_2CO in C_6H_6 (300 ml, Fr. II), 5% Me_2CO in C_6H_6 (300 ml, Fr. III), 10% Me_2CO in C_6H_6 (500 ml, Fr. IV) and pure MeOH (500 ml, Fr. V). Phytanylglycerol diether was purified from fraction III by TLC on Si gel H in $CHCl_3$–Et_2O (99:1, R_f 0.40) and on Al_2O_3 in $CHCl_3$–Et_2O (99.5:0.5, R_f 0.72). Geranylgeraniol was purified from fraction IV by TLC on Al_2O_3 in $CHCl_3$–Et_2O (99.5:0.5, R_f 0.46). The spots were detected by I_2 vapour. Phytanol was determined by applying column fraction IV to GLC on a 10% SP-2300 column (column temp.—180°, N_2 1 kg/cm², H_2 0.7 kg/cm², injector temp.—200°, detector temp.—230°). The relative retentions of phytanol, phytol and geranylgerniol were 2.08, 2.80 and 6.18, respectively (relative to methylpalmitate).

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IVALIN IN *GEIGERIA ASPERA*

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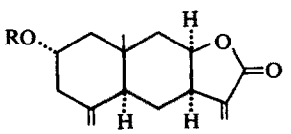
Key Word Index—*Geigeria aspera*; Compositae; eudesmanolide; ivalin; geigerinin; dihydrogriesenin.

Geigeria species, commonly known as the 'vomiting bush', are responsible for vomiting disease [1]. Several crystalline sesquiterpenoid lactones have been isolated from *G. aspera* Harv. and *G. filifolia* Mattf. From *G. aspera* were isolated geigerin [2, 3], vermeerin [4–6], geigerinin [7, 8] and dihydrogriesenin [9], while *G. filifolia* yielded gafrinin [10, 11], griesenin and dihydrogriesenin [12, 13]. Reinvestigation of *G. aspera* has resulted in the identification of ivalin (1). Dihydrogriesenin and geigerinin were also isolated.

Ivalin(1) has previously been isolated as the main sesquiterpene lactone from *Iva microcephala* Nutt. and *Iva imbricata* [14], collected in the Southern Coastal Plain of the USA. It was also shown to be present in *Zaluzania triloba* Pers [15] and *Polymnia leavigata*



(1) R = —H
(2) R = —OCOCH$_2$Br

Beadle [16]. The structure of ivalin(1), the first eudesmanolide isolated from *Geigeria* species, was identified and confirmed by mp, PMR, IR, MS, accurate mass determination and rotation. The X-ray crystallography, by another group [17], on the bromoacetate(2) was in agreement with the known structure. Ivalin(1) as well as the known guaianolides from *Geigeria* species were toxic