The release of metal ions from ash and rock samples by rhodotorulic acid after 21 days of equilibration

Sample[a]	Element (mg/g)	
	Fe	Mg
Ash 18–5	0.07 [0.01] (31.0)	0.44 [0.21] (11.5)
Ash 12–6	0.06 [0.01] (33.6)	0.35 [0.20] (12.6)
ACV–1	15.28 [0.34] (46.5)	1.93 [1.79] (9.1)
BCR–1	19.90 [0.07] (93.2)	0.39 [0.16] (20.9)
G–2	7.66 [2.31] (18.6)	0.04 [0.03] (6.1)
GSP–1	14.56 [5.49] (29.6)	0.10 [0.07] (5.97)
PCC–1	19.04 [0.29] (57.4)	0.00 [0.26] (261.0)
Sand	0.07 [0.01] (ND)	0.08 [0.07] (ND)

[a] The figures shown are the amounts of Fe and Mg released by RA corrected for the RA free controls (bracketed numbers), while the parenthetical numbers are the amount of that element present in the sample[10,13]. ND, not determined.

Niwana, Woodville, Texas, was freed of plant debris, washed with distilled water, and dried at 37 °C. The median grain diameter of this sand was within the fine sand range of the Wentworth size grade scale (0.25–0.125 mm). The dihydroxamic acid RA was crystallized from the medium of low iron cultures[12] of *Rhodotorula pilimanae* ATCC 26423. In a laboratory experiment 5 g of rock sample and 50 ml of 0.05 M pH 7.0 sodium phosphate+0.01 M RA were placed in 125-ml bottles on a shaker (150 3.7-cm reciprocating strokes per min) at 23 °C for 21 days. Following centrifugation (12,000 × g, 10 min) the supernatants were discarded if they deviated more than 0.1 pH units from that of the original solution, if the residue showed evidence (microscopic) of microbial contamination, or if there was any weight loss during shaking. Solutions were examined for metal ions by atomic absorption (Perkin-Elmer 360). Corrections were made for metal ions present in the extracting solution prior to exposure to the rock samples and for the control run without RA.

Results and discussion. The amounts of Fe and Mg released from the rock samples by 3 weeks exposure to buffer+RA is recorded in the table. Also recorded in the table is the original Fe and Mg content of the rock samples. Other elements were considered but less than the following amounts were removed from the samples by RA (units are mg metal/g rock sample): 0.01 Ca, 0.005 Co, 0.01 Cr, 0.015 Cu, 0.005 Mn, 0.005 Ni, 0.008 Sr, and 0.003 Zn. With longer shaking times or higher ash to chelator ratios increasing amounts of Mg and Fe were removed from the ash (data not shown).

The release of Mg from rock samples by RA was unexpected because siderophores are not involved in magnesium metabolism and hydroxamic acids are not particularly selective for Mg[13]. The amounts of Fe removed from the silicate standards were related to, but not a linear function, of the quantities of Fe present. This is not surprising because the rock samples differ in particle size distributions, chemical compositions, and grain size of individual rock forming minerals. Due to the 'glass-like' nature[14] of ash, less iron would be expected to be leached from the ash than from the crystalline rock standards[11].

In this study RA was used as the model chelator because it is the most readily available siderophore[12]. The RA concentration of 0.01 M used was substantially higher than the concentrations of siderophores present in soils[7]; however, this RA concentration has been observed in the medium of low iron cultures of *Rhodotorula pilimanae*[10].

When considering the grain size and weathered nature of the sand sample, the fact RA was effective at Mg and Fe removal suggests siderophores may have a role in rock decomposition.

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A biologically active diphenyl ether from the green alga *Cladophora fascicularis*[1]

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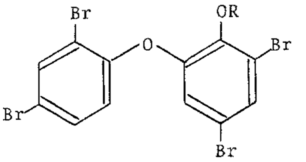
Summary. A new polybrominated diphenyl ether (**1**) has been isolated from the green alga *Cladaphora fascicularis,* and the structure was determined by spectral analysis and conversion to known compounds. It showed antibacterial and antiinflammatory activities.

Key words. Green alga; alga, green; *Cladophora fascicularis;* diphenyl ether, polybrominated; 2-(2′,4′-dibromophenoxy)-4,6-dibromoanisole.

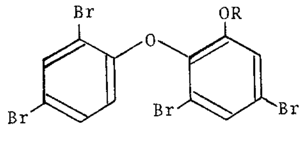
In the course of our chemical study of marine algae from Okinawan waters, we found a new diphenyl ether, 2-(2′,4′-dibromophenoxy)-4,6-dibromoanisole (**1**), in the green alga *Cladophora fascicularis.* The alga, collected from Miyako Island in April, 1983, was washed with fresh water, air-dried at room temperature for a week, and extracted by steeping in 95% ethanol for a month. The extract was concentrated and partitioned between water and chloroform. The chloroform-soluble material was chromatographed on a Sephadex LH-20 column by eluting with 1:1 chloroform-methanol. Fractions displaying NMR signals in the aromatic region were combined and purified by repeated chromatography on silica gel columns and finally on HPLC (Jascopack SS-05, 3:1 hexane-$CHCl_3$) to give the ether **1** as a colorless viscous oil in 0.06% yield of the wet alga.

The molecular formula $C_{13}H_8Br_4O_2$ was established by combustion analysis and supported by mass spectrometry which showed a molecular ion cluster at m/z 520 (44), 518 (94), 516 (100), 514 (93), and 512 (44% rel. intensity). Compound **1** showed λ_{max} (hexane) 239 (ε 11,600) and 284 (ε 1800) nm; ν_{max} (film) 3080, 3000, 2930, 1560, 1470, 1420, 1390, 1260, 1230, 1080, 1050, 1000, 930, 840, 810, and 760 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ7.77 (1H, d, J = 2.4 Hz), 7.48 (1H, d, J = 2.1 Hz), 7.37 (1H, dd, J = 8.7, 2.4 Hz), 6.90 (1H, d, J = 2.1 Hz), 6.73 (1H, d, J = 8.7 Hz), and 3.88 (3H, s). The absence of hydroxyl and carbonyl absorptions in the IR spectrum suggested that the two oxygen atoms in the molecule are in the ethereal linkages. The coupling pattern in the ^{1}H-NMR spectrum, as confirmed by decoupling experiments, revealed that the molecule contained two phenyl moieties, one 1,2,4-tri-substituted and the other 1,2,3,5-tetra-substituted. The presence of only one methoxy group indicated the other oxygen atom to be in a phenoxy linkage. Hydrogenolysis of **1** over 10% Pd/C gave 2-phenoxyanisole (**2**, m.p. 77–78°C; lit.[2], m.p. 76–78°C) which was identical in all respects with an authentic sample prepared by coupling of 2-bromoanisole with phenol under the conditions of Kime and Norymberski[3]. At this stage either **1** or **3** was the most plausible structure for the tetrabromo compound. The choice of structure **1** was based on comparisons of the melting point and spectral data of the demethylated (BBr_3/CH_2Cl_2)[4] product (m.p. 91.5–92.5°C) with those reported for **4** (m.p. 88–90°C) and **6** (m.p. 172.5–173°C)[5]. Furthermore, acetylation (Ac_2O/pyridine) of the demethylated derivative yielded a product which showed essentially identical melting point (121.5–122°C) and ^{1}H-NMR data with those reported for **5** (m.p. 118–119°C)[2].

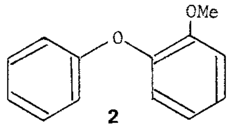
We have also isolated compound **1** from the digestive gland of the sea hare *Aplysia dactylomela* which was grazing the alga at the time of collection[6]. Isolation of polybrominated diphenyl ethers have been reported from only a few marine species, most notably from several collections of the sponge *Dysidea herbacea*[2,7], an unidentified sponge[5], and the acorn worm *Ptychodera flava laysanica*[8]. To our knowledge this is the first example of the isolation of a polybrominated diphenyl ether from a marine plant. Compound **1** exhibited antibacterial activity against *Escherichia coli, Bacillus subtilis,* and *Staphylococcus aureus.* It also showed potent antiinflammatory activity[9]. Although it was present in the sea hare, it did not exhibit feeding deterrent activity towards the omnivorous fish *Tilapia mosambica* in levels up to 5% in feed.



1, R=Me
4, R=H
5, R=Ac



3, R=Me
6, R=H



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9 Studies of the antiinflammatory activity will be reported elsewhere.

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Phytosterols from pressmud residue obtained after methanogenic fermentation

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Summary. Pressmud, a sugar factory waste, was fermented with methanogenic bacteria in an anaerobic fermenter for 40 days at 31 ± 2°C. The pressmud residue obtained after fermentation was used as a source for the extraction of phytosterols. The anaerobic digestion degraded the organic matter and resulted in enrichment of phytosterols from 0.33% in the pressmud to 3.05% in the residue. Refluxing of 100 g of residue with benzene, petroleum ether, and ethanolic KOH (10:5:1) yielded 8 g of soft cake, which on further fractionation with methylcyanide and isopropanol gave three fractions: 1) a crude mixture of phytosterols, 2) resin, and 3) undigested organic matter. The crude mixture of phytosterols after purification on neutral alumina followed by GLC analysis resulted in the separation of 68.7% of β-sitosterol, 18.4% of stigmasterol and 12.9% of campesterol and brassicasterol together. Phytosterols were extracted more easily from fermented than from unfermented samples, because of biodegration of lipophilic compounds by the methanogenic bacteria.
Key words. Pressmud; methanogenic fermentation; phytosterols.

Steroid hormones and drugs are as important for human beings as vitamins and antibiotics[2]. The starting materials for the production of steroidal drugs have been the plant steroids such as diosgenin and solasodine extracted from *Dioscoria* and *Solanum* sp., respectively. The increasing demand for steroidal drugs has resulted in the depletion of these natural resources. Hence, an