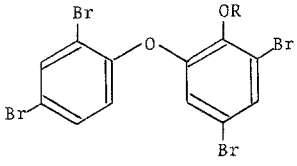
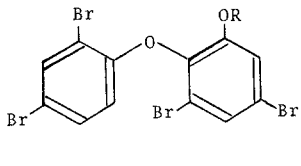
finally on HPLC (Jascopack SS-05, 3:1 hexane-$CHCl_3$) to give the ether **1** as a colorless viscous oil in 0.06% yield of the wet alga.

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}; ^1H 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 ^1H-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 ^1H-NMR data with those reported for **5** (m.p. 118–119 °C)[2].

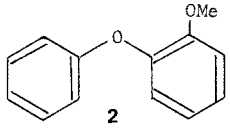
We have also isolated compound **1** from the digestive gland of the sea hare *Aplysia dactylomela* which was grazing the alga at the time of collection[6]. Isolation of polybrominated diphenyl ethers have been reported from only a few marine species, most notably from several collections of the sponge *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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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

alternative source for a starting material is imperative[3-6]. In this context, phytosterols, which are also used for the production of steroidal drugs, are of some importance. The already-known sources are not commercially viable because of poor recovery of sterols and an cumbersome process of extraction. In the present paper pressmud, which is a waste product of sugar factory, is proposed as an alternative source of phytosterols with a simple extraction method.

Experimental procedure and results. Phytosterols have been extracted from unfermented and fermented pressmud. For the former, a modified method[7] was followed. In the latter case phytosterols were extracted from the slurry obtained after fermentation of the pressmud with methanogenic bacteria at 31 ± 2 °C for 40 days. In this process extraction of phytosterols became easy because of biodegradation of lipophilic compounds by methanogenic bacteria.

To extract phytosterols from the residue obtained from the fermented pressmud, it was dried in an oven for 24 h at 100 °C to a moisture content of 2–3%, and was powdered and sieved (100 mesh) to remove bagasse.

One hundred grams of the powdered residue was then refluxed with a solvent mixture of benzene, petroleum ether and 2 N ethanolic KOH (10:5:1) for 12 h in a 5-liter Ca Soxhlet extraction flask. The extract so obtained, after decantation, was distilled in a 2-liter flask until 20 ml slurry was left. The slurry was transferred to a pre-weighed Petri-plate. After drying off the solvent at 80 °C in an oven, the slurry was cooled to room temperature. A soft cake weighing 8 g was thus obtained.

Extraction of phytosterols from the soft cake. For the selective solubilization of phytosterols, 8 g of the soft cake was refluxed with 80 ml of methylcyanide for 30 min. The hot extract was decanted into a pre-weighed Petri-plate and was allowed to cool at room temperature. The precipitate so obtained weighed 3.05 g and contained a mixture of phytosterols. This was again refluxed with 50 ml of isopropanol, and yielded 1.2 g of resin and 3.75 g of an undigested sticky slurry. The crude mixture of phytosterols separated by methylcyanide was purified on a column of neutral alumina using hexane, diethyl ether and methanol as solvents (table 1).

The percentage of individual sterols in the mixture was analyzed by GLC on a Gas Chromatograph Model Varian 3700 equipped with FID having a glass column packed with Chromosorb W(HP) 80–100 mesh and 3% OV-17 at column temperature of 275 °C. The chromatographic peaks were identified using authentic samples. The percentage composition of phytosterols obtained from the fermented and unfermented pressmud has been calculated (table 2).

Discussion. Pressmud is reported to contain 0.33% of phytosterols[8], but their low recovery and a cumbersome method of extraction has made its use uneconomical. However, its use after fermentation with methanogenic bacteria has made it a great potential source of phytosterols. During anaerobic fermentation of the pressmud carbohydrates including cellulose, proteins, and lipophilic compounds such as waxes were degraded into methane and CO_2. This resulted in approximately a 10-fold increase in the sterol content in the fermented samples over unfermented ones. The relative percentage of individual sterols in the mixture, however, remained unaltered. The GLC analysis of the purified mixture gave four peaks which were identified as those of brassicasterol, campesterol, stigmasterol and β-sitosterol. The amount of sterol was found to be 68.7% β-sitosterol, 18.4% stigmasterol, 12.9% of a mixture of campesterol and brassicasterol.

Pressmud has a distinct advantage over other sources of phytosterols in the country because of its cheap and plentiful availability (0.2 million tons per annum), besides being a potential raw material for the production of biogas.

Table 1. Amount of purified phytosterols obtained from pressmud by column chromatography using different solvents

Eluting agents	Compound eluted (g)	% of sterol
Hexane fraction	0.085	10.1
Diethyl ether fraction	3.469	87.6
Methanol fraction	0.078	3.0

Table 2. Percent composition of sterols obtained from unfermented and fermented pressmud

Mud	Individual sterol (% ± SD)		
	Brassicasterol and campesterol	Stigmasterol	β-Sitosterol
Unfermented pressmud	12.8 ± 0.04	18.3 ± 0.07	68.9 ± 1.90
Fermented pressmud	12.9 ± 0.06	18.4 ± 0.09	68.7 ± 1.85

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Chromatofocusing coupled with automated assay for β-hexosaminidase isoenzymes in GM_2 gangliosidosis[1]

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Summary. Good separations of the two major β-hexosaminidase forms from human leukocytes were achieved by chromatofocusing, a technique which separates proteins on the basis of their isoelectric points. The use of an automated and reliable method is described for the identification of homozygotes and carriers of the GM_2 gangliosidosis.
Key words. Chromatofocusing; β-hexosaminidase; GM_2 gangliosidosis.

The lysosomal enzyme hexosaminidase (β-2-acetamido-2-deoxy-D-hexoside acetamidodeoxy-hexohydrolase, EC 3.2.1.51, Hex) is widely distributed in nature[3-5]. The isoenzymes A and B have been characterized from different tissues of normal