structural data. A more elaborate structural model of this compound(s) would be only speculative at present.
Finally, the relationship between the 2 families of toxins is a particularly constructive working hypothesis, since it leads to the better understanding of their respective toxicities.

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Dimethyloxarsylethanol from anaerobic decomposition of brown kelp *(Ecklonia radiata)*: A likely precursor of arsenobetaine in marine fauna[1]

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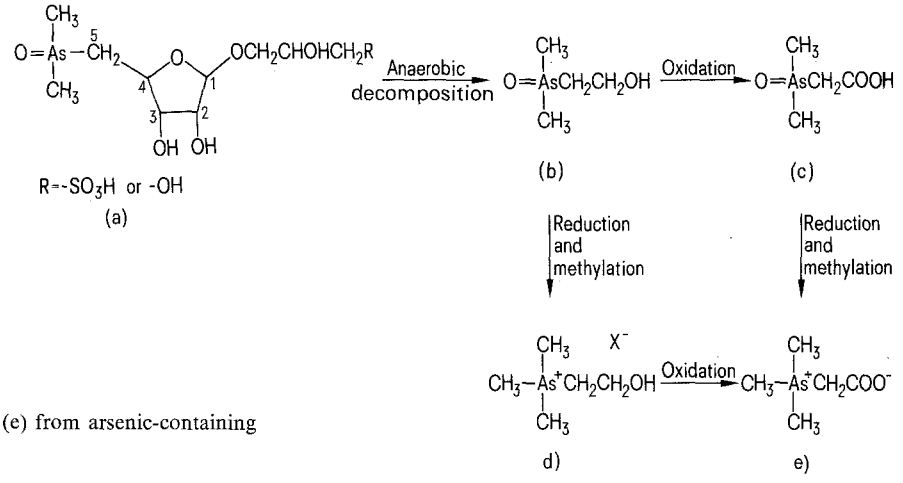
Summary. The novel arsenic compound, dimethyloxarsylethanol, has been isolated from anaerobically incubated *Ecklonia radiata.* It is proposed that this compound has a key position in the biosynthesis of arsenobetaine.

Arsenic is present as arsenobetaine (fig., e) at levels up to 40 ppm in a range of marine animals used as human food and taken from nonpolluted waters[2-5]. It has been shown that arsenic-containing sugars (fig., a) are present in the brown kelp *Ecklonia radiata* and that tnese are a possible source of arsenobetaine in the western rock lobster, *Panulirus cygnus,* and the school whiting, *Sillago bassensis,* associated with the nearshore waters of Western Australia supporting *Ecklonia*[6]. The conversion of the arsenic-containing sugars to arsenobetaine requires the cleavage of the C_3–C_4 bond of the sugar residue with subsequent oxidation at the C_4 position, and reduction and further methylation at the arsenic atom. Thus both reducing and oxidizing conditions are necessary and if cleavage of the C_3–C_4 bond occurs under reducing conditions (as found in kelp bed sediments or in large beach deposits of kelp) an accumulation of dimethyloxarsylethanol (fig., b) or arsenocholine (fig., d) might be expected. The latter would be produced if reduction and methylation of the arsenic atom occurs under the same reducing conditions. We here report the isolation of dimethyloxarsylethanol from anaerobically incubated *Ecklonia.* The position of this compound as a key intermediate in the biosynthesis of arsenobetaine is indicated in the figure.

Freshly collected *Ecklonia* (50 g) was incubated at room temperature under argon with unfiltered seawater (1 l) and sand (10 g) collected from the same site as the *Ecklonia.* The incubation flask was shaken in the dark for 11 days. Sulphide was first detected after 72 h and continued to be produced until the end of the incubation period. Methane was not detected. After a further 30 days the contents of the fermentation flask, smelling strongly of H_2S, were filtered and the filtrate boiled to dryness and the residue (60 g) extracted with methanol. Atomic absorption spectrometric analysis revealed the bulk of the arsenic (> 200 µg) in the filtrate and subsequently in the methanol extract. Only a trace of arsenic (< 10 µg) remained in the decomposed fragments of *Ecklonia.* TLC examination (n-butanol, acetic acid, water; 60: 15: 25; cellulose) of the crude methanol extract demonstrated the presence of a single major arsenic compound (R_f 0.65) and a trace of arsenic-containing material at R_f 0.2 (the value expected for unchanged arsenic-containing sugars). The major arsenical in the methanol extract was isolated by the following procedure: The methanol was evaporated and the solid residue (14 g) dissolved in water and extracted with phenol. The arsenic compound was recovered from the phenol by dilution with ether and extraction with water. This technique had previously been used to great advantage in the isolation of arsenobetaine from animal tissue[2]. After concentration, the aqueous solution (containing 200 µg As in 400 mg total solids) was twice subjected to gel filtration chromatography (Sephadex LH20, MeOH) to yield 200 µg As in 12 mg total solids. The arsenic compound was further purified by TLC (n-butanol, acetic acid, water; 60: 15: 25; cellulose; R_f 0.63) to yield a white solid (200 µg As in about 500 µg total weight). It was identified as dimethyloxarsylethanol by comparison of its 80 MHz ^1H NMR-spectrum, mass-spectrum and TLC behavior with those of a synthetic specimen. Dimethyloxarsylethanol, prepared by a modification of the method used by Wigren[7,8] to synthesize unsymmetrical dialkylarsyl compounds, crystallized from methanol/acetone as needles (m.p. 148–149.5 °C) which contained: C, 28.54%; H, 6.85%. $C_4H_{11}O_2As$ requires: C, 28.93%; H, 6.63%. IR: ν_{max} (film) 3080, 2800, 1473, 1350, 1264, 1077 s, 1008 s, 920, 893, 870 s, 640, 620 cm^{-1}. NMR (80 MHz ^1H, D_2O) δ 3.90, t, J 6.25 Hz, 2H, $AsCH_2$ CH_2 OH; 2.36, t, J 6.25 Hz, 2H, As CH_2 CH_2; 1.72, s, 6H, $(CH_3)_2$ As. MS (electron impact, 200°/35 eV) 165 (4.5%), 136 (52.5), 123 (13.1), 122 (49.1), 121 (36.8), 107 (100), 105 (9.4), 103 (10.2), 93 (41.1), 91 (35.1), 89 (24.8).

The conversion of arsenic-containing sugars to dimethyloxarsylethanol could occur through the metabolic activity of a number of anaerobic bacterial groups. Under anaerobic conditions, the complete microbial degradation of organic compounds is a sequential process with partial degradation by different microorganisms at each step. Fermentative bacteria utilize complex organic substrates releasing sim-



Biosynthetic scheme for arsenobetaine (e) from arsenic-containing sugars (a).

pler end-products. Specific fermentation end-products are then used as carbon substrates in anaerobic respiration by methanogenic and sulphate reducing bacteria. Because fermenters can metabolize a diverse and complex range of substrates, the formation of dimethyloxarsylethanol may be a product of their activity. However, McBride and Wolfe[10] have shown reduction and methylation of arsenate by Methanobacterium strain M.o.H. and, possibly, by *Desulfovibrio gigas*. Sulphate reducing and methanogenic species may therefore be involved at this step or other steps in arsenobetaine formation.

It is yet to be established if the route to arsenobetaine from dimethyloxarsylethanol is via arsenocholine or dimethyloxarsylacetic acid (fig. c). It is also not yet clear at what stage in the food chain (bacteria → detritovores → arsenobetaine-containing animals of higher trophic levels) the final conversions occur. We are currently studying these processes.

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Effect of diphosphonates on ATP and Pi content, Pi uptake and energy charge of cultured calvaria cells[1]

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Summary. In previous studies we have shown that glycolysis is strongly inhibited in cultured calvaria cells treated with ethane-1-hydroxy-1,1-diphosphonate (EHDP) or dichloromethanediphosphonate (Cl_2MDP). This study shows that the energy charge of the adenylate pool, and the ATP and Pi content, were not changed by treatment with diphosphonates except for a slight decrease of ATP and Pi at 0.25 mM Cl_2MDP. The uptake of Pi was diminished by 50% and 20% in cells treated for 6 days with Cl_2MDP or EHDP, respectively, but not when diphosphonates were present only during the uptake studies.

Diphosphonates are compounds which contain a P-C-P bond and are thus related to pyrophosphate, but they are resistant to metabolic destruction. They inhibit both formation and dissolution of calcium phosphate in vitro; in vivo they prevent ectopic calcification and bone resorption[3,4]. Recently these effects have been utilized clinically. Thus EHDP[2] has been found to decrease the development of ectopic ossification after total hip replacement[5] and in paraplegia[6]. Furthermore, various diphosphonates have proved useful in the management of Paget's disease[7], a disease in which bone turnover is increased, and of tumoral bone disease[8,9]. In high doses, however, certain diphosphonates also lead to an inhibition of normal mineralization. These effects of diphosphonates in vivo have been mainly attributed to their physicochemical interactions with calcium phosphate crystals. Recently, however, it has been found that the diphosphonates also influence cellular metabolism[10–15]. Thus, we found that in cultured rat calvaria and rabbit ear cartilage cells, both Cl_2MDP[2] and EHDP drastically decrease the production of lactate[10]. Since in these cells glycolysis is an important metabolic pathway for the production of ATP, the question arose whether the ATP content and the energy charge[16] might consequently be lowered. Another possibility might be that yet other metabolic pathways may be activated or that the cells utilize less energy. In this paper we describe the effect of diphosphonates on the ATP content, the energy charge and the content and uptake of Pi, in cultured calvaria cells.

Materials and methods. EHDP and Cl_2MDP were obtained as the sodium salts from Procter & Gamble Co., Cincinnati,