ous substance that is repugnant to potential predators[5]. Agersborg[6] likened the odour to that of oil of bergamot, and described in detail the glands from which the substance is secreted.

Specimens of *M. leonina* were collected during a reproductive congregation of the nudibranchs in a shallow kelp bed (−1 to −5 M) at Cates Park, Vancouver, B.C. The number of nudibranchs was extremely high (≈ 50 animals/m^2) and their odour could be detected in-situ by self contained underwater breathing apparatus (SCUBA) divers. Freshly collected whole specimens were immediately immersed in chloroform.

Silica gel column chromatography (hexane/chloroform) of the chloroform extracts yielded 2 pure metabolites. Mass spectrometry indicated that the least polar compound **1** had a molecular formula of $C_9H_{16}O$ (M^+, m/z, 140). An ^1H NMR-spectrum of this substance displayed resonances appropriate for 3 methyl groups at δ 1.70 (bs, 3 H), 1.61 (bs, 3H) and 1.11 (d, J = 7.1 Hz, 3H), for a single olefinic proton at 5.10 (m), and for an aldehyde proton at 9.62 (d, J = 1.9 Hz). The aldehyde functionality also displays an IR-absorption at 1723 cm^{-1} and a ^{13}C NMR-resonance at δ 205.0 (d). In the mass spectrum compound **1** undergoes a McLafferty rearrangement to give a base peak at m/z 82. All the spectral evidence[7] suggested that the non polar compound **1** was the degraded monoterpene 2,6-dimethyl-5-heptenal which had been previously reported as a component of one of the pheromones of the ant *Lasius carniolicus*[8] and which is used as the synthetic racemate in the perfume industry[9].

The more polar compound from *M. leonia* extracts had a molecular formula of $C_9H_{16}O_2$ (HRMS: M^+, m/z 156.1151, calc'd 156.1151) and its ^1H NMR-spectrum indicated that it was closely related to **1**[10]. IR-absorption bands (3500 → 2200 and 1700 cm^{-1}) characteristic of a carboxylic acid and the absence of an aldehyde proton in the ^1H NMR-spectrum suggested that the polar metabolite was 2,6-dimethyl-5-heptenoic acid (**2**). This was confirmed by preparing the methyl ester **3**. We have not been able to find any previous report of carboxylic acid **2** as a natural product[11].

In view of the postulated defensive role for the odiferous compound, we tested **1** and **2** for antifeedent activity in a standard goldfish bioassay[12]. The carboxylic acid **2** showed no activity at 100 µg/mg, while the aldehyde **1** was too volatile for reliable testing.

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Semi-synthesis of A23187 (calcimycin) analogs[1]

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Summary. The cleavage of A23187 to give a synthon (**4**) and the semi-synthesis from (**4**) of 2 Des-N-methylamino A23187 isomers (**7a, b**) are described. The antibiotic activities of the acids analogous to A23187 (**1**), (**2**), (**7a**), (**7b**) are compared.

A23187 (calcimycin) (**1**) has been isolated from a strain of *Streptomyces chartreusis* NRRL 3882[3]. It belongs to a large and growing class of natural carboxylic ionophores and presents a structure which is specifically adapted for the complexation of divalent cations in solution[4] and allows their transport through a membrane phase[5]. Extensive work has been done on the application of this ionophore to investigations of the involvement of Ca^{++} in the control of numerous physiological processes[6]. However, its mechanism of action as an antibacterial agent is not yet clearly understood[7].

In this communication, we report the first example of a semi-synthetic method for obtaining analogs with a modified benzoxazole ring which may be of interest for structure-activity studies, since benzoxazole appears to interact at 2 sites with Ca^{++}[8] or Mg^{++}[9] judging by X-ray structures of 2:1 complexes.

The N-methyl derivative (**2**) was prepared by a conventional method and has the following physical parameters: m.p. 113–115 °C, $(\alpha)^{25}_{578}$: −26° (c: 0.01, $CHCl_3$), mass spectrum ($M^+ = 537$), ^{13}C-NMR (15 MHz, $CDCl_3$) δ: 32.5 (C_9), 45.9 ($-N(CH_3)_2$), 98.7 (C_{14}), 165.3 (C_8), 168.3 (C_1), 194.6 (C_{20})[10].

We observed that this compound, unlike calcimycin, is rapidly cleaved in a proton-containing medium like DMF. The oxazole ring is opened to give the corresponding amide-phenol (**3**) (64% yield). m.p. 114 °C, $(\alpha)^{25}_{578}$: +79° (c: 0.01, $CHCl_3$) mass spectrum ($M^+ = 555$), ^{13}C NMR (15 MHz, $CDCl_3$) δ: 42.3 (C_9), 45.6 ($-N(CH_3)_2$), 98.4 (C_{14}), 170.5, 173.5 (C_1, C_8), 194.3 (C_{20}).

This reaction offers the possibility of performing semi-synthesis of new molecules related to A23187 after cleavage of the amide bond. This cleavage is achieved at room temperature with oxalyl chloride using a method proposed by Shiozaki et al.[11] for cephamycins. In this way we obtained the synthon (**4**)[12] (40% yield), m.p. 68–69 °C, $(\alpha)_{578}^{25}$: +131° (c: 0.01, $CHCl_3$), mass spectrum ($M^+ = 377$), ^{13}C NMR (15 MHz, $CDCl_3$) δ: 38.2 (C_9), 98.6 (C_{14}), 175.5 (−*C*OOH), 195.5 (C_{20}).

This compound which has been the object of a detailed study by ^1H NMR at a high field (400 MHz), was shown to have the same stereochemistry at its spiroketal center as A23187. The overall shape of the molecule is thus conserved and so replacement of the original benzoxazole moiety by slightly modified ones would be expected to yield molecules still able to complex divalent cations.

Initially we prepared the methyl-esters of commercially available aminohydroxy-benzoic acids to fix them onto this synthon.

A catalyst BOP[13a] described by Castro et al.[13b] for peptidic coupling can be used here with a good yield. Coupling is performed from the aminohydroxy-benzoic methyl ester, hydrochlorides in DMF at 50 °C and triethylamine with mechanical stirring for 4–6 h giving **5a** (60% yield), m.p. 151–153 °C. $(\alpha)_{578}^{25}$: +96° (c: 0.027, $CHCl_3$) mass spectrum ($M^+ = 526$), ^{13}C NMR (100 MHz, $CDCl_3$) δ: 42.2 (C_9), 52.4 (−$COOCH_3$), 98.5 (C_{14}), 168.4, 172.7 (C_8, −$COOCH_3$), 194.4 (C_{20}) and **5b** (100% yield), m.p. 111 °C, $(\alpha)_{578}^{25}$: +146° (c: 0.012, $CHCl_3$), mass spectrum ($M^+ = 526$), ^{13}C NMR (100 MHz, $CDCl_3$) δ: 41.3 (C_9), 52.0 (−$COOCH_3$), 99.2 (C_{14}), 167.4, 172.4 (C_8, −*C*OOCH$_3$), 195.6 (C_{20}).

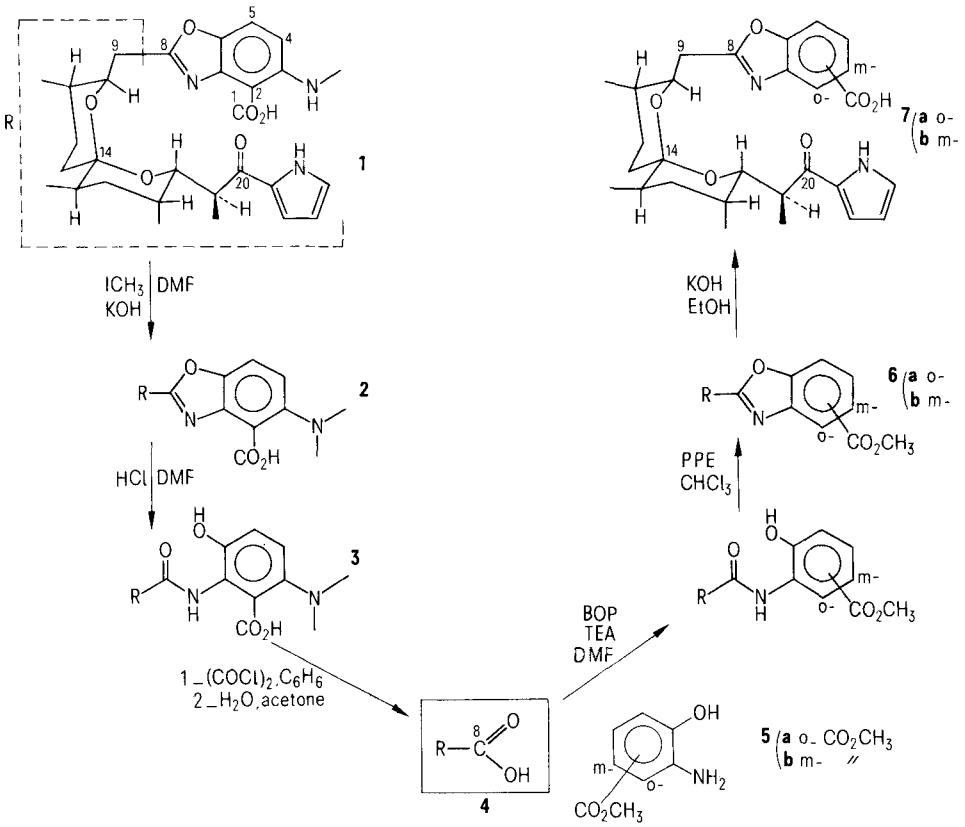
The next step was the benzoxazole ring closure. Ethyl polyphosphate in refluxing chloroform was used according to a method proposed by Kanaoka et al.[14] under nitrogen for 4–5 h to give **6a** (55% yield), m.p. 57–58 °C, $(\alpha)_{578}^{25}$: −27° (c: 0.01, $CHCl_3$), mass spectrum ($M^+ = 508$), ^{13}C NMR (100 MHz, $CDCl_3$) δ: 32.5 (C_9), 52.2 (−$COOCH_3$), 98.8 (C_{14}), 165.8, 167.3 (C_8, −$COOCH_3$), 194.7. (C_{20}) and **6b** (90% yield), m.p. 64 °C, $(\alpha)_{578}^{25}$: +47° (c: 0.01, $CHCl_3$), mass spectrum ($M^+ = 508$), ^{13}C NMR (100 MHz, $CDCl_3$) δ: 32.8 (C_9), 52.3 (−$COOCH_3$), 98.5 (C_{14}), 167.0, 167.1 (C_8, −$COOCH_3$), 194.3 (C_{20}).

The last step was the hydrolysis of ester with potassium hydroxide in ethanol (30 °C for 5 h). Water was added and the pH adjusted to 4.5 with 0.1 N HCl. Extraction with chloroform led to the corresponding acids **7a** (50% yield), m.p. 75–77 °C, $(\alpha)_{578}^{25}$: +151° (c: 0.012, $CHCl_3$), ^{13}C NMR (100 MHz, $CDCl_3$) δ: 32.5 (C_9), 98.0 (C_{14}), 165.2, 168.8 (C_8, −*C*OOH), 194.2 (C_{20}) and **7b** (72% yield), m.p. 95 °C, $(\alpha)_{578}^{25}$: +13° (c: 0.017, $CHCl_3$), mass spectrum ($M^+ = 494$), ^{13}C NMR (100 MHz, $CDCl_3$) δ: 32.7 (C_9), 98.4 (C_{14}), 165.4, 167.8 (C_8, C_1), 194.4 (C_{20}).

7a acid is identical in all respects to the cezomycin obtained in our laboratory[15] from cultures of the strain NRRL 3882 by addition of L-tryptophan to the culture medium, which

Tabelle

Compound	*Bacillus cereus* ATCC 14579	*Bacillus negaterium* ATCC 14581	*Micrococus luteus* ATCC 4698	*Streptomyces rimosus* ATCC 10970
1	0.025	0.003	0.03	1.56
2	6.25	0.25	3.12	25
7a	0.1	0.05	0.2	3.12
7b	25	6.25	12.5	50



inhibited production of A23187 and led to the formation of cezomycin.

The antibiotic activities of the different acids are reported below (minimum inhibitory concentration in µg/ml, obtained by the broth dilution method).

Evidently, the N-methyl derivative (**2**) is devoid of most of the calcimycin or cezomycin activity, presumably due to a steric effect of the N-dimethyl group. More physicochemical data would be necessary to confirm this explanation. Furthermore, comparison of **7a** and **7b** shows that the $-COOH$ group must be ortho to the benzoxazole nitrogen for antibiotic activity, as in calcimycin. This suggests that the activity is linked to a complexation step probably involved in magnesium or calcium transport. Analogs bearing other substituents on the benzoxazole ring are under investigation. Further results are to be published.

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Sterols of the clam *Chlamys tehuelcha*

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Summary. The sterols of the clam *Chlamys tehuelcha* were analyzed by gas-liquid chromatography (GLC) and GLC-mass spectroscopy confirming the presence of at least 9 Δ^5-sterols, including 1 C_{26} sterol.

Extensive examination of the sterols of mollusks[3–8] revealed that bivalves uniquely contain a great diversity of Δ^5-sterols. In view of these observations, we wish now to report the composition of the sterol mixture of the clam, *Chlamys tehuelcha,* which contains at least 9 Δ^5-sterols including 1 C_{26} sterol.

The clam *C. tehuelcha* is one of the most abundant species of the Argentine south Atlantic shore. The fatty acid composition of this clam has been published previously[2].

Frozen tissue of *C. tehuelcha* specimens (85 g) collected at Puerto Madryn, Argentina, was homogeneized in ethanol and the mixture filtered. The solid was extracted twice with ethanol, the combined filtrates were taken to dryness and the residue was dissolved in ethyl acetate. The original residue remaining in the filter was extracted twice at room temperature with ethyl acetate and the combined extracts plus the previous ethyl acetate extract were washed with water, dried over magnesium sulphate and evaporated to give a syrup (0.975 g), which was chromatographed on a silica gel column and eluted with mixtures of toluene-ethyl acetate of increasing polarity.

The crude sterol mixture (0.346 g) was recrystallized from

Composition of the sterols of *Chlamys tehuelcha*

Sterols	RRT[11]	Percent in the mixture by GLC	by SIM
22-Trans-24-nor-cholesta-5,22-dien-3β-ol	0.81	3.43	
22-Cholesta-5,22-dien-3β-ol	0.94	13.56	
Cholest-5-en-3β-ol	1.00	30.76	
22-Trans-24-methyl-cholesta-5,22-dien-3β-ol	1.04	19.16	
24-Methyl-cholest-5-en-3β-ol	1.11		13.65
24-Methylene-cholest-5-en-3β-ol	1.11		4.55
22-Trans-24-ethyl-cholesta-5,22-dien-3β-ol	1.15	5.87	
24-Ethyl-cholest-5-en-3β-ol	1.23	5.69	
24(28)-Ethyliden-cholest-5-en-3β-ol	1.25	5.31	