

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 $\mu\text{g/ml}$, 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 physico-chemical data would be necessary to confirm this explanation. Furthermore, comparison of **7a** and **7b** shows that the $-\text{COOH}$ 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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- All the reactions were protected from light. The crude products were purified by TLC (silica gel, eluting with several solvents: cyclohexane-ethylacetate 50-50 (**4,5b**) cyclohexane-ethylacetate 70-30 (**5a,6b**) ether (**6a**), ethylacetate (**7a,7b**). All the analysis conform to the proposed formula.
- a) Benzotriazol-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphat.
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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 mollusks³⁻⁸ 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 previously².

Frozen tissue of *C. tehuelcha* specimens (85 g) collected at Puerto Madryn, Argentina, was homogenized 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-3 β -ol	0.81	3.43	
22-Cholesta-5,22-dien-3 β -ol	0.94	13.56	
Cholest-5-en-3 β -ol	1.00	30.76	
22-Trans-24-methyl-cholesta-5,22-dien-3 β -ol	1.04	19.16	
24-Methyl-cholest-5-en-3 β -ol	1.11		13.65
24-Methylene-cholest-5-en-3 β -ol	1.11		4.55
22-Trans-24-ethyl-cholesta-5,22-dien-3 β -ol	1.15	5.87	
24-Ethyl-cholest-5-en-3 β -ol	1.23	5.69	
24(28)-Ethyliden-cholest-5-en-3 β -ol	1.25	5.31	

ethanol, giving a clean sterol mixture that gave 1 spot by AgNO_3 -TLC. The mixture was analyzed and quantified by GLC (SP-2100 capillary, 240–280 °C at 10 °C/min; 3% OV 17, 200–300 °C at 2 °C/min) and was characterized as indicated in the table by GLC-MS of the free sterol mixture and of their acetates (acetic anhydride:pyridine, 1:1). Mass spectrometric analysis of the trimethylsilyl ether derivatives (hexamethyldisilazane:trimethylchlorosilane:pyridine, 3:3:10) allowed confirmations of the results and an unequivocal assignment of Δ^5 -sterols. The assignments were done by comparison with authentic samples.

In the case of unresolved signals by GLC, quantification was done by single ion monitoring (SIM) of the sterols base peaks in the mass chromatogram of the mixture. Values obtained by this method for all the sterols were in good agreement with those obtained by GLC.

GLC studies have shown the presence of at least 9 different sterols in the mixture. Like results obtained with other mollusks, this clam contains a high proportion of cholesterol and also 22-trans-24-methyl-cholesta-5,22-dien-3 β -ol⁹, cholesta-5,22-dien-3 β -ol⁸ and 24-methylenecholesta-5-en-3 β -ol¹⁰, which are among the sterols found in other marine invertebrates. The rather uncommon C_{26} sterol: 22-trans-24-nor-cholesta-5,22-dien-3 β -ol, was first reported by Idler et al.⁶ in a scallop and has been detected in extracts from organisms representing many phyla, including marine diatoms, an important component at the base of the marine food chain and specially abundant in the zone where the specimens were collected. *C. tehuelcha* are plankton feeders and feed on diatoms. Since the sterols described occur in variable marine plants, part of them may be derived from phytoplankton.

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IR-spectra of amides of steroidal alkaloids with lactic acid

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Summary. IR-spectra of lactamides of 1,4-tomatadien-3-one (5), 4-solasoden-3-one (7), dihydrotomatidine (8) and tomatine (6) were taken and the frequency of the C=O amide band compared with that of the lactamides of piperidine (1) and its derivatives and N-acetyl tomatidine (9). It was found that the C=O bands of lactamides of steroidal alkaloids show an unusual but characteristic frequency at 1730–1733 cm^{-1} .

In the microbial transformation of tomatidine and tomatine by *Nocardia restricta* the conjugation of tomatidine² and tomatine (6)^{3,4} with lactic acid was observed as well as the dehydrogenation products. The transformation of 1,4-tomatadien-3-one (5) with the same microorganism gave a metabolite for which the conjugation of steroidal alkaloids with lactic acid was also assumed. The IR-spectra of these compounds show a C=O band at the frequency of 1730 cm^{-1} , which is more characteristic of esters than of amides^{5,6}. It is known⁷ that the C=O group of some carbamates with the structure $\text{R}_1\text{R}_2\text{N}-\text{C}-\text{OR}_3$ absorbs at



the frequency 1732–1738 cm^{-1} , if the substituents are alkyl groups. If the N-atom is cyclic, then they absorb at 1718–1731 cm^{-1} .

In order to identify these products, the amide of 1,4-tomatadien-3-one (5) with lactic acid was synthesized and its IR-spectrum compared to that of the conjugation product from *Nocardia restricta*. Both IR-spectra show the C=O

band absorption at 1730 cm^{-1} , which is higher than is known for amides^{5,6}.

To determine the influence of the N-atom in the molecule of steroidal alkaloids on the C=O absorption band, the IR-spectra of synthesized lactamides of piperidine (1), 2-methyl- (2), 3-methyl- (3) and 4-methyl-piperidine (4) were taken. The carbonyl group frequency appeared at 1630 or 1640 cm^{-1} . It was obvious that an N-atom in the ring does not cause the rise in frequency from 1640 to 1730 cm^{-1} .

We also synthesized the amide of 4-solasoden-3-one (7) with lactic acid. Its IR-spectrum shows a C=O group frequency at 1733 cm^{-1} . Therefore, we assumed that the proximity of the oxygen in ring E to the lactamide group of 1,4-tomatadien-3-one and 4-solasoden-3-one might possibly shift the frequency of the C=O group.

The shift of the C=O frequency of some synthesized and microbiologically produced steroidal alkaloids might be influenced by the spiroketal ring E. Therefore, we synthesized the lactamide of dihydrotomatidine B (8) with an