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 trimethylsylil 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-3β-ol[9], cholesta-5,22-dien-3β-ol[8] and 24-methylenecholest-5-en-3β-ol[10], which are among the sterols found in other marine invertebrates. The rather uncommon C_{26} sterol: 22-trans-24-nor-cholesta-5,22-dien-3β-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 speciallly 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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[1] Acknowledgments. We thank UMYMFOR (CONICET - FCEN) for the GLC and GLC-MS analysis and CONICET, SECYT and The Organization of the American States for financial support. We also thank Dr Héctor Saixo (Centro Nacional Patagónico, Puerto Madryn, Argentina) for providing the specimens of *C. tehuelcha*.

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IR-spectra of amides of steroidal alkaloids with lactic acid

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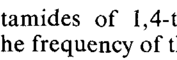
Summary. IR-spectra of lactamides of 1,4-tomatadien-3-one (**5**), 4-solasoden-3-one (**7**), dihydrotomatidine (**8**) and tomatine (**6**) were taken and the frequency of the C=O amide band compared with that of the lactamides of piperidine (**1**) and its derivatives and N-acetyl tomatidine (**9**).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

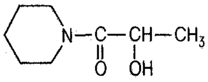
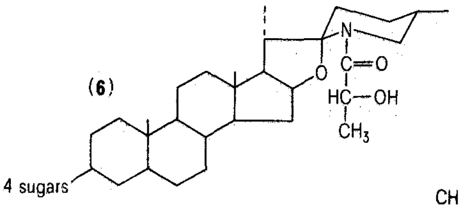
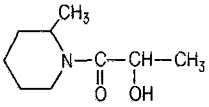
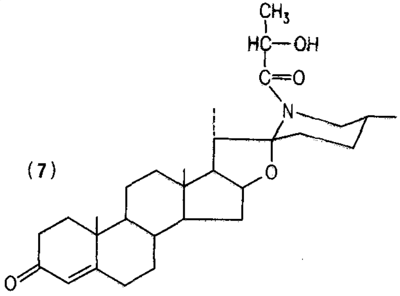
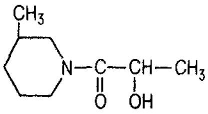
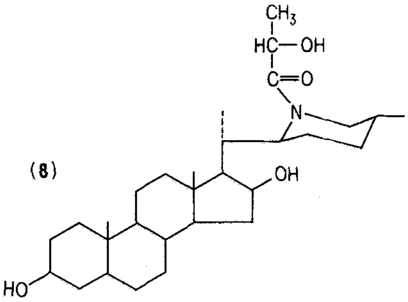
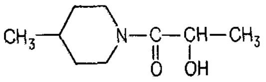
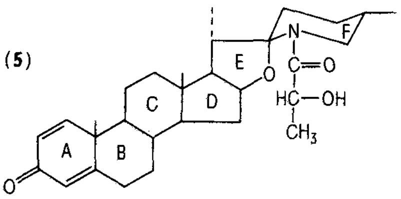
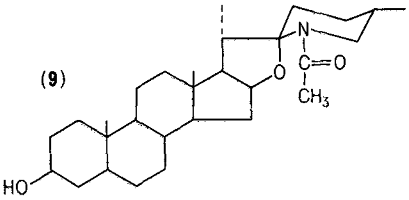
In the microbial transformation of tomatidine and tomatine by *Nocardia restricta* the conjugation of tomatidine[2] and tomatine (**6**)[3,4] with lactic acid was observed as well as the dehydrogenation products. The transformation of 1,4-tomatadien-3-one (**5**) with the same microorganism gave a metabolite 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 known[7] that the C=O group of some carbamates with the structure  $R_1R_2N-\underset{\underset{\textstyle O}{\|}}{C}-OR_3$ 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

Lactamide of		C=O frequency of amide in cm^{-1}			C=O frequency of amide in cm^{-1}
(1)		1640	(6)		1733
(2)		1630	(7)		1733
(3)		1640	(8)		1733
(4)		1640			
(5)		1730	(9)		1615

IR-spectra of amides of steroidal alkaloids with lactic acid.

open spiroketal ring E. In its IR-spectrum the carbonyl frequency absorbs at 1733 cm^{-1}. It seems that the opened spiroketal rings E do not influence the C=O absorption of lactamides.

N-acetyl tomatidine (9)[8] does not show this effect on the frequency of the C=O group in the IR-spectrum, which appears at 1615 cm^{-1}. Apparently, for this effect an α-OH group in the molecule of the amide of the steroidal alkaloids is necessary. Our work allows the conclusion that lactamides of steroidal alkaloids of the tomatidine type show the carbonyl frequency at 1730–1733 cm^{-1} and that oxygen proximity to the lactamide group may cause the shift from the generally observed frequency of 1640 cm^{-1} of lactamides.

1 Acknowledgments. The author thanks Prof. I. Belič, Biochemical Institute of the Medical Faculty, for his interest in this work and Prof. D. Hadži, Chemical Institute B. Kidrič, Ljubljana, for helpful discussions.
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