Main Sterols from the Echinoid *Encope emarginata*

MARINA G. PÉREZ,* ALEJANDRO J. ROCCATAGLIATA,* MARTA S. MAIER,*†
ALICIA M. SELDES* and JUAN M. DÍAZ DE ASTARLOA‡

*Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pabellón 2, Ciudad Universitaria, 1428 Buenos Aires, Argentina;
†Departamento de Ciencias Marinas, Facultad de Ciencias Exactas y Naturales, Universidad de Mar del Plata, Funes 3350, 7600 Mar del Plata, Buenos Aires, Argentina

Key Word Index: *Encope emarginata*; Clypeasteroida; Echinoidea; sand dollar; marine sterols; cholestan-3β-ol; cholest-4-en-3-one.

Abstract—The hitherto undescribed sterol composition of the masticatory apparatus and the body of the echinoid *Encope emarginata* was studied, revealing the presence of common 3β-hydroxy sterols. A comparison between the sterol composition of both mixtures shows the presence of very small amounts of cholestan-3β-ol and cholest-4-en-3-one in the masticatory apparatus only. These had not previously been detected in sand dollars. Copyright © 1996 Elsevier Science Ltd

Introduction
In continuation of our studies on the metabolites of echinoderms (Maier *et al.*, 1993; Roccatagliata *et al.*, 1994) we had occasion to examine the echinoid *Encope emarginata*, a very common sand dollar of the Atlantic South American coast. Previous work on this organism involved the isolation of echinochrome A, a red naphthoquinone pigment widely distributed in the Echinoidea (Stonik and Eliakov, 1988), but no study on its steroidal content has been reported.

Several important papers and a general review of sterols from the phylum Echinodermata have been published (Goad, 1978; Kerr and Baker, 1991) and it is known that from the five classes in the phylum, sea cucumbers (class Holothuroidea) and starfishes (class Asteroidea) contain a predominance of Δ^7 sterols, whereas sea lilies (class Crinoidea), sea urchins and sand dollars (class Echinoidea) as well as brittle stars (class Ophiuroidea) predominantly produce conventional Δ^5 sterols. Sand dollars live in or on soft sediments. Most of them are detritus and particulate feeders and their feeding depends in large part of the action of a highly modified masticatory apparatus (commonly called Aristotle's lantern) with nonprotractable teeth. In order to analyze the sterol composition of *Encope emarginata* and to establish the possible contributing sources to this content, i.e. *de novo* biosynthesis and assimilation or modification of dietary sterols, we have undertaken a careful study of the sterol composition of the masticatory apparatus and the body of this organism.

Materials and Methods
Fresh specimens of *Encope emarginata* (Leske 1778) (4.0 kg) were collected in 1993 off the coast near Mar del Plata, Buenos Aires, Argentina and were identified by Dr Alejandro Tablado of the Museo de Ciencias Naturales "Bernardino Rivadavia" where a voucher specimen (No. 31238) is preserved. The masticatory apparatus (0.1 kg) was separated from the body (3.8 kg) and each part analyzed separately.

†Author to whom correspondence should be addressed.

(Received 13 January 1995; accepted 9 June 1995)
After homogenization in MeOH and filtration, the remaining solid was extracted with AcOEt. The combined organic extracts were taken to dryness and the residue was dissolved in MeOH:H₂O (9:1) and extracted twice with hexane. After concentration under reduced pressure, the resulting residue was subjected to dry column flash chromatography on Davisil C₁₈ (35-75 μm) using mixtures of MeOH:H₂O (1:1–9:1), MeOH, MeOH:AcOEt (1:1) and AcOEt. The last two fractions provided the sterol mixture. This was chromatographed on a silica gel column (Cl₃CH₂:hexane (1:1), Cl₂CH₂ and Cl₂CH₂:MeOH (1:1) as eluants). The purified sterol mixture was fractionated by HPLC affording separated sterols which were then analyzed by GC-MS and ¹H-NMR spectroscopy.

GC was performed on a Hewlett Packard 5890A chromatograph equipped with a flame-ionization detector and a HP-5 column (25 m x 0.2 mm i.d.) containing crosslinked 5% PhMe silicone (carrier gas N₂, temperatures between 150 and 280°C at a rate of 15°C/min). The identities of the steroids were assigned by GC-MS using a TRIO-2 VG mass spectrometer coupled to a Hewlett Packard 5890A gas chromatograph. ¹H-NMR spectra were recorded on a Bruker ACE-200 spectrometer in CDCl₃. Preparative HPLC was carried out on a SP liquid chromatograph equipped with a Spectra Series P100 solvent delivery system, a Rheodyne manual injector and a refractive index detector using a YMC-Pack ODS-A column (25 cm x 20 mm i.d.) and MeOH as solvent.

Results
After purification of the sterol mixtures, they were separated using reversed-phase HPLC. The sterols were characterized on the basis of GC-MS, ¹H NMR spectroscopy (200 MHz) and comparison with reported spectra. As shown in Table 1, both sterol mixtures contained Δ⁵ mono- and di-unsaturated sterols, cholesterol accounting for ca. 70% of the sterol composition. Several di-unsaturated sterols were isolated, including (22E)-24-norcholesta-5,22-dien-3β-ol, 22-dehydrocholesterol and (22E)-24R-methylcholesta-5,22-dien-3β-ol (brassicasterol). The ¹H NMR spectra of the di-unsaturated compounds showed the expected chemical shifts for the C-18 and C-19 protons at 0.70 and 1.01 ppm, respectively, as well as the C-21 methyl doublet at 1.01 ppm due to the presence of the Δ²²-trans bond. The ¹H NMR spectrum of brassicasterol allowed the characterization of the C-24 stereochemistry since it presented the C-26, C-27 and C-28 methyl doublets at 0.836, 0.819 and 0.911 ppm, which was in accordance with those reported in literature (Rubinstein et al., 1976). We also isolated by HPLC a mixture of 24-methylcholesta-5,24(28)-dien-3β-ol (22%) and 22-dehydrocholesterol (77%) from both sterol mixtures. Signals at 4.65 and 4.70 ppm in the ¹H NMR spectrum of each mixture due to the C-28 hydrogens confirmed the presence of the former.

Both sterol mixtures contained (24R)-24-methylcholesta-5-en-3β-ol (camp-esterol) and 24-ethylcholesta-5-en-3β-ol in ca. 9% and 3% abundance, respectively, and very small amounts of 24-methyl-27-norcholesta-5,22-dien-3β-ol. The 24R (α) configuration assigned to campesterol is based on the pattern of signals corresponding to C-27 and C-28 protons (Rubinstein et al., 1976).

<table>
<thead>
<tr>
<th>Sterol</th>
<th>RRT</th>
<th>%</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>(22E)-24-Norcholesta-5,22-dien-3β-ol (1)</td>
<td>0.80</td>
<td>1.5</td>
<td>1.8</td>
</tr>
<tr>
<td>24-Methyl-27-norcholesta-5,22-dien-3β-ol (2)</td>
<td>0.91</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>22-Dehydrocholesterol (3)</td>
<td>0.94</td>
<td>6.6</td>
<td>7.1</td>
</tr>
<tr>
<td>Cholest-5-en-3β-ol (4)</td>
<td>1.00</td>
<td>70.2</td>
<td>71.4</td>
</tr>
<tr>
<td>(22E)-24R-Methylcholesta-5,22-dien-3β-ol (5)</td>
<td>1.06</td>
<td>8.1</td>
<td>5.4</td>
</tr>
<tr>
<td>24-Methylcholesta-5,24(28)-dien-3β-ol (6)</td>
<td>1.14</td>
<td>2.3</td>
<td>2.7</td>
</tr>
<tr>
<td>(24R)-24-Methylcholesta-5-en-3β-ol (7)</td>
<td>1.15</td>
<td>8.9</td>
<td>8.7</td>
</tr>
<tr>
<td>24-Ethylcholesta-5-en-3β-ol (8)</td>
<td>1.30</td>
<td>2.6</td>
<td>3.0</td>
</tr>
</tbody>
</table>

*R Relative retention time (RRT) of the sterols to cholest-5-en-3β-ol (4).
†Sterol composition of the masticatory apparatus.
‡Sterol composition of the body.
In addition to the sterols mentioned before, the masticatory apparatus contained 24-ethylcholesta-5,22-dien-3β-ol, cholestan-3β-ol and cholest-4-en-3-one as confirmed by GC-MS.

Discussion
The results obtained from *Encope emarginata* demonstrated that the principal sterols from this echinoid are cholesterol, campesterol, brassicasterol and 22-dehydrocholesterol with minor amounts of sitosterol, 24-methylcholesta-5,24(28)-dien-3β-ol, (22E)-24-norcholesta-5,22-dien-3β-ol and 24-methyl-27-norcholesta-5,22-dien-3β-ol. All these common 3β-hydroxy-Δ5-sterols, with the exception of 24-methyl-27-norcholesta-5,22-dien-3β-ol, have been previously isolated from echinoids. In the phylum Echinodermata the 24S epimer (occelasterol) was also detected in the ophiuroid *Ophiocoma longicaudum* (Riccio et al., 1985). The presence of this sterol along with 24-norcholesta-5,22-dien-3β-ol in large quantities in the dinoflagellate *Gymnodinium simplex* (Goad and Withers, 1982) is indicative of the dietary origin of these norsterols frequently found in marine invertebrates.

We could not detect any cholesta-5,24-dien-3β-ol (desmosterol) already found in *Echinus acutus* (Voogt, 1972), *Echinus esculentus* (Smith and Goad, 1974) and *Temnopleurus toreumatus* (Yasuda, 1974).

An interesting finding is the high abundance of brassicasterol (8.0%) in the sterol mixture of the masticatory apparatus with respect to the sterol mixture of the body (5.4%). This sterol is a component of phytoplankton and it is also suggested (Kobayashi and Mitsuhashi, 1975) that it could be involved in the biosynthesis of ocelasterol and (22E)-24-norcholesta-5,22-dien-3β-ol via two consecutive demethylations. In the present study, the abundance of the latter compound is only 0.3% higher in the sterol mixture of the body with respect to the corresponding level of the masticatory apparatus. Similar differences in composition were found for 22-dehydrocholesterol, 24-methylcholesta-5,24(28)-dien-3β-ol and sitosterol as well as 1.2% in the case of cholesterol which could be attributed to modification of dietary sterols.

We have also studied by GC-MS the presence of stigmasterol, cholestan-3β-ol and cholest-4-en-3-one only in the masticatory apparatus. Stigmasterol has been isolated previously from the echinoids *Echinus acutus* and *Paracentrotus lividus* (Voogt, 1972). With respect to cholestan-3β-ol and cholest-4-en-3-one, there are no previous reports on their presence in echinoids. Both compounds, as well as other steroidal ketones, have been isolated from dinoflagellates (Withers, 1983). Their presence in trace amounts in the masticatory apparatus only is in accordance with a dietary origin.

Acknowledgements—We are most grateful to Dr Alejandro Tablado (Museo de Ciencias Naturales "Bernardino Rivadavia", Buenos Aires) for the taxonomic identification. We also thank UMYMFOR (CONICET-FCEN) for spectroscopic analysis and the International Foundation for Science, the Universidad de Buenos Aires and Fundación Antorchas for partial financial support.

References


