STUDIES OF BRAZILIAN MARINE INVERTEBRATES.
IX*. COMPARATIVE STUDY OF ZOANTHID STEROLS 1.
THE GENUS ZOANTHUS

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ABSTRACT: The sterol composition of two Zoanthus species has been analysed and compared with literature data. The new marine sterol zoanthosterol (4α-methyl-5α-ergosta-24(28)-en-3β-ol) has been obtained from Z. sociatus. Biological implications are discussed.

KEYWORDS: Zoanthids *, Zoanthus species, Zoanthus sociatus, sterol composition *, cholesterol, chalinasterol, brassicasterol, zoanthosterol *, zooxanthella.

INTRODUCTION

The increased interest in marine sterols has led to the identification, during the last 15 years, of a number of new structures obtained principally from Molluscs, Echinoderms, Porifera and Coelenterates mostly of the class Anthozoa, i.e. Octocorallia and Hexacorallia (15). Whilst Octocorals (soft corals and sea fans) have been intensively investigated yielding more than 40 new sterols (15), little attention has been payed, in comparison, to sterols from Hexacorals (hermatypic corals, sea anemones and zoanthids) (8). This is not surprising for corals and sea anemones for which a generous part of the wet weight is inorganic matter (corals) or water (some sea anemones). In contrast, zoanthids yield large proportions of extractable organic material. Even so, zoanthid sterols have been much less studied than the coral or sea anemone ones (8). The rather confused biological systematics of Zoanthids, at species level, is probably responsible, in part, for this situation (10, 19). Indeed, after CARLGREN (7), "everyone examining the Zoantharia has learnt how difficult it is to determine the members of this group of Anthozoans".

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chromatograms being revealed by spraying a 3% ethanolic solution of phosphomolybdic acid followed by heating at 150°C.

**Animals:** fresh polyps of *Zoanthus sociatus* (Ellis, 1767) (Hexacorallia, Zoanthidea) and of an unidentified *Zoanthus* species were collected, on rocky bottom, along the coast of the state of Rio de Janeiro. They were preserved in 70% EtOH until work up. Data about the collection appear in Table 2.

**TABLE 2**

The *Zoanthus* collection

<table>
<thead>
<tr>
<th>Animal</th>
<th>Local</th>
<th>Date</th>
<th>Depth</th>
<th>Yield % extract</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Zoanthus</em> sp</td>
<td>Ilha Grande</td>
<td>15-03-80</td>
<td>1 — 2 m</td>
<td>18.3</td>
</tr>
<tr>
<td><em>Z. sociatus</em></td>
<td>Cabo Frio</td>
<td>29-05-80</td>
<td>0 — 1 m</td>
<td>10.5</td>
</tr>
</tbody>
</table>

*Zoanthus* sp: as sterile colonies  
*Z. sociatus*: as sterile or hermaphroditic colonies

**Extraction:** the aqueous ethanol in which the animals were preserved was filtered off, and the animals extracted several times with ethanol until a colorless extract was obtained. The polyps were then extracted with methylene chloride. The organic extracts were combined and evaporated under reduced pressure. Water was eliminated by azeotropic distillation with benzene. The extract was then triturated in methylene chloride to eliminate inorganic matter. Evaporation of the solvent afforded a methylene chloride residue from which the sterol fraction was isolated.

**Obtention of the sterol mixtures — Isolation of the sterols:**

a) The obtention, from *Zoanthus sociatus*, of the free monohydroxy sterol mixture and the separation of the sterols as their acetyl derivatives have been described earlier (12). The physico-chemical data of compounds Z-1, Z-2 and Z-3 (see figure 1.2) have been reported for the acetyl derivatives in cited reference.

b) Identical techniques have been used in the case of the unidentified *Zoanthus* species.

**MS** of the sterol mixture from *Zoanthus* sp: molecular ion at m/e 414 (2, C_{29}H_{49}O) — 400 (7, C_{28}H_{45}O) — 398 (42, C_{28}H_{45}O) — 386 (28, C_{27}H_{44}O) and 384 (8, C_{27}H_{44}O); characteristic fragment ions at m/e 399 (13, 414 — CH, 396 (1, 414 — H,O), 385 (4, 400 — CH, 383 (6, 398 — CH, 382 (2, 400 — H,O), 381 (2, 414 — CH, H,O), 380 (4, 398 — H,O), 371 (8, 386 — CH, 369 (4, 383 — CH), 368 (11, 366 — H,O), 367 (2, 400 — CH, H,O), 365 (4, 398 — CH, H,O), 355 (3.5), 353 (8, 386 — CH, H,O), 351 (1, 384 — CH, H,O), 336 (7), 314 (13, 398 — C_{6}H_{12}, i.e. Mac Lafferty rearrangement from a Δ^{24(28)} double bond), 300 (28), 299 (7, 314 — CH, 296 (2, 314 — H,O), 275 (13), 273 (17), 271 (30), 255 (42), 213 (22), ...

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The GC trace is described in the text (Figure 1.b. and Table 3).

From 41 mg of the steryl acetate mixture of Zoanthus sp (obtained in the usual conditions), we isolated as described earlier (12), five fractions: S-1 (2 mg), a mixture of S-1/S-2 and S-3 (10 mg), S-4 (15 mg), S-5 (4 mg) and S-6 (5 mg).

S-1 (as the acetate) : \( RRT = 1.00 \), 83% pure by GC, co-chromatographs with an authentic sample of acetyl-cholesterol; \( MS : M^+ - 60 = 368, 353 (368 - \text{CH}_3)_2, \ldots \)

S-1 / S-2 / S-3 (as the acetates) : \( RRT = 1.00 / 1.25 \) and 1.46 respectively, relative proportions of 73 / 17 and 10% ; S-1 co-chromatographs with acetyl-cholesterol and S-2 with an authentic sample of (24S)-24-methylcholesterol (IV with the methyl \( \beta \) oriented). \( MS \) of the mixture : \( M_1^+ - 60 = 368 \) (S-1), \( M_2^+ - 60 = 382 \) (S-2) and \( M_3^+ - 60 = 396 \) (S-3).

S-4 (as the acetate) : \( RRT = 1.12 \), over 94% pure, co-chromatographs with an authentic sample of brassicasterol acetate ; \( m.p. : 150.5-152.2^\circ \) (lit. 152^0, ref 9) ; \( [\alpha]_D = -55.9^0 \) (c = 1.12 in CHCl_3) (lit. -62.2^0, ref. 9) ; \( MS : M^+ - 60 = 380 \) (32, C_{20}H_{41}O_2 - ACOH), 365 (6, 380 - CH_3), 337 (6, 380 - C_3H_7), 296 (2), 282 (5), 267 (3), 255 (8, 380 - side chain), 253 (8, 380 - side chain + 2H), 239 (3), 228 (6), 213 (10, 380 - side chain and 42) ; \( IR : 1720, 1255, 1035 \) and 980 cm\(^{-1}\).

S-5 (as the acetate) : \( RRT = 0.96 \), over 96% pure, co-chromatographs with an authentic sample of 22(E)-22-dehydrocholesterol acetate; \( MS : M^+ - 60 = 366 \) (17, C_{20}H_{41}O_2 - ACOH), 351 (2.5, 366 - CH_3), 323 (1, 366 - C_3H_7), 295 (1), 255 (15, 366 - side chain), 253 (2.5, 366 - side chain + 2H), 228 (2), 213 (4, 366 - side chain and 42).

| TABLE 3 |

| GC analysis of free sterol mixtures from some Zoanthus spp |

<table>
<thead>
<tr>
<th>RRT</th>
<th>SAMPLE</th>
<th>0.66</th>
<th>0.96</th>
<th>1.00</th>
<th>1.12</th>
<th>1.25</th>
<th>1.27</th>
<th>1.39</th>
<th>1.46</th>
<th>1.64</th>
<th>C (28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z. soc.</td>
<td>t</td>
<td>—</td>
<td>3</td>
<td>—</td>
<td>—</td>
<td>87</td>
<td>8</td>
<td>—</td>
<td>t</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td>Z. sp.</td>
<td>—</td>
<td>34</td>
<td>51</td>
<td>14</td>
<td>—</td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>Z. pag. *</td>
<td>—</td>
<td>—</td>
<td>19</td>
<td>41</td>
<td>30</td>
<td>10</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>81</td>
<td></td>
</tr>
<tr>
<td>Z. prot. **</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>100</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Z. soc. (Al)§</td>
<td>—</td>
<td>t</td>
<td>35</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>t</td>
<td></td>
</tr>
</tbody>
</table>

Z. soc. = Z. sociatus; Z. pac. = Z. pacificus; Z. prot. = Z. proteus; (Al) = alga
§ after KOKKE et al. Tet. Letters 3601 (1979)
S-6 (as the acetate) : $RRT = 1.27$, over 99% pure, co-chromatographs with an authentic sample of chinalasteryl acetate; $MS : M^+ - 60 = 380$ (27, C$_{30}$H$_{49}$O$_3$ = AcOH), 365 (8, 380 - CH$_3$), 296 (15, 380 - C$_{8}$H$_{15}$, i.e. Mac Lafferty rearrangement of a $\Delta^{24(28)}$ double bond), 281 (296 - CH$_3$), 272 (2), 259 (5), 255 (6, 380 - side chain), 253 (9, 380 - side chain + 2H), 228 (5), 213 (10); $IR : 1720, 1640, 1260$ and 895 cm$^{-1}$.

RESULTS

The GC traces of the sterol mixtures from the two Zoanthus species are compared in Figure 1 with the one reported for Zoanthus confertus (now named Z. pacificus Walsh & Bowers 1971 (20)). Calculated data appear in Table 3, where retention times are relative to cholesterol and where compositions (%) are deduced from the area of the GC peaks.

The three major sterols produced by Z. sociatus were isolated and identified as cholesterol (Z-1, I), chalinasterol (or 24-methylenecolesterol, Z-2, V) and zoanthosterol (4a-methyl-5a-ergosta-24(28)-en-3p-ol, Z-3, XI) by gas chromatographic, physico-chemical and spectroscopic data (12).

GC analysis of the sterol mixture obtained from the unidentified Zoanthus species (Fig. 1.b.) showed it to be more complex than that of Z. sociatus. The MS of the sterol fraction displayed at least five molecular ions at m/e = 414, 400, 398, 386 and 384. The characteristic fragment ion at m/e = 314 was indicative of the presence of a sterol with an exomethylene group at C-24 (Mac Lafferty rearrangement of a $\Delta^{24(28)}$ double bond) (9). Separation of the sterols, as their acetates, by argentific silica gel column chromatography, afforded four sterol acetates over 92% pure (by GC) and a mixture of three steryl acetates. The retention times relative to cholesteryl acetate were: 1.00 (S-1, 2 mg), 1.00-1.25-1.46 (S-1, S-2 and S-3, 10 mg), 1.12 (S-4, 15 mg), 0.96 (S-5, 4 mg) and 1.27 (S-6, 5 mg).

S-1 was identified as cholesteryl acetate with which it co-chromatographs in GC, on the basis of its retention time (GC) and of its MS identical with that of an authentic sample.

S-2 and S-3 were tentatively identified as 24p-methyl and 24p-ethyl-cholesteryl acetates by their RRT identical with published data obtained in similar operating conditions (14). The MS of the mixture S-1/S-2/S-3 showed fragmentations (M$^+ - 60$) at m/e = 368 (i.e. parent sterol C$_{27}$H$_{48}$O), 382 (i.e. parent sterol C$_{28}$H$_{50}$O) and 396 (i.e. parent sterol C$_{29}$H$_{52}$O) which were in perfect agreement with our assumption. The absence of molecular ions strongly suggested a 3p-OAc-$\Delta^6$ moiety (17). That the extra-carbon(s) of S-2 and S-3 were located in the side chain came from their MS showing fragment ions corresponding to the tetracyclic nucleus at the same m/e ratios as in cholesteryl acetate. In addition, S-2 co-chromatographed with an authentic sample of 22,25-dihydrobrassicasteroyl acetate (IV with the methyl p oriented), suggesting the extra-carbon of S-2 to be located at C-24, the biogenetic favoured position.
l. a. *Z. sociatus*  
l. b. Zoanthus sp  
l. c. *Z. pacificus*  

Fig. 1 — GC traces of sterol mixtures from Zoanthus spp.*  

* after GUPTA & SCHEUER (9): reconstructed; in the original paper the zoanthid is named *Z. convolutus*
Fig. 2 — Sterol structures: Distribution of sterols in Zoanthus spp. 1 = Z. sociatus; 2 = Zoanthus sp.; 3 = Z. pacificus; 4 = Z. proteus; 5 = symbiotic algae of Z. sociatus.
Fig. 8 — Sterols of Zoanthus sp., biosynthetic pathway
S-4 was identical with brassicasteryl acetate (9) by m.p., [α]D, IR, MS and GC (RRT and co-injection with an authentic sample).

S-5 was found identical with 22(E)-22-dehydrocholestereryl acetate by MS and GC (RRT and co-injection with an authentic sample).

S-6 was identified with chalinalsteryl acetate (24-methylencholestereryl acetate) by MS, IR and GC (RRT and co-injection with an authentic sample).

DISCUSSION

Although our knowledge on zoanthid sterols of the genus Zoanthus is restricted to only two published papers (5, 9) dealing with two species, some general conclusions appear.

1. C25-sterols are the major sterols of Zoanthus species as they are of the class Anthozoa in general (11).

2. All the Zoanthus species studied up to now contain chalinalsterol (V). It is the sole sterol of Z. proteus, the major one of Z. sociatus and a minor component of Z. pacificus and of the unidentified Zoanthus species.

3. Cholesterol (I), a typical animal sterol, and brassicasterol (III), widely distributed in marine invertebrates (8), seem to be common sterols in Zoanthus species.

4. As Z. proteus is now considered synonymous with Z. sociatus (19), it is possible that the presence of small amounts of cholesterol (I) and zoanthosterol (XI) in the sterol fraction isolated by BERGMANN et al. (5) could not be detected by these authors due to the low performance analytical techniques existing prior to 1960.

5. The sterol composition of the unidentified Zoanthus species was found quite similar to that reported by GUPTA & SCHEUER (9) for Z. pacificus with, in addition to the reported cholesterol (I), brassicasterol (III), chalinalsterol (V) and 24α-methylcholesterol (IV), the common (8) marine sterol 22(E)-22-dehydrocholesterol (II) and probably 24α-ethylcholesterol (VI). Considering the biosynthetic pathway shown in Fig. 3, the presence of brassicasterol (III) together with S-2 and S-3 allows us to suggest for the two latter sterols absolute configuration "24S", i.e. the alkyl at C-24 is probably β.

6. Z. sociatus was found to contain the rare zoanthosterol (XI), a new 4α-methyl-5α-stanol (12). Sterols containing a 4α-methyl-5α-stanol moiety have been obtained from various marine organisms including a red alga (4), a sea star (18) and an alcyonarian (6) but are most frequently encountered in free living dinoflagellates (1, 2, 3, 21 and 22). As zoanthids contain large amounts of symbiotic dinoflagellate algae known as zooxanthellae, it is tempting to speculate about the algal origin of zoanthosterol (XI). Indeed, the cultured alga isolated from the Jamaican Z. sociatus furnished, together with the Δ⁵ sterols I and II, a series of 4α-methyl-5α-stanols (VII, VIII, IX, X, and XII) (13). But when about 96% of the sterol mixture of the Brazilian Z. sociatus is composed of sterols with a Δ₂⁴(28) double bond, such compounds are completely


