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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, Poriferas 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.°.

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

Animal	Local	Date	Depth	Yield % extract
Zoanthus sp	Ilha Grande	15-03-80	1 — 2 m	18.3
Z. sociatus	Cabo Frio	29-05-80	0 — 1 m	10.5

Zoanthus sp: as sterile colonies

Z. sociatus: as sterile or hermaphrodite 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.ª) 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, $C_{29}H_{50}O$) — 400 (7, $C_{28}H_{48}O$) — 398 (42, $C_{28}H_{46}O$) — 386 (28, $C_{27}H_{46}O$) and 384 (8, $C_{27}H_{44}O$); characteristic fragment ions at m/e = 399 (13, 414 — CH_3), 396 (1, 414 — H_2O), 385 (4, 400 — CH_3), 383 (6, 398 — CH_3), 382 (2.5, 400 — H_2O), 381 (2, 414 — CH_3, H_2O), 380 (4, 398 — H_2O), 371 (8, 386 — CH_3), 369 (4, 383 — CH_3), 368 (11, 386 — H_2O), 367 (2, 400 — CH_3, H_2O), 365 (4, 398 — CH_3, H_2O), 355 (3.5), 353 (8, 386 — CH_3, H_2O), 351 (1, 384 — CH_3, H_2O), 336 (7), 314 (13, 398 — C_6H_{12}, *i.e.* Mac Lafferty rearrangement from a $\Delta^{24(28)}$ double bond), 300 (28), 299 (7, 314 — CH_3), 296 (2, 314 — H_2O), 275 (13), 273 (17), 271 (30), 255 (42), 213 (22), ...

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* : $M^{\cdot +}$— 60 = 368, 353 (368 — CH_3),...

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 β 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 brassicasteryl acetate ; *m.p.* : 150.5-152.0° (lit. 152°, ref 9); $[\alpha]_D$ = —55.9° (c = 1.12 in $CHCl_3$) (lit. —62.2°, ref. 9) ; *MS* : $M^{\cdot +}$— 60 = 380 (32, $C_{30}H_{48}O_2$ — AcOH), 365 (6, 380 — CH_3), 337 (6, 380 — C_3H_7), 296 (2), 282 (5), 267 (3), 255 (37, 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 and authentic sample of 22(E)-22-dehydrocholesteryl acetate; *MS* : $M^{\cdot +}$— 60 = 366 (17, $C_{29}H_{46}O_2$ — 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

RRT SAMPLE	0.66	0.96	1.00	1.12	1.25	1.27	1.39	1.46	1.64	C (28)
Z. soc.	t	—	3	—	—	87	8	—	t	87
Z. sp	—	$\underbrace{\quad\quad}_{34}$		51	$\underbrace{\quad\quad}_{14}$		—	1	—	65
Z. pag. *	—	—	19	41	30	10	—	—	—	81
Z. prot. **	—	—	—	—	—	100	—	—	—	100
Z. soc. (Al) §	—	t	35	—	—	—	—	—	—	t

Z. soc. = *Z. sociatus*; *Z. pac.* = *Z. pacificus*; *Z. prot.* = *Z. proteus*; (Al) = alga

* after GUPTA & SCHEUER Steroids *13*:343 (1969)

** after BERGMANN *et al.* J. Org. Chem, *16*:1337 (1951)

§ 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 chalinasteryl acetate ; *MS* : $M^{\cdot +} - 60 = 380$ (27, $C_{30}H_{48}O_2$ — AcOH), 365 (8, 380 — CH_3), 296 (15, 380 — C_6H_{12}, *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-methylenecholesterol, Z-2, V) and zoanthosterol (4α-methyl-5α-ergosta-24(28)-en-3β-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 argentic silica gel column chromatography, afforded four steryl 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 24ξ-methyl and 24ξ-ethylcholesteryl 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 ($M^{\cdot +} - 60$) at m/e = 368 (*i.e.* parent sterol $C_{27}H_{46}O$), 382 (*i.e.* parent sterol $C_{28}H_{48}O$) and 396 (*i.e.* parent sterol $C_{29}H_{50}O$) which were in perfect agreement with our assumption. The absence of molecular ions strongly suggested a 3β-OAc-Δ^5 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 22,23-dihydrobrassicasteryl acetate (IV with the methyl β oriented), suggesting the extra-carbon of S-2 to be located at C-24, the biogenetic favoured position.

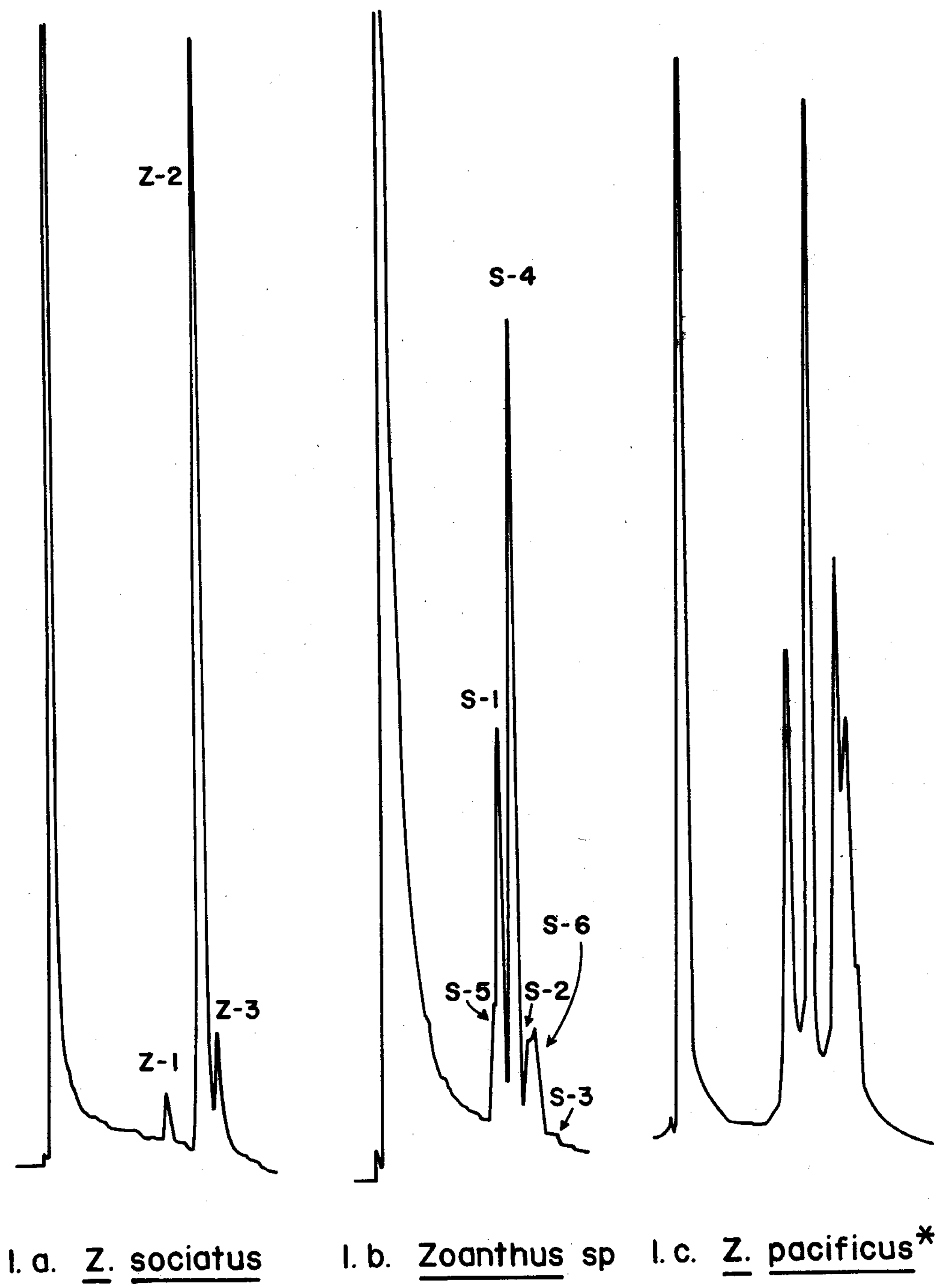

Fig. 1 — GC traces of sterol mixtures from *Zoanthus* spp *.

* after GUPTA e SCHEUER (9): reconstructed; in the original paper the zoanthid is named *Z. confertus*

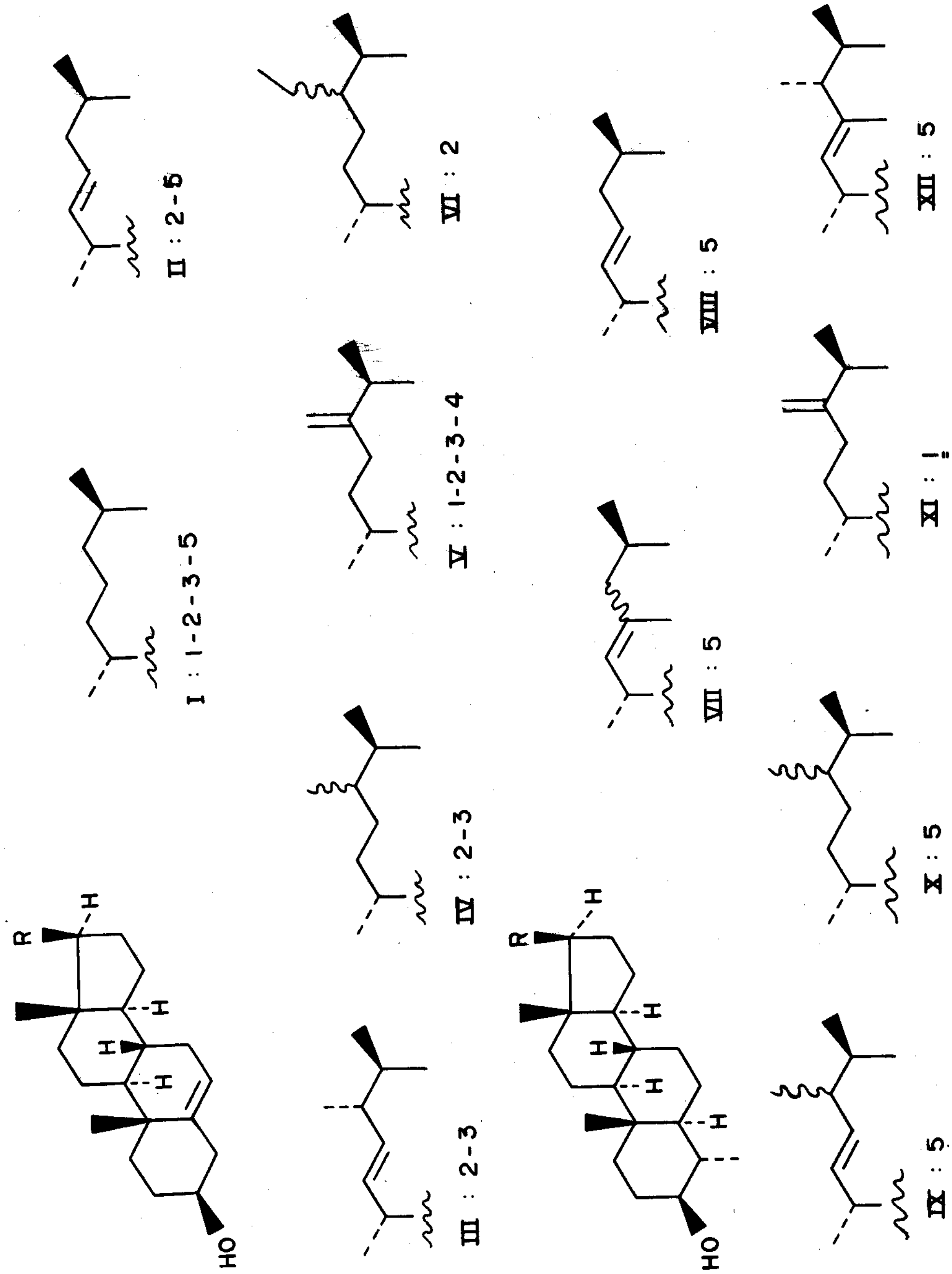

Fig. 2 — Sterol structures: Distribution of sterols in *Zoanthus* spp. 1 = *Z. sociatus;* 2 = *Zoanthus* sp; 3 = *Z. pacificus;* 4 = *Z. proteus;* 5 = symbiotic alga of *Z. sociatus.*

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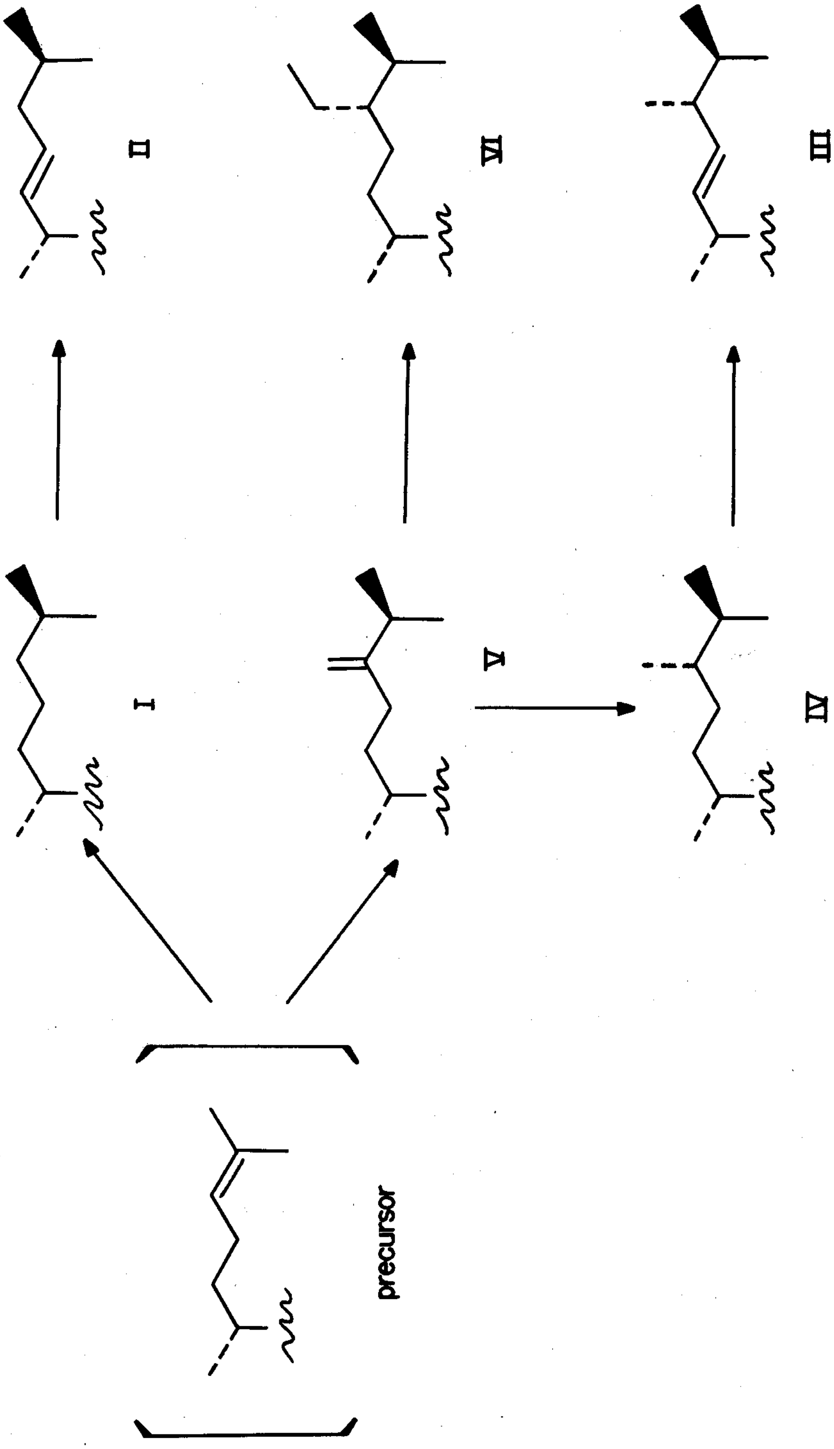

Fig. 3 — Sterols of *Zoanthus* sp, biosynthetic pathway

S-4 was identical with brassicasteryl acetate (9) by m.p., $[\alpha]_D$, IR, MS and GC (RRT and co-injection with an authentic sample).

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

S-6 was identified with chalinasteryl acetate (24-methylenecholesteryl 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. C_{28}-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 chalinasterol (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 synonimous 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), chalinasterol (V) and 24ξ-methylcholesterol (IV), the common (8) marine sterol 22(E)-22-dehydrocholesterol (II) and probably 24ξ-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 Δ^5 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 $\Delta^{24(28)}$ double bond, such compounds are completely

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