Comp. Biochem. Physiol. Vol. 72B, No. 4, pp. 677 to 682, 1982
Printed in Great Britain

0305-0491/82/080677-06$03.00/0

COMPARATIVE STUDY OF ZOANTHID STEROLS. THE GENUS *PALYTHOA* (HEXACORALLIA, ZOANTHIDEA)*

ALPHONSE KELECOM
Núcleo de Pesquisas de Produtos Naturais, Universidade Federal do Rio de Janeiro, Bloco H, 21941 Ilha do Fundão, Rio de Janeiro, Brazil

and

ANTONIO M. SOLÉ-CAVA
Laboratório de Bioquímica, Fundação Universidade do Rio Grande, 96200 Rio Grande, Brazil

(*Received* 18 *December* 1981)

Abstract—1. The sterol composition of three *Palythoa* species has been analysed and compared with literature data.

2. The mixture of sterols known as "palysterol" has been observed in all our samples suggesting that palysterol might be considered as a fingerprint of *Palythoa* species.

3. The sterols of *Palythoa caribaeorum* did not show marked geographic, seasonal or sex variations and its zooxanthella showed about the same sterol composition as the host–symbiont association.

4. Biological implications are discussed.

INTRODUCTION

Chemical studies of zoanthids have dealt principally with palytoxin (Moore & Bartolini, 1981), zoanthoxanthins (Prota, 1980; Chevolot, 1981) and mycosporins (Hirata *et al.*, 1979). In comparison, very little attention has been payed to zoanthid sterols (Goad, 1978).

Pioneer in zoanthid sterol chemistry, Bergmann (1949) isolated "palysterol" from the caribbean *Palythoa mammilosa*. Based on molecular rotation studies, palysterol has been first believed to be the C-20 epimer of γ-sitosterol (I) (Bergmann *et al.*, 1951), than to be a new sterol of unknown structure (Bergmann, 1962). Later Gupta & Scheuer (1969) isolated the sterol fraction of *P. tuberculosa* from Eniwetok and showed it to be identical to Bergmann's palysterol. Analytical gas chromatography (GLC) proved the presence of five sterols which were separated by preparative GLC and identified as cholesterol (II), brassicasterol (III), 22,23-dihydrobrassicasterol (IV), β-sitosterol (V) and gorgosterol (VI). From an unidentified *Palythoa* collected near Okinawa island, Kanazawa *et al.* (1977) also isolated sterols II, III, IV and VI co-occurring with chalinasterol (VII), small amounts of cholesta-5,22(E)-dien-3β-ol (VIII) and traces of the very peculiar 23,24ξ-dimethylcholesta-5,22-dien-3β-ol (IX). With this exception, the latter sterol is found only in octocorals (Kanazawa *et al.*, 1977; Kelecom *et al.*, 1980) and is supposed to be an intermediate in the biosynthesis of gorgosterol (VI) (Ling *et al.*, 1970). Contrasting with the aforementioned results, another unidentified *Palythoa* from Tahiti furnished only chalinasterol (VII) (Gupta & Scheuer, 1969).

As a part of our general screening on marine invertebrates from the Brazilian coast, we became interested in zoanthid sterols. In the two previous papers of this series (Kelecom, 1981; Kelecom & Solé-Cava, 1981), we studied the sterol composition of two species from the *Zoanthus* genus. We now report on the sterol composition of some Brazilian *Palythoa* species. In one case, the sterols of the associated alga have also been analysed.

MATERIALS AND METHODS

Equipment

In addition to the equipment described previously (Kelecom, 1981), we used for centrifugation of the associated zooxanthellae a Heraeus-Christ Minifuge 2 instrument operating at 15°C.

Animals

Three *Palythoa* species were collected on rocky bottom, along the Brazilian coast. *Palythoa caribaeorum* were obtained from various locations, at different periods of the year and also as sterile, male, female and hermaphrodite colonies. For convenience, samples have been distinguished by their collection number. Animals were then sun-dried or stored over 70% aqueous ethanol until examination. Data about the collection appear in Table 1. The associated alga have been obtained keeping the freshly collected *Palythoa caribaeorum* (sample CF-72) for 3 days at 45–50°C. An abundant mucus is produced which revealed, under microscopic examination, to be extremely rich in intact zooxanthellae. The mucus suspended in saline water has been filtered over a 100 μm sieve. Repetitive suspension of the filtrate in saline water followed by centrifugation yielded a zooxanthella fraction practically not contaminated by zoanthid cellular detritus. The host was freeze-dried before extraction.

* Part X of the series: *Studies of Brazilian Marine Invertebrates.*

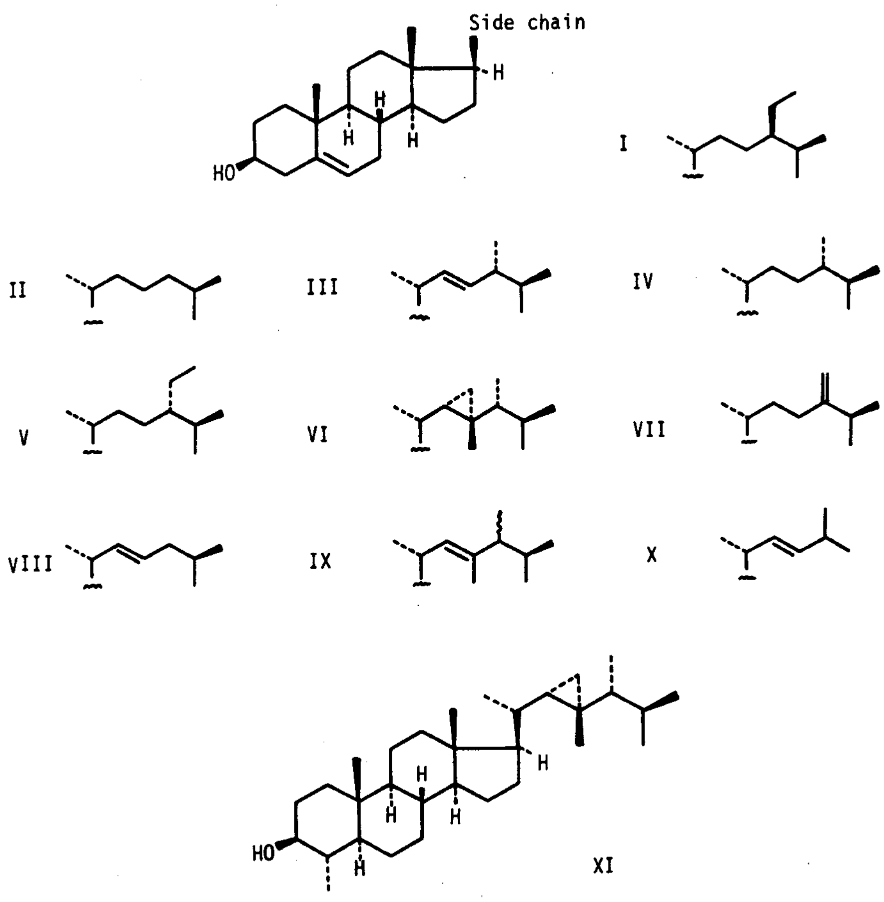

Fig. 1.

Table 1. The *Palythoa* collection

Animal & local		Sample code	Date	Depth	conservation	yield in % of CH_2Cl_2 solubles
P. caribaeorum						
Cabo Frio	(RJ)	CF-31	07.02.77	0-1 m	dry	3.4
Cabo Frio	(RJ)	CF-41	05.07.77	0-1 m	dry	3.5
Cabo Frio	(RJ)	CF-72	29.05.80	0-1 m	*	2.0
Abrolhos	(BA)	A-001	02.12.77	0-2 m	dry	2.9
Parati	(RJ)	D-036	13.03.80	1 m	70% aq EtOH	3.1
Angra dos Reis	(RJ)	D-043	31.01.80	0-1 m	70% aq EtOH	5.2
P. variabilis						
Abrolhos	(BA)	A-015	09.12.77	0-2 m	70% aq EtOH	2.9
Palythoa sp						
Guarapari	(ES)	G-12	16.12.76	8-12 m	dry	1.7
Alga from CF-72		-	29.05.80	-	-	15.7

P. caribaeorum from Cabo Frio: male or sterile colonies, from Abrolhos: female or hermaphrodite colonies.
P. variabilis: hermaphrodite colonies.
* See Materials and Methods.

variations; however for all *P. caribaeorum* samples but CF-72, no important sex, geographic or seasonal variations have been detected (see Table 2).

The sterol mixtures isolated from *P. caribaeorum* sample CF-72 and from the unidentified *Palythoa* species (sample G-12) have been found quite similar, particularly in the high concentration of brassicasterol (III) and the low one of 22,23-dihydrobrassicasterol (IV). The former animal was poor in symbiotic algae and the latter had been collected in deeper waters (see Table 1). The higher concentration in brassicasterol together with the lower one in derivative IV might thus reflect either low amounts of associated algae or reduced photosynthetic activity. Both animals also yielded lower amounts of extractable organic material (Table 1). On the contrary, the yield in organic matter isolated from the zooxanthella of sample CF-72 was four times that from the host (Table 1). The sterol mixture obtained from the isolated alga appeared very similar to that of the host–symbiont association, but the concentration of gorgosterol is double in the alga as compared to the host. This will be discussed further.

Finally, the GLC trace of the sterol mixture from *P. variabilis* (A-015) also showed considerable resemblance with the one of palysterol from *P. tuberculosa* (Gupta & Scheuer, 1969) but with a somewhat higher concentration in sterol IV and a lower one in sterol VI.

The sterol fraction of sample CF-72 was the only one obtained in sufficient amounts to allow examination of trace sterols. Acetylation of this sterol mixture, in usual conditions, followed by argentic silica gel column chromatography afforded the steryl acetates of dominant sterols II, III, IV and VI, and, in addition, the acetyl derivatives of chalinasterol (VII), cholesta-5,22(E)-dien-3β-ol (VIII) and 24-*nor*-cholesta-5,22(E)-dien-3β-ol (X), identified by their physico–chemical properties identical to published data (Sheikh & Djerassi, 1974). Sterols VII and VIII had already been reported by Kanazawa *et al.* (1977) for an unidentified *Palythoa* species; sterol X had never been reported as a zoanthid sterol, however being found in many marine organisms (Goad, 1978).

DISCUSSION

As it has been observed for the genus *Zoanthus* (Kelecom & Solé-Cava, 1981) and for Antozoans in general (Kanazawa *et al.*, 1977), C_{28}-sterols are also the major sterols of the genus *Palythoa*. However, contrasting with the results obtained for *Zoanthus* species, the sterol composition of *Palythoa* species appeared much more uniform. Considering the dominant sterols, one may conclude that *Palythoa variabilis* and *Palythoa caribaeorum* (except sample CF-72 commented separately) elaborate a mixture of sterols practically identical to that of *P. tuberculosa* reported by Gupta & Scheuer (1969). All along this discussion, we shall use the name palysterol for this sterol mixture.

Palysterol has also been reported for the Jamaican *P. mammilosa* (Silberberg, cited by Ciereszko & Karns, 1973, p. 189) and for the Pacific zoanthids *P. psammophilia* and *P. vestitus* (Quinn *et al.*, 1974). Remarkably, the latter species was formerly known as *Zoanthus vestitus* (Verrill, 1928) and has been recently reclassified as *Palythoa vestitus* on the basis of biological considerations (Walsh & Bowers, 1971). Hence, albeit a small number of zoanthid species have been investigated up to now, all colonies of the genus *Palythoa* seem to produce the same sterol mixture (at least for what concerns the major sterols). In addition, the specimens of *P. caribaeorum* did not show marked sex, seasonal or geographic variations. Similarly, no geographic variations had been observed by Gupta & Scheuer (1969) for the sterol mixture of *P. tuberculosa*. It is thus tempting to consider palysterol as a fingerprint for zoanthids of the genus *Palythoa*. Indeed, from the more than 80 marine invertebrates screened in our laboratory (among them sea stars, holothurians, sponges and gorgonians), only the sterol mixtures obtained from animals of the *Palythoa* genus presented the GLC trace of palysterol. On the contrary, no species of the closely related genus *Zoanthus* contained palysterol, since gorgosterol has never been found in this genus (Kelecom & Solé-Cava, 1981).

However, an unidentified Tahitian *Palythoa* species (Gupta & Scheuer, 1969) and the Hawaiian *P. toxica* (Quinn *et al.*, 1974) have been reported to contain essentially a single sterol, chalinasterol (VII). This is in apparent contradiction with our assumption that palysterol might be a fingerprint of the genus *Palythoa*. As there seem to be no doubt about the biological identifications of both specimens, these observations may be in agreement with the suspicion that the genus *Palythoa* might well be comprised of two different genera or sub-genera (Quinn *et al.*, 1974). The question raises then whether the presence of palysterol can be used to divide the genus *Palythoa* into two groups, in the same way as the presence of cholesterol characterises the division of red algae into the sub-classes Bangiophycidae and Florideophycidae (Brothers & Dickson, 1980). Since palysterol did not show sex, seasonal or geographic dependence and since palysterol is neither related to the liberae (i.e. digitated) vs immersae (i.e. incrusted) shape of the colony nor to the presence of palytoxin, known to be associated with female colonies (Kimura *et al.*, 1972; Kelecom *et al.*, 1982) (Table 3), it seems that the presence of palysterol might well be suitable to distinguish both genera or sub-genera comprised into *Palythoa*.

Since many marine invertebrates are known to be unable to biosynthetise sterols *de novo* (Goad, 1978), the problem merges to know whether zoanthid sterols are from exogenous origin or not. Indeed, zoanthids contain large amounts of intracellular dinoflagellate algae named zooxanthellae. The role of these algae is still not well understood, but it is known that zooxanthellae furnish nutritive organic material to the host (Muscatine, 1973). It is thus reasonable to question about the algal origin of palysterol. But if palysterol was strictly from algal origin, one should expect the various palysterol producing *Palythoa* species to be associated to identical or very similar zooxanthellae. This seems not to be the fact since two samples of *P. mammilosa* collected from two locations around Bermudas have been shown to be associated with two different strains of zooxanthellae (isoenzyme studies) and that the similarity coefficient between both strains was very low (Schoenberg & Trench, 1980). The same observations have been made with two

Extraction

Animals stored over 70% ethanol were extracted as described earlier (Kelecom, 1981). Dried animals were exhaustively extracted with methylene chloride. The yield in methylene chloride solubles appear in Table 1.

Obtention of the sterols

Obtention of the sterol mixtures and isolation of the sterols as their steryl acetates by argentic silica gel column chromatography have been carried out by previously described techniques (Kelecom, 1981; Kelecom & Solé-Cava, 1981). As crystallizations of the sterol mixtures altered the relative proportions of the sterols, sterol mixtures for GLC comparative analysis have been crystallized only once.

Palysterol from P. caribaeorum (CF-31)

m.p. 139.5–141.0° [lit. 139.0–141.0° (Gupta & Scheuer, 1969), 140–141° (Bergmann *et al.*, 1951)]; $[\alpha]_D^{25} = -47.0°$ in $CHCl_3$ ($c = 1.7$) (lit.: −48.5° (Gupta & Scheuer, 1969), −46.7° (Bergmann *et al.*, 1951); MS of the mixture: molecular ions at m/e 426 (6, $C_{30}H_{50}O$), 412 (1, $C_{29}H_{48}O$), 400 (57, $C_{28}H_{48}O$), 398 (9, $C_{28}H_{46}O$) and 386 (14, $C_{27}H_{46}O$), characteristic fragment ions at m/e = 385 (15, 400 − CH_3), 383 (8, 398 − CH_3), 382 (22, 400 − H_2O), 380 (1, 398 − H_2O), 371 (3, 386 − CH_3), 368 (8, 386 − H_2O), 367 (14, 400 − CH_3, H_2O), 365 (2, 398 − CH_3, H_2O), 355 (3), 353 (4, 386 − CH_3, H_2O), 339 (3), 336 (4), 328 (3), 315 (22), 314 (14, MacLafferty rearrangement from a $\Delta^{24(28)}$ double bond and/or cleavage of the cyclopropane ring of gorgosterol), 300 (11), 289 (22), 273 (18), 271 (18), 255 (27), 213 (26), ...; GLC: see Table 2.

Identical physico–chemical data have been recorded for the other sterol mixtures and will not be repeated here.

From *P. caribaeorum* sample CF-72, we obtained by argentic silica gel column chromatography sterols III, VII, VIII and X as the acetates, all of them over 90% pure by GLC. Physico–chemical data have been found essentially identical to reported data (Sheikh & Djerassi, 1974). Sterols II, IV and VI were obtained no better than 75% pure.

RESULTS

Biological identification of our collection appeared to be a delicate task. First, there is no available systematic biological study of Brazilian zoanthids. Second, the sole strictly South American zoanthid, *Palythoa braziliensis* Heider, 1895, had his holotype lost (Pax & Müller, 1957, p. 22) and could not be identified as none of our specimens due to the incomplete original description. Finally, systematics of zoanthids is rather confused at species level (Walsh, 1967). Based on gorgonian (Bayer, 1961) and porifera distributions (Solé-Cava *et al.*, 1981), it can be assumed that the lower invertebrate marine fauna of the Brazilian tropical region can be best compared with the West Indies one. Consequently, final identifications of our specimens came from their comparison with zoanthids reported for the West Indies.

Sterol mixtures obtained from male or sterile colonies of *Palythoa caribaeorum* (except CF-72) have been found by m.p., [α], MS and GLC practically identical to each other and also to the sterol mixture known as palysterol (Bergmann *et al.*, 1951; Gupta & Scheuer, 1969) (see Table 2). The only differences were the absence of the trace sterol β-sitosterol (V) (no molecular ion at $m/e = 414$) and the presence in some of our samples of minute amounts of sterols with relative retention times (RRT) to cholesterol of 0.70 and 1.97.

The sterols of RRT of 1.00–1.13 and 2.09 have been identified by co-chromatography with authentic samples of cholesterol (II), brassicasterol (III) and gorgosterol (VI). Sterol of RRT 1.27 (molecular ion at $m/e = 400$) has been identified as 22,23-dihydrobrassicasterol (IV) on the basis of its RRT identical with literature data obtained in the same operating conditions (Popov *et al.*, 1976) and by analogy with reported data for palysterol (Gupta & Scheuer, 1969). The sterols of RRT 1.50 and 1.97 could not be identified, by our techniques, due to their too low concentrations in the total sterol mixture.

Female or hermaphrodite colonies of *P. caribaeorum* (sample A-001) showed slightly higher concentration of gorgosterol (VI) than observed for male colonies, which may reflect some sex or geographic

Table 2. GLC analysis of the sterol fractions from the *Palythoa* spp

Animals \ RRT	0.70 X	1.00 II	1.13 III	1.27 IV	1.50	1.97	2.09 VI	others	total C_{28}
P. caribaeorum									
CF-31	-	6	9	66	< 1	t	18	-	75
CF-41	t	6	8	66	< 1	2	17	∿ 2	74
CF-72	t	6	27	58	< 1	t	9	t	85
D-036	-	8	7	65	1	t	19	t	72
D-043	-	6	4	72	t	-	17	t	76
A-001	t	5	2	70	< 1	t	23	t	72
P. variabilis A-015	t	10	3	73	1	t	13	t	76
Palythoa sp G-12	-	8	21	52	< 1	t	16	t	73
Alga of CF-72	< 1	3	8	69	1	t	18	t	77
P. tuberculosa *	-	13	3	64	1	-	19	-	67
Palythoa sp **	-	15	4	66	-	-	9	∿ 6 ‡	75

* After Gupta & Scheuer, 1969.
** After Kanazawa *et al.*, 1974.
‡ See Introduction.

variations; however for all *P. caribaeorum* samples but CF-72, no important sex, geographic or seasonal variations have been detected (see Table 2).

The sterol mixtures isolated from *P. caribaeorum* sample CF-72 and from the unidentified *Palythoa* species (sample G-12) have been found quite similar, particularly in the high concentration of brassicasterol (III) and the low one of 22,23-dihydrobrassicasterol (IV). The former animal was poor in symbiotic algae and the latter had been collected in deeper waters (see Table 1). The higher concentration in brassicasterol together with the lower one in derivative IV might thus reflect either low amounts of associated algae or reduced photosynthetic activity. Both animals also yielded lower amounts of extractable organic material (Table 1). On the contrary, the yield in organic matter isolated from the zooxanthella of sample CF-72 was four times that from the host (Table 1). The sterol mixture obtained from the isolated alga appeared very similar to that of the host–symbiont association, but the concentration of gorgosterol is double in the alga as compared to the host. This will be discussed further.

Finally, the GLC trace of the sterol mixture from *P. variabilis* (A-015) also showed considerable resemblance with the one of palysterol from *P. tuberculosa* (Gupta & Scheuer, 1969) but with a somewhat higher concentration in sterol IV and a lower one in sterol VI.

The sterol fraction of sample CF-72 was the only one obtained in sufficient amounts to allow examination of trace sterols. Acetylation of this sterol mixture, in usual conditions, followed by argentic silica gel column chromatography afforded the steryl acetates of dominant sterols II, III, IV and VI, and, in addition, the acetyl derivatives of chalinasterol (VII), cholesta-5,22(E)-dien-3β-ol (VIII) and 24-*nor*-cholesta-5,22(E)-dien-3β-ol (X), identified by their physico-chemical properties identical to published data (Sheikh & Djerassi, 1974). Sterols VII and VIII had already been reported by Kanazawa *et al.* (1977) for an unidentified *Palythoa* species; sterol X had never been reported as a zoanthid sterol, however being found in many marine organisms (Goad, 1978).

DISCUSSION

As it has been observed for the genus *Zoanthus* (Kelecom & Solé-Cava, 1981) and for Antozoans in general (Kanazawa *et al.*, 1977), C_{28}-sterols are also the major sterols of the genus *Palythoa*. However, contrasting with the results obtained for *Zoanthus* species, the sterol composition of *Palythoa* species appeared much more uniform. Considering the dominant sterols, one may conclude that *Palythoa variabilis* and *Palythoa caribaeorum* (except sample CF-72 commented separately) elaborate a mixture of sterols practically identical to that of *P. tuberculosa* reported by Gupta & Scheuer (1969). All along this discussion, we shall use the name palysterol for this sterol mixture.

Palysterol has also been reported for the Jamaican *P. mammilosa* (Silberberg, cited by Ciereszko & Karns, 1973, p. 189) and for the Pacific zoanthids *P. psammophilia* and *P. vestitus* (Quinn *et al.*, 1974). Remarkably, the latter species was formerly known as *Zoanthus vestitus* (Verrill, 1928) and has been recently reclassified as *Palythoa vestitus* on the basis of biological considerations (Walsh & Bowers, 1971). Hence, albeit a small number of zoanthid species have been investigated up to now, all colonies of the genus *Palythoa* seem to produce the same sterol mixture (at least for what concerns the major sterols). In addition, the specimens of *P. caribaeorum* did not show marked sex, seasonal or geographic variations. Similarly, no geographic variations had been observed by Gupta & Scheuer (1969) for the sterol mixture of *P. tuberculosa*. It is thus tempting to consider palysterol as a fingerprint for zoanthids of the genus *Palythoa*. Indeed, from the more than 80 marine invertebrates screened in our laboratory (among them sea stars, holothurians, sponges and gorgonians), only the sterol mixtures obtained from animals of the *Palythoa* genus presented the GLC trace of palysterol. On the contrary, no species of the closely related genus *Zoanthus* contained palysterol, since gorgosterol has never been found in this genus (Kelecom & Solé-Cava, 1981).

However, an unidentified Tahitian *Palythoa* species (Gupta & Scheuer, 1969) and the Hawaiian *P. toxica* (Quinn *et al.*, 1974) have been reported to contain essentially a single sterol, chalinasterol (VII). This is in apparent contradiction with our assumption that palysterol might be a fingerprint of the genus *Palythoa*. As there seem to be no doubt about the biological identifications of both specimens, these observations may be in agreement with the suspicion that the genus *Palythoa* might well be comprised of two different genera or sub-genera (Quinn *et al.*, 1974). The question raises then whether the presence of palysterol can be used to divide the genus *Palythoa* into two groups, in the same way as the presence of cholesterol characterises the division of red algae into the sub-classes Bangiophycidae and Florideophycidae (Brothers & Dickson, 1980). Since palysterol did not show sex, seasonal or geographic dependence and since palysterol is neither related to the liberae (i.e. digitated) vs immersae (i.e. incrusted) shape of the colony nor to the presence of palytoxin, known to be associated with female colonies (Kimura *et al.*, 1972; Kelecom *et al.*, 1982) (Table 3), it seems that the presence of palysterol might well be suitable to distinguish both genera or sub-genera comprised into *Palythoa*.

Since many marine invertebrates are known to be unable to biosynthetise sterols *de novo* (Goad, 1978), the problem merges to know whether zoanthid sterols are from exogenous origin or not. Indeed, zoanthids contain large amounts of intracellular dinoflagellate algae named zooxanthellae. The role of these algae is still not well understood, but it is known that zooxanthellae furnish nutritive organic material to the host (Muscatine, 1973). It is thus reasonable to question about the algal origin of palysterol. But if palysterol was strictly from algal origin, one should expect the various palysterol producing *Palythoa* species to be associated to identical or very similar zooxanthellae. This seems not to be the fact since two samples of *P. mammilosa* collected from two locations around Bermudas have been shown to be associated with two different strains of zooxanthellae (isoenzyme studies) and that the similarity coefficient between both strains was very low (Schoenberg & Trench, 1980). The same observations have been made with two

Table 3. Presence of palysterol and palytoxin in *Palythoa* spp

species	type	presence of palysterol	presence of palytoxin	references
P. mammilosa	immersae	+	+	a - b
P. tuberculosa	immersae	+	+	c - d
P. toxica	liberae	-	+	d - d
P. psammophilia	liberae	+	-	d - d
P. vestitus	liberae	+	+	d - d
P. variabilis	liberae	+	+	e - f
P. caribbaeorum				
male colonies	immersae	+	-	e - f
female colonies	immersae	+	+	e - f

a. Bergmann *et al.*, 1951.
b. Hashimoto, 1979.
c. Gupta & Scheuer, 1969.
d. Quinn *et al.*, 1974.
e. This work.
f. Kelecom *et al.* 1982.

samples of another zoanthid, *Protopalythoa grandis* (Schoenberg & Trench, 1980).

Are zoanthid sterols thus strictly produced by the host, or are they from mixed (host–algal) origin? The following considerations on gorgosterol may answer this question.

As pointed out before, gorgosterol is in *P. caribaeorum* (sample CF-72) twice as abundant in the sterol mixture isolated from the alga as compared with that from the host. The same observation had already been reported by Ciereszko *et al.* (1968) for gorgosterol from gorgonians. These authors concluded that the occurrence of gorgosterol in coelenterates is associated with the occurrence of zooxanthellae, and it has been shown since then that zooxanthellae-free gorgonians did not contain gorgosterol at all (Kokke *et al.*, 1981). Recently, gorgosterol has been isolated from the cultured non-zooxanthella dinoflagellate *Peridinium foliaceum* (Withers *et al.*, 1979), but it has never been found in a *cultured* zooxanthella isolated from an host known to contain gorgosterol, since cultured zooxanthellae produce principally 4α-methyl-sterols (Kokke *et al.*, 1981). This might indicate that zooxanthellae are unable to produce gorgosterol or that a modification of the algal metabolism takes place when the zooxanthella is isolated from the host. More probably it means that gorgosterol is effectively produced by the host from an algal precursor. A candidate for precursor might be 4α-methyl-5α-gorgostanol (XI), a sterol which has been isolated from the cultured marine alga *Peridinium foliaceum* (Withers *et al.*, 1979). Hence, gorgosterol should be from mixed (host–algal) origin. The same may be proposed for palysterol which contains gorgosterol as the second major sterol, but in the absence of any biosynthetic experiments envolving zoanthids, no final hypothesis can be proposed.

Further experiments are needed to solve the problem of the origin of palysterol in zoanthids; but it remains that palysterol is only produced by most of the zoanthids of the genus *Palythoa* and that palysterol might be useful to divide the genus *Palythoa* into two genera or sub-genera.

Acknowledgements—The authors are indebted to the Laboratórios Silva-Araujo Roussel S. A. (S.A.R.S.A.) for equipment facilities, to Prof. B. Tursch and colleagues (Free University of Brussels, Belgium) for a sample of gorgosterol and to the Conselho Nacional de Desenvolvimento Cientifico e Tecnológico (CNPq).

REFERENCES

Bayer F. M. (1961) The shallow-water octocorallia of the West-Indian region. In *Studies of the Fauna of Curaçao and other Caribbean Islands*. Vol. 12, pp. 1–373. Martinus Nijhoff, The Hague.

Bergmann W. (1949) Comparative biochemical studies on the lipids of marine invertebrates, with special reference to the sterols. *J. mar. Res.* **8**, (2), 137–176.

Bergmann W., Feeney R. J. & Swift A. N. (1951) Contribution to the study of marine natural products XXXI. Palysterol and other lipids components of sea anemones. *J. org. Chem.* **16**, (9), 1337–1344.

Bergmann W. (1962) *Comparative Biochemistry*. Vol. III, part A (Edited by Florkin M. & Mason H. S.), pp. 103–162. Academic Press, New York.

Brothers S. L. & Dickson L. G. (1980) Sterols of *Goniotrichum elegans*. *Phytochemistry* **19**, (11), 2357–2358.

Chevolot L. (1981) Guanidine derivatives. In *Marine Natural Products—Chemical and Biological Perspectives*, Vol. IV, chap. 2, (Edited by Scheuer P. J.), pp. 53–91. Academic Press, New York.

Ciereszko L. S. & Karns T. K. B. (1973) Comparative biochemistry of coral reef coelenterates. In *Biology and Geology of Coral Reefs*. Vol. II, *Biology* 1, chap. 6 (Edited by Jones O. A. & Endean R.) pp. 183–203. Academic Press, New York.

Goad L. J. (1978) The sterols of marine invertebrates: composition, biosynthesis, and metabolites. In *Marine Natural Products—Chemical and Biological Perspectives*, Vol. II. chap. 2. (Edited by SCHEUER P. J.) pp. 75–172. Academic Press. New York.

GUPTA K. C. & SCHEUER P. J. (1969) Zoanthid sterols. *Steroids* **13** (3). 343–356.

HASHIMOTO Y. (1979) *Marine Toxins and other Bioactive Marine Matabolites*. chap. 4. p. 248. Jap. Scient. Soc. Press. Tokyo.

HIRATA Y., UEMURA D., UEDA K. & TAKANO S. (1979) Several compounds from *Palythoa tuberculosa* (Coelenterata). *Pure appl. Chem.* **51** (9). 1875–1883.

KANAZAWA A., TESHIMA S.-I. & ANDO T. (1977) Sterols of Coelenterates. *Comp. Biochem. Physiol.* **57B** (2). 317–323.

KELECOM A. (1981) Studies of Brazilian marine invertebrates VIII. Zoanthosterol, a new sterol from the zoanthid *Zoanthus sociatus* (Hexacorallia, Zoanthidea). *Bull. Soc. chim. Belg.* **90** (9). 971–976.

KELECOM A., SOLÉ-CAVA A. M. & KANNENGIESSER G. J. (1980) Occurrence of 23,24ξ-dimethylcholesta-5,22-dien-3β-ol in the Brazilian gorgonian *Phyllogorgia dilatata* (Octocorallia, Gorgonacea) and in its associated zooxanthella. *Bull. Soc. chim. Belg.* **89** (11). 1013–1014.

KELECOM A. & SOLÉ-CAVA A. M. (1981) Studies of Brazilian marine invertebrates IX. Comparative study of zoanthid sterols 1. The genus *Zoanthus*. *Mem. Inst. Butantan*. In press.

KELECOM A., SOLÉ-CAVA A. M. & BOAVENTURA A. M. (1982) Palytoxin in Brazilian zoanthids. In *Marine Natural Products—Chemical and Biological Perspectives*, Vol. V, *Known Compounds*, (Edited by SCHEUER P. J.). Academic Press, New York.

KIMURA S., HASHIMOTO Y. & YAMAZATO K. (1972) Toxicity of the zoanthid *Palythoa tuberculosa*. *Toxicon* **10** (6), 611–617.

KOKKE W. C. M. C., FENICAL W., BOHLIN L. & DJERASSI C. (1981) Sterol synthesis by cultured zooxanthella; implications concerning sterol metabolism in the host-symbiont association in Caribbean gorgonians. *Comp. Biochem. Physiol.* **68B**, (2). 281–287.

LING N. C., HALE & DJERASSI C. (1970) The structure and absolute configuration of the marine sterol gorgosterol. *J. Am. chem. Soc.* **92** (17). 5281–5282.

MOORE R. E. & BARTOLINI G. (1981) Structure of palytoxin. *J. Am. chem. Soc.* **103**, (9) 2491–2494.

MUSCATINE L. (1973) Nutrition of corals. In *Biology and Geology of Coral Reefs*. Vol. II. *Biology* I. chap. 4. (Edited by JONES O. A. & ENDEAN R.) pp. 77–115. Academic Press. New York.

PAX F. & MÜLLER I. (1957) Zoantharia aus Viet-Nam *Mém. Mus. natn. Hist. nat., série A. Zool.* **16** (1). 1–40.

POPOV S., CARLSON R. M. K., WEGMANN A. & DJERASSI C. (1976) Minor and trace sterols in marine invertebrates 1. General methods of analysis. *Steroids* **28** (5). 699–732.

PROTA G. (1980) Nitrogenous pigments in marine invertebrates. In *Marine Natural Products—Chemical and Biological Perspectives*, Vol. III, chap. 3, (Edited by SCHEUER P. J.) pp. 141–178. Academic Press, New York.

QUINN R. J., KASHIWAGI M., MOORE R. E. & NORTON T. R. (1974) Anticancer activity of zoanthids and the associated toxin, palytoxin, against Ehrlich ascites tumor and P-388 lymphocitic leukemia in mice. *J. Pharm. Sci.* **63** (2), 257–260.

SHEIKH Y. M. & DJERASSI C. (1974) Steroids from sponges. *Tetrahedron* **30** (23/24). 4095–4103.

SHOENBERG D. A. & TRENCH R. K. (1980) Genetic variation in *Symbiodinium* (= *Gymnodinium*) *microadriaticum* Freudenthal, and specificity in its symbiosis with marine invertebrates. I. Isoenzymes and soluble protein patterns of axenic cultures of *Symbiodinium microadriaticum Proc. R. Soc. Lond. B* **207** (2). 405–427.

SOLÉ-CAVA A. M., KELECOM A. & KANNENGIESSER G. J. (1981) Systematic study of some sponges (Porifera, Demospongiae) from the infralitoral of guarapari (Espírito Santo, Brazil). *Iheringia, Zool.* **60** (12). 125–150.

VERRILL A. E. (1928) Hawaiian shallow water anthozoa. *Bull. Bernice P. Bishop Mus.* **49** (1) 3–30.

Walsh G. E. (1967) An annotated bibliography of the families Zoanthidae, Epizoanthidae and Parazoanthidae (Colenterata, Zoantharia). Hawaii Inst. Mar. Biol. Technical Report no. 13, pp. 1–77.

WALSH G. E. & BOWERS R. L. (1971) A review of Hawaiian zoanthids with description of three new species. *Zool. J. Linn. Soc.* **50** (2), 161–188.

WITHERS N. W., KOKKE W. C. M. C., ROHMER M., FENICAL W. H. & DJERASSI C. (1979) Isolation of sterols with cyclopropyl-containing side chains from the cultured marine alga *Peridinium foliaceum*. *Tetrahedron Lett.* **38**, 3605–3608.