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SECONDARY METABOLITES OF THE YELLOW AND GRAY MORPHS OF THE SOFT CORAL *PARERYTHROPODIUM FULVUM FULVUM*: COMPARATIVE ASPECTS

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ABSTRACT.—The yellow and gray morphs of the soft coral *Parerythropodium fulvum fulvum* (Cnidaria: Octocorallia) have been investigated during a three-year period. Fifteen secondary metabolites have been identified; of these, one (**8**) is a new acetate of lemnacarnol, and six others (**3**, **11**, **12**, **14**, **16**, and **17**) are new sesquiterpenes of known skeletons. The structure of the yellow morph's volatile dye, designated fulfulvene [**3**], has been established. It has been found that fulfulvene, together with other sesquiterpenes and other yet unidentified compounds, is lost during freeze-drying. Although some of the 15 identified compounds appeared in all extracts, others were found to be specific to either the gray or yellow morph, and some were rarely present. Gc studies of the volatile compounds have indicated significant differences for the various colonies, but no significant variation of the 15 identified compounds was evident when the chemistry of the male, female, and unidentified colonies was compared. The gc-ms analysis of the volatile compounds of the yellow morph suggests it to contain, inter alia, alkylated benzenes. Comprehensive ^{1}H- and ^{13}C-nmr assignments of compounds **1**, **2**, and **18** are described.

The soft coral *Parerythropodium fulvum fulvum* (Forskäl) (family Alcyoniidae) was originally described from the Red Sea, but its present zoogeographical distribution extends to the reefs of Madagascar and east to Indonesia (1). Colonies of *Pare. fulvum fulvum* have an encrusting membranaceous growth form and are among the most abundant soft corals on the coral reefs of the Red Sea. The colonies exist in two color morphs: yellow-brown and gray, although there are no taxonomic differences between them (1). The yellow-brown morph is found at a wide range of depths, and the gray morph is common usually below 20 m (1).

Pare. fulvum fulvum is a dioecious species. Young oocytes appear annually in August and within 10–11 months reach maturity. The sperm sac starts to develop a few months later and matures after 7–9 months. Spawning occurs at dusk, and it fully synchronizes with lunar periodicity, occurring a few days after the new moon and few days preceding its last quarter from the end of June to the beginning of August.

Pare. fulvum fulvum is oviparous, yet its eggs cleave on the surface of the female colonies while embedded in a mucoid suspension. This mode of planulae brooding is termed surface brooding (1). It is interesting to note that the color of the gonads corresponds to the colony morph: i.e., yellow-brown colonies have yellow gonads and gray colonies have gray gonads. Both morphs have a similar reproductive seasonality and breeding characteristics.

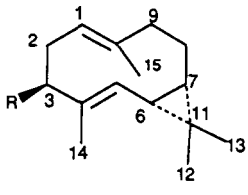
Earlier studies disclosed the structure of four sesquiterpenes, compounds **4**–**7**, from the Australian yellow *Pare. fulvum fulvum* (2).

The co-existence of two morphs of this soft coral in the Red Sea, and especially the thorough study of *Pare. fulvum fulvum* including its sexual reproduction by one of us (Y.B.) (1), triggered the present comparative study. During this investigation we have determined the structures of 15 compounds, of which one (**8**) is a new acetate of lemnacarnol and six others are new sesquiterpenes of known skeletons.

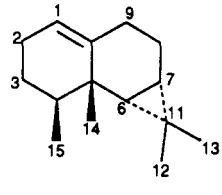
Comparison of males and females of *Pare. fulvum fulvum* with oocytes (by which they can be differentiated) was of special interest in light of earlier observations that eggs

either contained compounds that were not present in the releasing colonies or compounds that were present in the releasing colonies but at greatly elevated levels (×10) (3). The egg-specific compounds reported by Coll *et al.* (3) were generally terpenoids and are believed to play a role in the ovulation process. That messenger molecules will be lipophilic does not come as a surprise as the membrane receptors for messenger molecules are lipophilic, and it can be assumed that most lipophilic organic molecules will have some solubility in sea water (4). Indeed, in pioneer algal sperm chemotaxis studies, Müller (5) has disclosed the important role of dictyopterene D′, a hydrocarbon olefin (1-but-1′-enylcyclohepta-2,5-diene), as a brown alga sperm attractant (6).

The high content of volatile compounds (mainly sesquiterpenes) in soft corals has been reported by several groups (4,7). In some cases, such as *Heteroxenia guardaquensis*, the volatile sesquiterpenes represent more than 10% dry wt, all of which is lost during freeze drying (8). Special care has to be taken to avoid the loss of such volatile compounds by either lyophilization or evaporation, since they may, as discussed above, play



1 R=H
4 R=OAc
5 R=OAc



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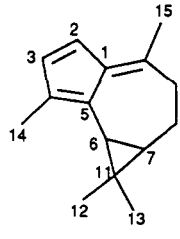

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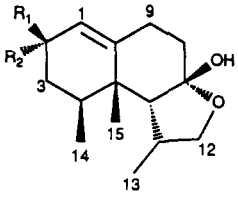
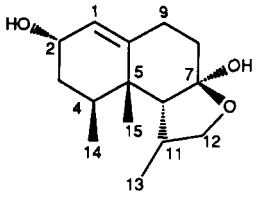


6 R_1=H, R_2=OH
7 R_1,R_2=O
8 R_1=H, R_2=OAc



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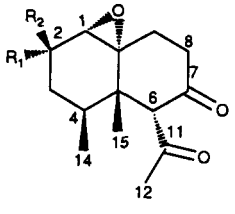
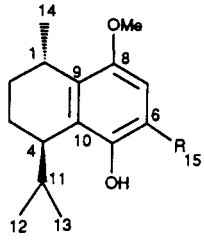
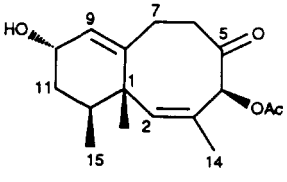


10 R_1=R_2=H
11 R_1=OH, R_2=H
12 R_1=OAc, R_2=H



13 R=Me
14 R=CHO



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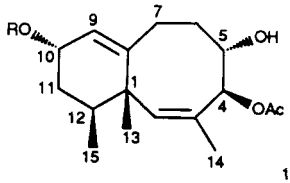
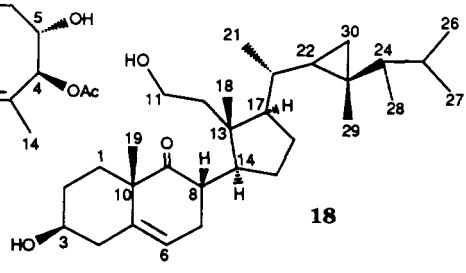


16 R=H
17 R=Ac



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important roles in interactions between marine organisms. In the case of *Pare. fulvum fulvum* we have isolated secondary metabolites either by extraction of the wet organism or by extraction after freeze drying.

Comparison of the gc of the less polar fraction of the wet extracts (see Experimental) of males and females of the yellow and gray morphs revealed both changes in the composition and differences in the relative concentrations of common compounds (Figure 1). Three of the more volatile compounds have been identified; *ent*-bicyclogermacrene [**1**] and (−)-1(10)-aristolene [**2**] from the gray morph and a new yellow compound designated fulfulvene [**3**] which is most probably responsible for the color of the yellow morph. Freeze drying of the latter morph causes bleaching of the dried soft coral and strong yellow coloring of the lyophilization water. Fulfulvene [**3**] is highly volatile, and careless evaporation (H_2O bath temperature over 30°) causes the evaporation of compound **3** with sequential strong yellow coloring of the entire evaporator.

The structures of compounds **1** and **2** ($C_{15}H_{24}$, *m/z* $[M]^+$ 204) were determined from the ^{13}C- and ^1H-nmr spectra taken in $CDCl_3$, using COSY and ^1H-^{13}C correlations (see Experimental for the line assignments). The nmr data (the first reported comprehensive ones) were fully consistent with the structure of bicyclogermacrene (9,10) and 1(10)-aristolene (11) [both with the cis substituted cyclopropane configuration

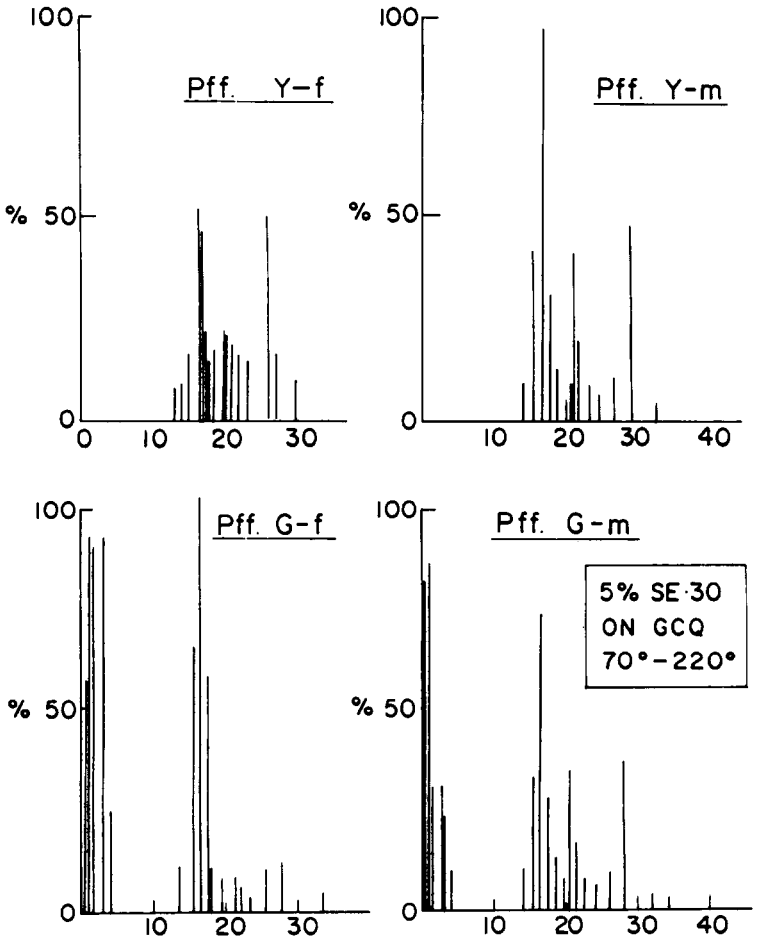

FIGURE 1. Drawings of gas chromatograms of male (m) and female (f) colonies of the yellow (Y) and gray (G) morphs of *Parerythropodium fulvum fulvum*.

(J_{cis} = 8.6 Hz) (2)]. However, the negative [α]D of compound **1** determined it to be the enantiomer of the terrestrial one. Aristolene known from marine organisms was also reported from terrestrial plants under two other names, that is, β-gurjunene and calarenene (12), with partial nmr data only.

The molecular formula of compound **3**, $C_{15}H_{20}$ (*m/z* 200 $[M]^+$), suggested that it was also a sesquiterpene. The ^{13}C-nmr spectrum of **3** (6 double bond equivalents) containing six sp^2 carbons (four singlets and two doublets) determined **3** to be tricyclic. A vicinal coupling constant of 5.5 Hz between the two olefinic protons suggested this double bond to be in a five-membered ring (13). The only other functionalities were two vinylic methyls (δ 1.89 s and 2.11 s) and a *gem*-dimethyl substituted cyclopropane (Table 1).

Comprehensive nmr work, COSY, one bond and long range ^1H-^{13}C correlations (HMQC and HMBC experiments) as well as nOe measurements (Table 1) led to the complete structure determination of fulfulvene [**3**]. Compound **3** possesses the alloaromadendrane skeleton and incorporates in its structure a fulvene moiety which is responsible for its yellow color. The alloaromadendrane skeleton is well known from terrestrial sources and was also earlier reported for marine sesquiterpenes (14); however, this is the first fulvene moiety to be disclosed from a non-terrestrial source (15).

Isolation of *ent*-bicyclogermacrene [**1**] was not unexpected in light of the previous isolation of 3-hydroxy bicyclogermacrene [**4**] and 3-acetoxy bicyclogermacrene [**5**] from *Pare. fulvum fulvum* (2), and indeed we have also isolated compound **5** from the yellow morph.

Three other compounds which have been isolated from the soft coral were compounds **6–8**. Two of the latter compounds, **6** and **7** were identified as lemnacarnol (16) and its 2-keto derivative, respectively, both of which have earlier been reported by

TABLE 1. ^1H- and ^{13}C-nmr Data[a] of Fulfulvene [**3**].

Position	δ_c	δ_H[b]	COSY to H	nOe to H	HMBC[c] to C		
					^2J	^3J	^4J
1	142.5 s						
2	132.3 d	6.25 d	9ax, Me-15		1	4,5	
3	119.4 d	6.23 d	6, Me-14		4	5,1	
4	155.0 s						
5	147.5 s						
6	25.0 d	1.32 brdq	3, 7, Me-14	7	7	Me-12	9
7	29.3 d	1.07 ddd	6, 8ax, 8eq	6	6,11	Me-12	
8	26.6 t	1.44 dddd (ax)	7, 8eq, 9ax	8eq	9,7		
		2.02 dddd (eq)	7, 8ax, Me-12		7		1
9	36.1 t	2.69 ddd (ax)	8ax, 8eq, 9eq, Me-15	9eq, 8eq, 7	8,10	1,7	6
		2.21 ddd (eq)	9ax	8eq, 9ax	8,10	1,7	6
10	128.5 s						
11	22.4 s						
12	28.3 q	1.16 s (3H)	Me-13	Me-13	11	Me-13,6,7	
13	16.5 q	0.88 s (3H)	Me-12	8ax, Me-12	11	Me-12,6,7	
14	13.7 q	1.89 brd (3H)	6, Me-15	3	4	5	
15	25.0 q	2.11 brs (3H)	1, 9eq, Me-14		10	1	

[a]360 MHz in $CDCl_3$.

[b]$J_{2,3}$ = 5.5, $J_{6,7}$ = 9.2, $J_{6,Me\text{-}14}$ = 1.7, $J_{7,8ax}$ = 10.8, $J_{7,8eq}$ = 6.7, $J_{8ax,8eq}$ = 14.3, $J_{8ax,9ax}$ = 11.7, $J_{8ax,9eq}$ = 1.3, $J_{8eq,9eq}$ = 6.3, $J_{8eq,9ax}$ = 1.0, $J_{9ax,9eq}$ = 17.8, $J_{6,Me\text{-}14}$ = 1.7.

[c]500 MHz in C_6D_6; for the ^1H and ^{13}C data see Experimental.

Bowden *et al.* (2) from *Pare. fulvum fulvum*. In one collection we could also find in minute amounts the 2-acetate derivative **8** of lemnacarnol. Mild basic hydrolysis (0.1% K_2CO_3, MeOH) converted **8** to lemnacarnol [**6**].

Compounds **6–8** might be considered as metabolites of 1(10)-aristolene [**2**]; whether they are obtained by oxidative cleavage of the cyclopropane ring in compound **2** has to be established.

From one collection we have also obtained the 7-isomer **9** of lemnacarnol [**6**]. As traces of TFA in $CHCl_3$ afforded the **6** to **9** isomerization, the isolation of **9** might have been a result of an unexplained higher acidity of the one organism from which it was isolated. The major changes in the ^{1}H-nmr spectrum of compound **9**, in comparison to that of lemnacarnol [**6**] (see Experimental), are the resonances of the three methyls and methylene-12.

Two additional compounds isolated are the 2-hydroxy **11** and the 2-acetoxy **12** derivatives of the known epoxy, diketo norsesquiterpene [**10**], earlier isolated by Izac *et al.* (17) from *Paralemnalia thrysoides*. Compound **11** was isolated from both morphs and **12** only from the yellow morph of *Pare. fulvum fulvum*. Interestingly, *Paralemnalia* and *Lemnalia*, unlike *Pare. fulvum fulvum*, both belong to the family Nephtheidae.

The molecular formula of compound **11**, $C_{14}H_{20}O_4$ (ms and ^{13}C data) suggested a norsesquiterpene of type **10**. Ir absorptions at 3476, 1725, and 1699 cm^{-1} were appropriate for a hydroxyl and two ketone groups. Furthermore, the ^{13}C-nmr spectrum of **11** contained resonances which closely corresponded to those observed for the ring-B carbon atoms (including the unique characteristic shift of C-6, δc 72.7), the methyls, and the epoxide functionalities of compound **10** (Experimental). Additionally, a W-arrangement between H-6 [δ_H (C_6D_6) 3.63 d, J = 1.5] and H-8eq [δ_H (C_6D_6) 2.30 ddt, J = 13.5, 5.8, 1.5 Hz] ($J_{\text{"W"}}$ = 1.5 Hz) suggested the same stereochemistries of C-6 for **11** as in **10**. The differences in the nmr data of compound **11** in comparison to those of compound **10**, were in the C-1 to C-3 site. The change of the epoxide proton H-1 to a doublet (3.24, J = 4.5 Hz) suggested an additional hydroxyl on C-2, the fourth oxygen of the molecule (δ_H 4.05 brt, J = 4.5 Hz, δ_c 63.0 d). From the coupling constants of H-2 we tentatively suggest the 2α orientation for the 2-hydroxyl. This was based on the assumption that ring A has a conformation in which C-1, -2, -3, -5, and -10 are almost in the same plane and C-4 beneath it [in accordance with an nOe between H-3β (pseudo axial) and Me-15ax (3%)]. In the latter conformation, H-2β has a ca. 40° dihedral angle with both H-1 and H-3β, and a ca. 90° angle with H-3α, in good agreement with the observed triplet. A second 2-epimer was obtained in minute amounts in a 3:1 mixture with **11** from one of the specimens. Both **11** and its 2-epimer afforded upon a Jones oxidation the same 2-one. As in this 2-epimer the pattern of H-2 (4.10, brt, J = 4.5 Hz) is very similar to that in **11**, the stereochemistry of C-2 in **11** is tentative.

Compound **12** was isolated in minute amounts, on one occasion only, from the yellow morph, whereas **11** was isolated from all the collections (Table 2). The major difference in the ^{1}H-nmr spectrum of **12** was the downfield shift of H-2 to 5.12 ddd (J = 7.5, 3.1, 1.5 Hz). Acetylation of **11** (Ac_2O/pyridine) afforded compound **12**, establishing the relationship between the two.

Two other compounds which have been isolated from the yellow morph were calamenene derivatives, compound **13** and **14**. Compound **13**, 5-hydroxy-8-methoxycalamenene, has earlier been reported by us together with 8-methoxycalamenene from *Subergorgia hicksoni* (18).

Comparison of the ^{1}H- and ^{13}C-nmr spectra of compounds **13** and **14** pointed to a great similarity between the two. The major difference in compound **14** was the disappearance of the aromatic methyl of **13** and the appearance of an aldehyde function (δ_H 9.78 s, δ_c 195.5 d). Furthermore, the phenolic O*H* of **14** was strongly downfield

TABLE 2. The Distribution of the Secondary Metabolites in *Parerythropodium fulvum fulvum*.

Compound	Gray morph[a]				Yellow morph[a]			
	b	m	f	a	b	m	f	a
1		+	+	+				
2		+	+	+				
8	+							
9	+							
13	+	+	+	+				
14	+	+	+	+				
16	+							
17		+		+				
3					+	+	+	+
5					+	+	+	+
12					+			
6	+	+	+	+	+	+	+	+
7	+	+	+	+	+	+	+	+
11	+	+	+	+	+	+	+	+
18	+	+	+	+	+	+	+	+

[a]m = male, f = female, b = before reproductive period, a = after reproductive period. Periods of collection: b = March, m and f = May–June, a = August (1988–1990).

shifted, to δ_H 11.3 ppm, establishing its vicinity to the aldehyde, with which it is proposed to form a strong hydrogen bond.

A 1*S*,4*R* stereochemistry is suggested for compound **14** ([α]D +104°) on the basis of the following argument. Bunko *et al.* (19) have shown that calamenenes with the 1*R*,4*R* or 1*S*,4*R* absolute configuration have a positive [α]D, whereas their enantiomers possess negative rotations; compound **14** thus must have the 4*R* configuration. Furthermore, the quintet for H-1 (Experimental) requires a pseudo equatorial conformation with a ca. 90° dihedral angle between this proton and H-2α or H-2β in case of the 1*S* or the 1*R* configurations, respectively. The absence of an expected nOe between the pseudo axial 1-Me and the pseudo axial 3β-proton in case of the 1*R* configuration suggested for **14** the 1*S*,4*R* absolute configuration. This absolute configuration is in full agreement with the stereochemistry of compound **13**, [α]D +32°, which has exactly the same coupling patterns of H-1 and -4; its 1*S*,4*R* stereochemistry was suggested following the synthesis of its 1*R*,4*S* enantiomer from dihydroxyserrulatic acid (19).

The last pair of sesquiterpenes which were determined were the new 5-hydroxy (**16**) and 4,5,10-triacetoxy (**17**) derivatives of the previously reported 5-ketoneolemnane [**15**] (20).

Compound **16**, ν max 3450, 1720 cm^{-1}, analyzed for $C_{17}H_{26}O_4$ by ms (*m/z* 276 $[M-H_2O]^+$) and ^{13}C-nmr data: four methyls, three methylenes, six methines (two sp^2, three methineoxy groups, and one sp^3), and four non-protonated carbons (one-CO_2-, two sp^2, and one sp^3).

The ^{1}H nmr of **16** suggested an acetate, one vinyl-methyl, two other methyls (a singlet and a doublet), two vinyl-protons, a secondary acetate, and two secondary alcohol-methines (Experimental). The latter two OH groups were confirmed by acetylation to a triacetate which was found to be identical with the natural triacetate **17** (δ_H 1.92, 1.97, and 2.22 ppm, $CDCl_3$). A COSY experiment [connectivities between H-2/Me-14, H-4; H-4/H-5; H-5/H-6, -6′ and H-7 ("W" arrangement); H-7/H-9; H-9/H-10 and -11; H-10/H-11, -11′; H-12/H-11, -11′ and Me-15] suggested the C-2 to C-12 (with Me-15) sequence of **16** to be the same as in neolemnane [**15**] except for the C-5 carbonyl of **15** which in **16** is reduced to a hydroxyl.

The structure of **16** was completed by the angular 13-methyl group on C-1, which closes simultaneously a six- and an eight-membered ring. This determination was based on an nOe between Me-13 (in the β orientation) and H-2, H-4β, and H-7β. Furthermore, the latter nOe's suggested the 4α orientation of the acetate and proposed a similar conformation to the one in **15** (20) for the eight-membered ring. An enhancement between Me-15 and H-10 determined the 10α configuration of the 10-OH group.

The low field resonance of H-4, $\delta_H = 6.81$ ppm (or even 7.32 in C_6D_6) interestingly had the same value as in **15**. This low value, earlier attributed to the C-5 keto group of **15** (20), seems to be best rationalized by paramagnetic effects from the two spatially close double bonds which are approximately in the same plane as H-4. This low value of H-4 seems therefore to be characteristic for the eight-membered ring of the neolemnanes.

The last compound which was isolated from all the *Pare. fulvum fulvum* collections was 9-oxo-9,11-secogorgost-5-ene-3β,11-diol [**18**], previously reported from *Pseudopterogorgia americana*, whose structure was determined by an X-ray diffraction analysis (21). A complete ^1H and ^{13}C resonance assignment of **18** based on COSY, CH-correlation experiments, and comparison with the proper parts of the *Sinularia* sp. (22) and *Xenia* sp. (23) sterols, 3β,11-dihydroxy-24-methylene-9,11-secocholest-5-en-9-one and xeniasterol, has been accomplished (Experimental).

The distribution of the more abundant identified secondary metabolites in the yellow and gray morphs of *Pare. fulvum fulvum* during different periods of the year between 1988 and 1990 is given in Table 2. Colonies of both morphs were collected by scuba diving throughout the study. Sex determination of male or female colonies of *Pare. fulvum fulvum*, based on the color of their gonads (1), was performed underwater only prior to the breeding season (May–June) when the gonads are distinguishable. Therefore, Table 2 presents results of unsexed colonies collected ahead of gonad maturation (March) and a

TABLE 3. The Major Ms Fragments of the Peaks Given in Figure 2.

Peak	Sex			*m/z* (rel. int.)
	f	m	u	
1	+	+	+	202 (40), 159 (100), 145 (80), 131 (100), 117 (75), 105 (90), 91 (95), 77 (60)
2	+	+	+	204 (5), 189 (20), 161 (100), 147 (22), 133 (30), 119 (40), 105 (45), 91 (45), 77 (25)
3	+	+	+	161 (60), 121 (100), 105 (70), 93 (100), 91 (80), 79 (80), 67 (60)
4	+	+	+	202 (1), 187 (1), 159 (30), 132 (90), 105 (100), 91 (45), 77 (25)
5	+	+	−	200 (30), 185 (35), 157 (100), 142 (60), 141 (60), 129 (70), 128 (65), 115 (60), 105 (20), 91 (40), 77 (25)
6	+	+	−	200 (65), 185 (75), 157 (85), 143 (80), 129 (100), 128 (90), 115 (70), 105 (25), 91 (40), 77 (45)
7	+	+	−	200 (20), 173 (75), 157 (50), 143 (100), 128 (80), 115 (40), 105 (10), 91 (25), 77 (35)
8	+	+	+	200 (100), 185 (55), 157 (75), 142 (50), 128 (40)—fulfulvene [**3**]
9	−	+	−	401 (20), 355 (100), 281 (15), 221 (15), 147 (10)
10	−	+	−	475 (5), 429 (100), 355 (35), 341 (20), 281 (15), 221 (20), 145 (20)
11	+	−	+	214 (95), 199 (72), 171 (55), 145 (100), 128 (65), 115 (82), 91 (70)
12	+	+	+	109 (30), 43 (100)
13	−	+	+	429 (100), 355 (90), 281 (80), 221 (80), 115 (85)
14	+	+	+	216 (1), 202 (75), 187 (82), 145 (80), 119 (80), 105 (90), 91 (100)
15	+	+	+	220 (M-42, 85) 109 (100)—compound **5**

few weeks after spawning (August) when they had no gonads at all. From Table 2 it can be seen that there are compounds specific for the yellow and the gray morphs while others are common to both; some are rare while others always appear. The work on another compound, specifically the gray morph which appears in tiny amounts, is ongoing.

While no real difference in the 15 identified compounds was found between the male, female and unidentified colonies of one morph, clear differences (vide supra) were observed in the gc of the less polar components of the various colonies. The gc-ms analysis of the less polar fraction of the yellow morph colonies is given in Figure 2 and Table 3. Several classes of compounds can be suggested, including alkylated benzenes (peaks 1 to 7, Figure 2). The alkylatyed benzenes are proposed on the basis of the fragmentation pattern, e.g., PhC_9H_{19}, *m/z* 77, 91, 105, 119 . . . 204 (peak 2) and PhC_9H_{17} *m/z* 77, 91, 105, 117, 131 . . . 202 (peak 1). Further studies are required for collection of enough of the volatile low-concentration components in order to determine their structure and their possible role during the breeding season and its associated processes.

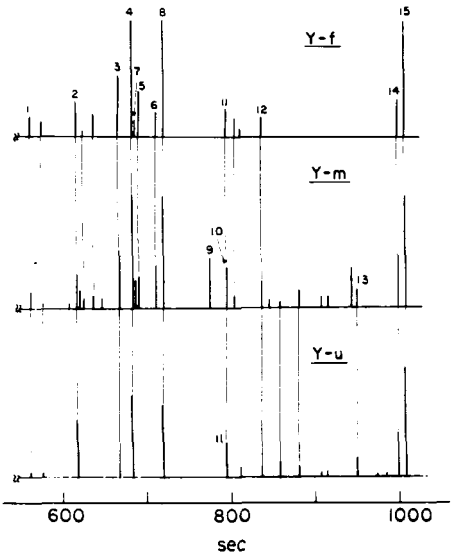

FIGURE 2. Gc-ms spectra of female (Y-f), male (Y-m), and unidentified (Y-u) yellow morph of *Parerythropodium fulvum fulvum*.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra were recorded on a Perkin-Elmer Model 177 spectrophotometer. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter using a 1 dm microcell. Low-resolution mass spectra were recorded on a Finnigan-4021 mass spectrometer. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are reported uncorrected. ^{1}H- and ^{13}C-nmr spectra were recorded on a Bruker AM-360 spectrometer, equipped with an Aspect 3000 computer and operated at 360 MHz and 90.5 MHz for ^{1}H and ^{13}C, respectively, and on an AM-500 spectrometer. All chemical shifts are reported with respect to TMS ($\delta = 0$).

EXTRACTION AND ISOLATION.—*Pare. fulvum fulvum* gray and yellow morphs were collected in the Gulf of Eilat, The Red Sea, during March and August 1988–1990 (unidentified sex) and during May–June 1988–1990 (males and females). Voucher specimens are located at the TA Zoological Museum (# CO27781–27784).

Compounds **1–3** *and* **5**.—A sample of the yellow morph (450 g wet wt) was extracted with CH_2Cl_2-

MeOH (1:1) (300 ml) *at room temperature overnight. After separation of the aqueous phase from the organic layer*, the latter was dried and evaporated on a cold bath (10–15°) to afford an oil (1.5 g). Vacuum liquid chromatography (vlc) through a short Silica H column eluted with petroleum ether afforded a fraction of non-polar compounds (R_f 0.58 and 0.27, petroleum ether) (130 mg). Hplc of the first fraction (100 mg) on a Si gel 60 column eluted with *n*-heptane gave fulfulvene [**3**] (50 mg), and hplc of the second fraction (100 mg) on the same column, eluted with *n*-heptane, gave compound **5** (10 mg). A similar hplc separation of the less polar fraction of the gray morph (100 g wet wt) afforded compound **1** (5 mg) and compound **2** (10 mg).

Compounds **6–9**, **11–14**, *and* **16–18**.—Freeze-dried yellow morph (80 g), soaked in EtOAc (100 ml) for 24 h, afforded after evaporation a brown oil (800 mg). Vlc of the latter oil on a Silica-H column eluted with petroleum ether with increasing percentages of EtOAc afforded, in order of polarity, compounds **6** (5 mg), **12** (2 mg), **7** (10 mg), **9** (2 mg), **11** (7 mg), and **18** (15 mg).

In a similar way compounds **13** (15 mg), **14** (10 mg), **16** (4 mg), **17** (3 mg), and **8** (3 mg) have been obtained from the gray morph.

Gc-ms was taken with a Varian 3400 gas-chromatograph equipped with a DB5 capillary column and a Finnigan ITD 800 mass spectrometer using iso-butane as the ionization gas (programming: 100° 2 min, then 10°/min to 200°, 10 min and then 30°/min to 250°). Gc was taken with a Varian chromatograph model 3700 equipped with a 5% SE-30 on GCQ (programming: 70° 3 min, then 15°/min to 250°, 30 min).

The petroleum ether extracts were the ones analyzed by gc, and the petroleum ether fractions from vlc of the wet CH_2Cl_2 extract were analyzed by gc-ms.

ent-*Bicyclogermacrene* [**1**].—Compound **1** was isolated as a colorless oil from the petroleum ether extract of the gray morph: $[\alpha]_D$ −87° (*c* = 0.6, CH_2Cl_2) [lit. (9) +61°]; cims *m/z* (rel. int.) $[M]^+$ 204 (22), $[M-Me]^+$ 189 (9), $[M-43]^+$ 161 (30), 121 (60), 43 (100); ^{1}H nmr ($CDCl_3$) δ 4.83 ddt (*J* = 10.8, 5.3, 1.0, H-1), 2.07 m (2H, H-2, -2′), 2.22 dt (*J* = 12.0, 3.5, H-3), 1.71 dt (*J* = 12.0, 4.0, H-3), 4.34 dd (*J* = 11.5, 1.0, H-5), 1.28 dd (*J* = 11.5, 8.6, H-6), 0.61 ddd (*J* = 11.5, 8.6, 2.7, H-7), 1.18 brdd (*J* = 13.7, 4.7, H-8), 1.82 m (H-8′), 2.42 brdt (13.0, 3.0, H-9), 1.88 m (H-9′), 1.03 s (Me-12), 1.10 s (Me-13), 1.66 d (*J* = 1.0, Me-14), 1.48 brs (Me-15), in agreement with the partial data from Nishimura (9); ^{13}C nmr ($CDCl_3$) δ 124.8 d (C-1), 41.1 t (C-2), 26.0 t (C-3), 128.0 s (C-4), 126.5 d (C-5), 26.9 d (C-6), 30.0 d (C-7), 27.1 t (C-8), 37.2 t (C-9), 140.9 s (C-10), 20.7 s (C-11), 15.4 q (Me-12), 29.2 q (Me-13), 16.5 q (Me-14), 20.8 q (Me-15). The following COSY-45° correlations have been observed (360 MHz in $CDCl_3$): H-1/H-2 and H-2′, Me-15; H-2 and H-2′/H-3 and H-3′; H-5/H-6, Me-14; H-6/H-5, H-7; H-7/H-6, H-8; H-8/H-9 and H-9′; H-8′/H-9 and H-9′.

(−)-1(10)-Aristolene [**2**].—Compound **2** was isolated from the gray morph as a colorless oil: $[\alpha]_D$ −41° (*c* = 0.3, CH_2Cl_2); enantiomer of the reported β-gurjunene [lit. (12) $[\alpha]_D$ +44°]; cims *m/z* (rel. int.) $[M]^+$ 204 (10), $[M-Me]^+$ 189 (10), $[M-43]^+$ 161 (70), 43 (100); ^{1}H nmr ($CDCl_3$) δ 5.20 brt (*J* = 2.3, H-1), 2.22 and 1.75 (each m, H-2, H-2′); 1.99 and 1.94 (each m, H-3, H-3′); 1.75 m (H-4), 0.56 d (*J* = 9.5, H-6), 0.74 dt (*J* = 3.6, 9.5), 1.99 and 1.39 (each m, H-8, -8′); 1.41 and 1.75 (each m, H-9, -9′), 0.98 and 1.02 (Me-12 and -13), 0.98 d (*J* = 7.3, Me-14), 1.07 s (Me-15); ^{13}C nmr ($CDCl_3$) δ 144.1 s, 120.3 d, 36.6 d, 33.4 d, 29.8 t, 27.1 t, 25.6 t, 22.9 q, 21.5 s, 20.8 t, 19.5 q, 16.5 q, 16.1 q. The following COSY-45° correlations (360 MHz in $CDCl_3$) have been obtained: H-1/H-2 and H-2′; H-2 and H-2′/H-3 and H-3′; H-3/H-2 and H-2′, H-4; H-4/Me-14; H-6/H-7; H-7/H-8, Me-12.

Fulfulvene [**3**].—Compound **3** was isolated from the extract of the yellow morph as a yellow-orange oil as described above: $[\alpha]_D$ −437° (*c* = 0.2, CCl_4); uv λ max (MeOH) nm (ε) 264 (19840), 390 (440); ir ν max (CCl_4) 3000–2850, 1677, 1627, 1454, 1376, 670 cm^{-1}; *m/z* found 200.1570, $C_{15}H_{20}$ requires 200.1565; cims *m/z* (rel. int.) $[M]^+$ 200 (100), $[M-Me]^+$ 185 (20), $[M-43]^+$ 157 (18); ^{1}H and ^{13}C nmr ($CDCl_3$) see Table 1; ^{1}H nmr (C_6D_6, 500 MHz) δ 6.40 (H-2), 6.38 (H-3), 1.39 (H-6), 0.95 (H-7), 1.46 (H-8ax), 1.89 (H-8eq), 2.48 (H-9ax), 1.86 (H-9eq), 1.14 (Me-12), 0.99 (Me-13), 1.85 (Me-14), 1.96 (Me-15); ^{13}C nmr (C_6D_6, 125 MHz) δ 142.5 s (C-1), 132.5 d (C-2), 120.3 d (C-3), 151.9 s (C-4), 141.8 s (C-5), 25.5 d (C-6), 29.5 d (C-7), 26.9 t (C-8), 36.1 t (C-9), 128.2 s (C-10), 22.4 s (C-11), 28.4 q (C-12), 16.6 q (C-13), 13.9 q (C-14), 24.6 q (C-15).

3-O-Acetylbicyclogermacrene [**5**].—Compound **5** was isolated from the yellow morph as a colorless oil: $[\alpha]_D$ −4° (*c* = 0.05, CCl_4); ir ν max (CH_2Cl_2) 1740, 1240 cm^{-1}; cims *m/z* (rel. int.) $[M]^+$ 262 (5), $[M-42]^+$ 220 (20), $[M-59]^+$ 203 (85), 43 (100); ^{1}H nmr (500 MHz C_6D_6) 4.68 ddt (*J* = 11.5, 5.0, 1.5, H-1), 2.28 q (*J* = 12, H-2), 2.39 dt (*J* = 12.0, 5.0, H-2′), 5.21 dd (*J* = 10.5, 4.5 H-3), 4.48 ddq (*J* = 12.0, 2.5, 1.5, H-5), 1.23 dd (*J* = 12.0, 8.5, H-6), 0.46 ddd (*J* = 12.5, 8.5, 3.0, H-7), 0.95 ddd (*J* = 12.5, 4.5, 1.5, H-8), 1.68 m (H-8′), 1.55 dq (*J* = 13.0, 4.0, H-9), 2.29 dt (*J* = 13.0, 3.5, H-9′), 0.92 and 0.99 s (Me-12 and -13), 1.66 d (*J* = 1.0, Me-14), 1.44 dd (*J* = 1.5, 0.5, Me-15), 1.74 s (16-Ac), in good agreement with the reported data (2); ^{13}C nmr (C_6D_6) δ 121.9 d (C-1), 31.9 t (C-2), 80.9 d

(C-3), 126.7 s (C-4), 125.4 d (C-5), 26.4 d (C-6), 30.6 d (C-7), 27.1 t (C-8), 37.3 t (C-9), 142.3 s (C-10), 20.7 s (C-11), 15.4 q (C-12), 29.1 q (C-13), 11.7 q (Me-14), 21.1 q (Me-15), 169.0 s and 20.7 q (Ac).

Lemnacarnol [**6**].—Compound **6** was isolated as an amorphous powder. Its ^{1}H nmr, mass, and ir spectra were virtually identical to the reported data (8).

2-Oxolemnacarnol [**7**].—Compound **7** was isolated as an oil and identified by comparing its ^{1}H- and ^{13}C-nmr data with reported values (2).

2-O-Acetyllemnacarnol [**8**].—Compound **8** was isolated in trace amounts as an oil from the gray morph: $[\alpha]_D$ −20° (c = 0.05, CH_2Cl_2); ir ν max ($CHCl_3$) 1720, 1260 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 5.63 d (J = 5, H-1), 5.13 m (H-2), 3.90 t (J = 8, H-12), 3.49 t (J = 8, H-12′), 2.06 s (OAc), 1.05 s (Me-15), 1.10 d (J = 6, Me-13), 0.90 d (J = 6, Me-14).

Hydrolysis of compound **8**.—Compound **8** (2 mg) was left in 0.1% K_2CO_3 in MeOH (1 ml) overnight at room temperature. The solution was neutralized with dilute HOAc, and the solvent was removed under vacuo. Filtration through a small amount of silica afforded compound **6**.

7-epi-*Lemnacarnol* [**9**].—Compound **9** was obtained in trace amounts only: ^{1}H nmr ($CDCl_3$) δ 5.54 d (J = 6, H-1), 4.06 m (H-2), 4.25 t (J = 8, H-12), 3.91 dt (J = 2, 8, H-12′), 1.20 d (J = 6, Me-13), 1.11 d (J = 6, Me-14), 1.10 s (Me-15). Treating compound **6** (1 mg) in $CDCl_3$ (0.5 ml) with TFA (5 μg) afforded compound **9** identical with the natural compound.

6α-Acetyl-4β,5β-dimethyl-1(10)-α-epoxy-2α-hydroxy-7-oxodecalin [**11**].—Compound **11** was isolated as an oil: $[\alpha]_D$ −313° (c = 0.25, CCl_4); ir ν max (CCl_4) 3476, 1725, 1699 cm^{-1}; *m/z* found 252.1368, $C_{14}H_{20}O_4$ requires 252.1361; cims (CH_4) *m/z* (rel. int.) $[M+1]^+$ 253 (100), $[M-H_2O]^+$ 235 (70), $[M-2H_2O]^+$ 217 (52), 193 (80), 175 (76); ^{1}H nmr ($CDCl_3$) δ 3.24 d (J = 4.5, H-1), 4.05 brt (J = 4.5, H-2eq), 1.50 m (H-3 and H-3′), 2.23 quintent (J = 6.6, H-4), 3.81 brs (H-6eq), 3.16 dt (J = 13.5, 7.5, H-8ax), 2.40 m (H-8eq), 2.49 m (H-9eq), 1.61 ddd (J = 14.5, 13.5, 5.5, H-9ax), 2.29 s (Me-12), 0.84 d (J = 6.8, Me-13), 0.93 s (Me-14). Repeating the ^{1}H nmr in Me_2CO-d_6 resolved the following overlapping resonances: δ 1.47 ddd (J = 14.5, 8.0, 1.6, H-3ax), 1.34 ddt (J = 14.5, 4.0, 1.3, H-3eq), 3.63 d (J = 1.5, H-6eq), 2.30 ddt (J = 13.5, 5.8, 1.5, H-8eq), 2.53 ddd (J = 14.5, 7.5, 6.8, H-9eq); ^{13}C nmr ($CDCl_3$) 59.4 d (C-1), 63.0 (C-2), 36.3 t (C-3), 26.0 d (C-4), 43.0 s (C-5), 72.7 d (C-6), 201.5 s (C-7), 36.4 t (C-8), 30.1 t (C-9), 65.0 s (C-10), 203.5 s (C-11), 32.8 q (C-12), 15.3 q (C-13), 17.7 q (C-14).

2-O-Acetyl derivative of **11** *(compound* **12**).—Compound **12** was isolated as an oil from the yellow morph: $[\alpha]_D$ −244° (c = 0.1, CH_2Cl_2); ^{1}H nmr ($CDCl_3$) δ 3.27 d (J = 3.1, H-1), 5.12 ddd (J = 7.5, 3.1, 1.5, H-2eq), 3.78 d (J = 1, H-6eq), 2.25 s (Me-12), 2.10 s (Ac), 0.93 s (Me-14), 0.81 d (J = 6.8, Me-13); ^{13}C nmr ($CDCl_3$) δ 58.5 d (C-1), 62.4 d (C-2), 32.3 t (C-3), 30.0 d (C-4), 36.3 s (C-5), 73.0 d (C-6), 32.6 t (C-8), 29.6 t (C-9), 66.7 s (C-10), 27.1 q (C-12), 15.2 q (C-13), 17.8 q (C-14). Acetylation of **11** (2 mg) with Ac_2O-pyridine (1:1) (0.5 ml) overnight at room temperature afforded, after evaporation, compound **12** quantitatively.

5-Hydroxy-8-methoxycalamanene [**13**].—Compound **13** was isolated as an oil and was found to be identical (ir, mass, and nmr) with **13** isolated from *Subergorgia hicksoni* (16).

5-Hydroxy-8-methoxycalamanen-15-al [**14**].—Compound **14** was isolated as a foaming oil: $[\alpha]_D$ +104° (c = 0.5, CCl_4); ir ν max (CCl_4) 2900, 1650, 1450, 1310 cm^{-1}; cims (CH_4) *m/z* $[M]^+$ 262 (15), $[M-iPr]^+$ 219 (100); ^{1}H nmr ($CDCl_3$) δ 3.25 quintet (J = 6.8, H-1), 1.97 dt (J = 13.4, 3.0, H-2), 1.97 m (H-2′), 1.70–2.01 m (H-3 and H-3′), 2.95 dt (J = 2.2, 5.2, H-4), 6.74 s (H-7), 2.06 oct (J = 7.0, H-11), 0.92 d (J = 7.0, Me-12), 0.95 d (J = 7.0, Me-13), 1.13 d (J = 7.0, Me-14), 9.78 s (C*H*O-15), 3.84 s (OMe), 11.3 s (O*H*); ^{13}C nmr ($CDCl_3$) δ 27.4 d (C-1), 25.4 t (C-2), 18.5 t (C-3), 36.4 d (C-4), 143.5 s (C-5)[a], 121.0 s (C-6), 108.9 d (C-7), 145.5 s (C-8)[a], 129.9 s (C-9)[b], 134.0 s (C-10)[b], 31.6 d (C-11), 21.9 q (Me-12)[c], 21.6 q (Me-13)[c], 21.1 q (Me-14)[c], 195.5 d (C-15), 55.4 q (OMe, C-16) (assignments with the same superscript are interchangeable).

4-Acetoxy-5,10-dihydroxyneolemna-2,8-diene [**16**].—Compound **16** was isolated from the gray morph: $[\alpha]_D$ +17° (c = 0.8, CCl_4); ir ν max (CCl_4) 3450, 2950, 1720, 1440, 1370, 1250, 1020 cm^{-1}; *m/z* found 276.1741, ($C_{17}H_{24}O_3$ $[M-H_2O]$ requires 276.1725); cims *m/z* (rel. int.) $[M-H_2O]^+$ 276 (10), $[M-HOAc]^+$ 234 (100), $[M-HOAc-Me]^+$ 219 (39), $[M-H_2O-HOAc]^+$ 216 (90); ^{1}H nmr (360 MHz in C_6D_6) δ 5.23 brs (H-1), 7.32 d (J = 9.2, H-4), 4.00 brdt (J = 9.2, 4.0, H-5), 1.85 and 2.05 (each m, H-6 and H-6′), 2.80 dt (J = 13.6, 4.3, H-7ax), 2.41 brd (J = 13.6, H-7eq), 6.20 brs (H-9), 4.55 m (H-10), 2.21 ddd (J = 13.6, 7.8, 3.5, H-11), 2.10 m (H-11), 1.86 m (H-12), 1.04 s (Me-13), 1.70 (Me-14), 1.00 d (J = 6.8, Me-15), 1.72 s (Ac); ^{13}C nmr ($CDCl_3$) δ 44.3 s (C-1), 138.4 d (C-2), 127.0 s (C-3), 76.0 d (C-4), 72.2 d (C-5), 31.6 t (C-6), 31.3 t (C-7), 144.3 s (C-8), 131.3 d (C-9), 66.0 d (C-10), 37.6 t (C-11), 39.4 d (C-12), 23.7 q (Me-13), 21.0 q (Me-14), 15.0 q (Me-15), 21.0 and 171.9 s (Ac).

5,10-Di-O-acetyl derivative of **16** *(compound* **17***).*—Compound **17** was isolated from the gray morph in trace amounts. The ^{1}H-nmr spectrum of **17** was identical with that of **16** except for the following changes: the signals of H-5 and H-10 (in $CDCl_3$) were downfield shifted to 5.05 brdt (J = 9.2, 4.0) and 5.20 m, respectively, and two additional singlets for the 2 Ac groups appeared at 2.22 and 1.97 ppm. Acetylation of **16** (2 mg) with Ac_2O-pyridine (1:1) (0.5 ml) overnight at room temperature afforded, after evaporation, compound **17** quantitatively.

*9-Oxo-9,11-secogorgost-5-ene-3*β*,11-diol* [**18**].—Compound **18** was isolated as fine needles: mp 128–130° (from Me_2CO); [α]D −27° (c = 0.35, $CHCl_3$); cims (CH_4) *m/z* (rel. int.) $[M+1]^+$ 459 (100), $[M]^+$ 458 (13), $[M+1-H_2O]^+$ 441 (89), $[M+1-2H_2O]^+$ 423 (12), 263 (2); ir ν max ($CHCl_3$) 3600, 2990, 1720, 1710 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 1.83 m (H-1 and H-1′), 1.89 m (H-2eq), 1.52 (H-2ax), 3.50 m (H-3ax), 2.38 m (H-4eq), 2.20 m (H-4ax), 5.47 brd (J = 5.0, H-6), 2.00 m (H-7), 2.38 m (H-7′), 3.00 dt (J = 12.0, 6.5, H-8ax), 3.85 m (H-11), 3.72 m (H-11′), 1.75 m H-12), 1.28 m (H-12′), 2.66 brq (J = 7, H-14), 1.28 m (H-15), 1.52 m (H-15′), 2.00 m (H-16), 1.29 m (H-16′), 1.70 m (H-17), 0.63 s (Me-18), 0.86 s (Me-19), 1.00 m (H-20), 1.01 d (J = 6.8, Me-21), 0.23 m (H-22), 0.23 m (H-24), 1.53 m (H-25), 0.86 d (J = 6.8, Me-26), 0.91 d (J = 6.8, Me-27), 0.84 d (J = 6.8, Me-28), 1.35 s (Me-29), 0.45 dd (J = 9.0, 4.0, H-30), −0.16 dd (J = 6.0, 4.0, H-30); ^{13}C nmr ($CDCl_3$) δ 31.0 t (C-1), 30.7 t (C-2), 72.0 d (C-3), 40.6 t (C-4), 140.0 s (C-5), 122.0 d (C-6), 32.2 t (C-7), 43.2 (C-8), 217.4 s (C-9), 36.0 s (C-10), 60.0 t (C-11), 40.4 t (C-12), 45.0 s (C-13), 41.7 d (C-14), 24.4 t (C-15), 27.5 t (C-16), 50.3 d (C-17), 17.0 q (C-18), 21.4 q (C-19), 34.5 d (C-20), 20.6 q (C-21), 31.8 d (C-22), 25.8 s (C-23), 50.6 d (C-24), 32.0 d (C-25), 14.2 q (C-26), 15.3 q (C-27), 21.6 q (C-28), 22.9 q (C-29), 21.2 t (C-30).

LITERATURE CITED

1. Y. Benayahu and Y. Loya, *Biol. Bull.*, **165**, 353 (1983), and references cited therein.
2. B.F. Bowden, J.C. Coll, S.J. Mitchell, J.L.E. Nemorin, and S. Sternhell, *Tetrahedron Lett.*, **21**, 3105 (1980).
3. J.C. Coll, B.F. Bowden, A. Heaton, P.J. Scheuer, M.K.W. Li, J. Clardy, G.K. Schulte, and J. Finer-Moore, *J. Chem. Ecol.*, **15**, 4 (1989).
4. J.C. Coll, B.F. Bowden, and M.N. Clayton, *Chem. Britain*, 761 (1990).
5. D. Muller, *Z. Pflanzenphysiol*, **80**, 120 (1976).
6. R.E. Moore, *Acc. Chem. Res.*, **10**, 40 (1977).
7. Y. Kashman, Y. Loya, M. Bodner, A. Groweiss, Y. Benayahu, and N. Naveh, *Mar. Biol.*, **55**, 255 (1980).
8. Z. Kimchi, "Isolation and Structure Elucidation of Natural Products from Soft Corals," M.Sc. Thesis, Tel Aviv University, 1982, p. 38.
9. K. Nishimura, *Tetrahedron Lett.*, 3097 (1968).
10. P. Ciminiello, E. Fattorusso, S. Magno, and L. Mayol, *J. Nat. Prod.*, **48**, 64 (1985).
11. A.J. Weinheimer, P.H. Washecheck, D. Van der Helm, and M.B. Hossain, *Chem. Commun.*, 1070 (1968).
12. C.S. Narayanan, K.S. Kulkarni, A.S. Vaidya, S. Kanthamani, G.L. Kumari, B.V. Bapat, S.K. Paknikar, S.N. Kulkarni, G.R. Kellar, and S.C. Bhattacharyya, *Tetrahedron*, **20**, 963 (1964), and references cited therein.
13. P.D.E. Pretsch, J. Seibl, W. Simon, and T. Clerc, "Spectral Data for Structure Determination of Organic Compounds," Springer-Verlag, Berlin, 1983, p. H230.
14. P. Ciminiello, E. Fattorusso, S. Magno, and L. Mayol, *Can. J. Chem.*, **65**, 518 (1987).
15. A.F. Barrero, J.F. Sánchez, Ma.J. Zafra, A. Barrón, and A.S. Feliciano, *Phytochemistry*, **26**, 1531 (1987).
16. B. Tursch, M. Colin, D. Daloze, D. Losman, and R. Karlsson, *Bull. Soc. Chim. Belg.*, **84**, 81 (1975).
17. R.R. Izac, P. Schneider, M. Swain, and W. Fenical, *Tetrahedron Lett.*, **23**, 817 (1982).
18. Y. Kashman, *Tetrahedron*, **35**, 263 (1979).
19. J.D. Bunko, E.L. Ghisalberti, and P.R. Jefferies, *Aust. J. Chem.*, **34**, 2237 (1981).
20. R.R. Izac, W. Fenical, B. Tagle, and J. Clardy, *Tetrahedron*, **37**, 2569 (1981).
21. E.L. Enwall, D. Van Der Helm, I.N. Hsu, T. Pattabhiraman, F.J. Schmitz, R.L. Spraggins, and A.J. Weinheimer, *Chem. Commun.*, 215 (1972).
22. R. Kazlauskas, P.T. Murphy, B.N. Ravi, R.L. Sanders, and R.J. Wells, *Aust. J. Chem.*, **35**, 69 (1982).
23. I. Kitagawa, M. Kobayashi, Z. Cui, Y. Kiyota, and M. Oknishi, *Chem. Pharm. Bull.*, **34**, 4590 (1986).

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