Tetrahedron Vol. 35, pp. 263–266

0040-4020/79/0115-0263/$02.00/0

8-METHOXY AND 5-HYDROXY-8-METHOXY-CALAMENENES FROM THE MARINE GORGONIAN *SUBERGORGIA HICKSONI*

Y. KASHMAN*
Department of Chemistry, Tel-Aviv University, Tel-Aviv, Israel

(*Received in UK* 3 *July* 1978; *Accepted for publication* 8 *August* 1978)

Abstract—The isolation and structure determination of two oxygenated calamenene sesquiterpenes from a gorgonian is reported. The NMR (^1H and ^{13}C) and CD spectra of these new compounds are interpreted and the stereochemistry of the system discussed.

We describe the isolation and structure elucidation of two new sesquiterpenes (**1** and **2**) which were obtained from the horny coral *Subergorgia hicksoni* (Coelenterata, Octocorallia, Gorgonacea) collected in the Gulf of Eilat (The Red Sea).

Compound **1** was isolated from the ether extract of the aqueous emulsion obtained during freeze drying of the coral. It was purified from accompanying olefins by chromatography on a silica-gel column. The compound $C_{16}H_{20}O$, *m/e* (%) 232 (M^+, 20), 217 (M-CH_3^+, 5) and 189 (M-iPr^+, 100), is an oil with b.p. 110°/0.1 mm Hg and ν_{max}^{neat} 3035, 1615, 1580 cm^{-1} (aromatic ring). It shows λ_{max} of 280 nm ($\epsilon = 1500$), which may indicate a benzenoid chromophore consistent with a substituted anisole.[1] Additional support for the latter moiety was obtained from the ^{13}C NMR spectrum in which the OMe group resonances at δ 55.5q indicative of an aromatic, or vinyl, OMe group.[a] The ^1H NMR spectrum of **1** shows signals at 6.52d and 6.62d for two weakly coupled aromatic protons ($J \simeq 1.5$ Hz), most likely in the *meta* position to each other. A singlet for an aromatic Me appears at δ 2.28 (3H), and an OMe group resonances at δ 3.80 ppm. The remaining two positions of the benzene ring are occupied by benzylic MeC<u>H</u>< and Me_2CHC<u>H</u>< groups (the underlined protons being vicinal to the phenyl ring, Table 1). The greater than expected downfield position of the former methine (δ 3.15) can be attributed to an adjacent OMe group which causes the paramagnetic shift. Four of the five degrees of unsaturation in **1** are accounted for in the aromatic ring, requiring a bicarbocyclic system.

Assuming normal biosynthesis, the above data suggested a cadinane skeleton for **1** (Fig. 1). The proposed substitution pattern of **1** was confirmed with the help of the ^{13}C NMR spectrum (Table 2). According to empirical parameters for the calculation of carbon-chemical shifts in substituted benzenes, which are approximately additive,[2,3] the δ-values for C_5–C_7 could be estimated for the different possible structural isomers. Estimate of the C_8–C_{10} atoms shifts (being in the *ortho* position to each other) was more speculative; however, approximate values neglecting the steric crowdedness effect could also be calculated for these atoms, and they are given in Table 2. It can be seen from Table 2 that the $\Delta\delta$ (obs-calc) values for the C_8–C_{10} atoms are not significantly larger than those for the C_5–C_7 ones. Thus despite the *ortho* substituent interactions, additive substituent parameters can be used to assign ^{13}C spectra correctly and to distinguish uniquely between similar isomers.[4] The calculated values in Table 2 are for the 8-methoxy-calamenene isomer, the one for which the δ-values were in best agreement with the measured ones. (For example, the calculated values for C-5 and C-6 in the 7-methoxy-calamenene isomer,[b] 131 and 120 ppm, respectively, differ by 9 and 8 ppm form the measured values, whereas in the 8-OMe isomer the differences do not exceed 3–4 ppm). It is interesting to compare the spectral data of **1** with the data of dihydroxy-serrulatic acid (**3a**),[5] an isoprenologue of the cadinene series which was obtained from the leaves of the *Eremophila serrulato* shrub, and with the data of appropriate derivative of this acid—**3b**.[5]

The ^1H NMR spectrum of **3b** closely resembles that of **1** including the $W_{1/2}$ values of H-1 and H-4 (Table 1) *vide infra*. Furthermore, in both **1** and **3b**, the base peak in the mass spectrum was found to be *m/e* 189, resulting from the C-4 side chain cleavage.

The second sesquiterpene (**2**) was isolated from the *Subergorgia hicksoni* petrol-ether extract following sequential chromatographies on LH-20 and silica-gel columns. Compound **2** is a crystalline material with m.p. 84° (after sublimation), $\nu_{max}^{CCl_4}$ 3480 (OH), 3030, 1610, 1585 cm^{-1} (aromatic ring), with the elemental formula $C_{16}H_{24}O_2$, *m/e* 248 (an additional O atom in comparison to compound **1**). The main change in the ^1H NMR spectrum of **1** compared to **2** is the remaining of only one aromatic proton at δ 6.52 and the appearance of a hydroxylic proton at δ 4.24. Except for these changes, there is a great similarity in their NMR spectra (Table 1), pointing to closely related structures. The λ_{max} of **2** bathochromic shifted to 290 nm ($\epsilon = 2400$), together with the other spectral data, suggested that **2** is a phenol derivative of **1**. However, no UV maxima shift could be noticed on basifying the ethanolic solution of **1** nor could a colourification of compound's **1** solution in ethanol by the addition of $FeCl_3$ be observed. The existence of an OH-group was nevertheless confirmed by acetylation with acetic anhydride-pyridine to give the mono acetate **4**, and by methylation with either Me_2SO_4/K_2CO_3 or MeI/K_2CO_3 (both methylations were rather sluggish as monitored by the appearance of the additional OMe signal at δ 3.90, giving only partial methylation after 24 hr at ambient temp).

It is known from the literature[6] that sterically hindered

[a] A sp^3 linked methoxy group appears usually at ~51 ppm.[2]

[b] The 7-methoxycalamenene is known in the literature.[12] Comparison of the reported IR and UV spectra clearly distinguishes between **1** and this 7-isomer.

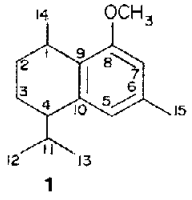
Table 1. ^{1}H NMR data (90 MHz)

Comp.	Solvent	H-1	H-4	Me_{12}	Me_{13}	Me_{14}	Me_{15}	OMe	H-arom
1	a	3.15(17)[d]	2.50(10)	0.98	0.80	1.13	2.28	3.80	6.52 6.62
	c	3.30	2.48	0.98	0.80	1.24	2.30	3.74	
	$\Delta\delta_{c-a}$	0.15	0.02	0	0	0.11	0.02	-0.06	
2	a	3.25(15)	2.71(13)	0.89	0.85	1.07	2.20	3.77	6.52 4.24(OH)
	b	3.40	2.70	0.90	0.82	1.25	1.92	3.36	6.30
	c	3.40	3.40	1.06	0.97	1.29	2.48	3.76	6.75
	$\Delta\delta_{c-a}$	0.15	0.69	0.17	0.12	0.22	0.28	-0.01	0.23
	c	0.15	0.25	0.10	0.20	0.10	0.26	0.11	0.11
3b[5]	a	3.20(18)	2.58(10)			1.13	2.30	3.78	6.55 6.65

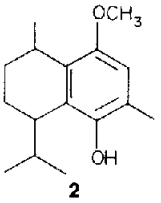
Solvents: a $CDCl_3$; b C_6D_6 c C_5D_5N.

d Numbers in parentheses are for $W_{1/2}$ - values (Hz).

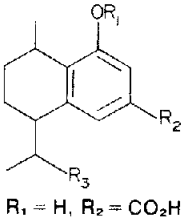
e $\Delta\delta$-values observed for the addition of 1.5eq. of $Eu(fod)_3$ to compound's 2 solution in $CDCl_3$. With the same amount of shift reagent compound's 1 spectrum remains unchanged.



1



2



3a: $R_1 = H$, $R_2 = CO_2H$
$R_3 = (CH_2)_2CH{=}C(CH_3)CH_2OH$
3b: $R_1 = R_2 = CH_3$, $R_3 = C_6H_{13}$

Fig. 1.

2,6-disubstituted phenols do not undergo complexation with $FeCl_3$; this may explain the above mentioned failure of compound's **2** colourification. As for the absence of the λ_{max} shift when KOH is added, this may be accidental (usually[1] two OR groups in the *ortho* position to each other give a bathochromic shift by basification, whereas in the *para*-position a hypsochromic shift is expected). Once the existence of the phenol was accepted, the substitution pattern of compound's **2** aromatic ring had to be established. This was achieved with the aid of the ^{13}C NMR spectrum (Table 2), in a manner similar to that described for the 8-methoxycalamenene (**1**). As with compound **1**, the estimated values immediately excluded several possible isomers—in fact, all the structures except for the 5-OH (or OMe)-8-OMe (or OH) Calamenenes:[c] e.g. the calculated values for C-5 in either the 7-OMe, 8-OH or the 7-OH, 8-OMe isomers, being 122 and 124 ppm, respectively, differ by 12 and 14 ppm from the measured values, and even larger deviations (*ca.* 20 ppm) are obtained for the 5,7 oxygenated isomers. Differentiation between the 5-OMe, 8-OH and the 5-OH, 8-OMe isomers was achieved by complexation of **2** with either $Eu(fod)_3$[7] or pyridine[8] (Table 1). Although, the $\Delta\delta$-values measured in the LIS experiment were surprisingly low (in comparison to values cited in the literature for a series of substituted phenols),[7] the

[c] We are aware of the ortho-effect on the δ-values; however, taking into consideration large $\Delta\delta$-values only, this seems to justify the comparisons, *vide supra*.

Table 2. ^{13}C chemical shift data of **1** and **2**[a]

Carbon No.	1		2		
	b	c	b	d	e
C - 1	26.6		26.6		
C - 2	27.2		25.7		
C - 3	f		22.2		
C - 4	43.1		37.9[i]		
C - 5	122.6	119.5	145.6	149.5	153.5
C - 6	134.6	135.5	119.6	123.0	121.5
C - 7	108.6	111.5	110.3	113.5	114.0
C - 8	157.2	160.5	150.9	153.0	149.0
C - 9	128.6	125.0	127.4	128.0	129.0
C - 10	140.7	144.0	130.0	132.0	129.0
C - 11	33.3		32.2		
C - 12	19.2[g]		19.5[g]		
C - 13	19.6[g]		21.1[g]		
C - 14	22.1[h]		21.5		
C - 15	21.5[h]		16.4[i]		
OCH_3	55.1		55.6		

a ^{13}C NMR spectra were recorded on a Bruker WH-90 spectrometer at 22.63 MHz. Chemical shifts in ppm, relative to carbons of TMS. Spectra were taken in $CDCl_3$ solutions using 10 mm spinning tubes; errors of δ are about ± 0.1. ^{13}C signals were assigned using known chemical-shift rules, off-resonance decoupling technique and calculation of the aromatic carbons (see text)[2,3]

b Measured values

c Calculated values for the 8-OMe isomer

d Calculated values for the 5-OH, 8-OMe isomer

e Calculated values for the 5-OMe, 8-OH isomer

f Overlaps with one of the Me groups

g h These assignments may be interchanged

i C - 4 and C - 15 are diamagnetically shifted as expected from an additional γ-effect of the hydroxy group

results prefer the 5-OH, 8-OMe structure. The same is true for the pyridine complexation (Table 1). This structure is also in accordance with the lack of complexation by $FeCl_3$ *vide supra*, which is prevented by two ortho to -OH substituents.[6] Furthermore, it seems to us that more structural information can be deduced from the above mentioned behaviour; namely, that the hindrance in complexation requires a pseudo-equatorial i-Pr group which will encounter great difficulty on the approach to the phenol group.

The configuration assignment of C-1 and C-4 could have been achieved by the following methods: (a) by comparison of $W_{1/2}$ values of H-1 and H-4 in **1** and **2** with the corresponding values in dihydroxy-serrulatic acid (**3a**) (the *trans* configuration of which was unequivocally determined by an X-ray analysis), and/or (b) by CD-measurements as was done by Anderson[9] for the calamenes themselves.[d] The high resolution NMR spectra of compounds **1** and **2** have shown that although the $W_{1/2}$ values of these compounds are almost the same as in **3a** and **3b**, the measured coupling constants (Experimental) did not agree with the serrulatic acid derivative conformation determined by the X-ray analysis.[e] This discrepancy may origin from the molecule flexibility of compounds **1** and **2**, as can be seen from a Dreiding model, and casts doubt on a configuration-conformation assignment based on partial NMR data only. Furthermore, a CD-assignment[f] assuming a specific conformation (determined by NMR) for the *cis* and *trans* configuration, as was done by Anderson for the calamenenes[9] seems to us to be uncertain, at least in our case. Thus the stereochemistry of **1** and **2** remains unresolved for the moment.

It is worth mentioning that (+)-calamenene was reported to be isolated from another gorgonian, namely *Pseudoplexaura porosa*.[11]

[d] An assignment based on the ^1L_b band Cotton-effect.[13]

[e] Additional supporting evidence for the conformational problem is obtained from the Me groups' chemical shift of the iPr in **1**. One would expect the ^1H NMR δ-value for the iPr in **1** and in one of the calamenene isomers[9,10] to be identical (the 8-OMe should not influence this remote group); this is not the case however.

[f] Both **1** and **2** exhibit positive ^1L_b (and ^1L_a) Cotton effects: 276 nm ($\Delta\epsilon + 0.22$) and 233 nm ($\Delta\epsilon + 0.70$) c = 0.08 for **1** and 291 nm ($\Delta\epsilon + 0.32$) and 233 nm ($\Delta\epsilon + 0.47$) c = 0.09 for **2**—both in MeCN solution.

EXPERIMENTAL

M.ps were taken on a Thomas Hoover capillary m.p. apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer Infracord model 257, and UV spectra on a Perkin-Elmer 137 UV. NMR spectra were taken on Jeol JNM-C-60HL, Bruker WH-90 and Bruker HX-270 spectrometers using 5–10% soln in $CDCl_3$ with TMS as an internal standard. Mass spectra were recorded with a DuPont 21-491B instrument. $[\alpha]_D$ were taken on a Bellingham and Stanley polarimeter in $CHCl_3$ solns.

Isolation procedure of compounds **1** *and* **2**

Subergorgia hicksoni was collected in the Gulf of Eilat (The Red Sea) near Ophira at a depth of 20 m, and was immediately frozen. Freeze-drying of the horny coral gave 950 g (dry weight) material. The aqueous emulsion collected during the drying process (*ca.* 500 ml) was extracted three times with $CHCl_3$ (100 ml); the combined organic phase gave after drying (Na_2SO_4) and careful evaporation a colourless oil (450 mg). Chromatography of this oil on a silica gel column (Merck 7734) showed two main fractions: the first was eluted with petrol ether and was a complicated mixture of olefins (220 mg), and the second was eluted with petrol-ether chloroform (9:1) and was compound **1** (55 mg). The homogeneity of **1** was confirmed by: (a) Tlc on silica gel (Merck 5735), in which elution with benzene gave $R_f = 0.8$, blue spot by vaniline-phosphoric acid development; and (b) HPLC on Porasil-A. Compound **1** is an oil with b.p. 110°/0.1 mm Hg, $\alpha_D^{25°} + 30°$ (*c*, 0.1 $CHCl_3$); λ_{max} (MeOH) 280 nm (1500), 273 (1380), 222s (11,000) and 207 (27,000) ν_{max}^{neat} 3035, 2960, 2870, 2830, 1615, 1580, 1465, 1420, 1385, 1370, 1350, 1335, 1315, 1275, 1220, 1170, 1125, 1105, 1050, 1005, 960, 895, 835, 810 and 760 cm^{-1}; *m/e* (%) 232 (M^+, 20), 217 ($M-CH_3^+$, 5), 189 (M-iPr$^+$, 100), 175 (4), 174 (4), 161 (6), 154 (m* 232→189) and 135 (2); with an ^1H and ^{13}C NMR as seen in Tables 1 and 2. ^1H NMR (270 MHz): 6.62 d ($J \simeq 1.5$), 6.52d ($J \simeq 1.5$), 3.80s (OMe), 3.14 quin (H-1, $J = 6.5$), 2.45dt (H-4, $J \simeq 1$ and 4), 2.30s (3H), 1.92m (H-11), 1.89 (H-2β), 1.81–1.75m (2H, H-3α and H-3β) and 1.46dt (H-2α, $J_{gem} = 14$ and 3 Hz). Freeze-dried *Subergorgia hicksoni* (250 g) was extracted during 48 hr with petrol ether in a Soxhlet to give 1.8 g crude extract. Chromatography of this extract (1.5 g) on a silica gel column (Merck 7734), following elution with petrol ether chroroform (1:1), gave a mixture of sterols (340 mg) and then compound **2** (80 mg). The homogeneity of **2** was confirmed as described for **1** (with the same tlc and development system, in which a yellow spot with $R_f = 0.65$ was obtained). Apart from the sterols and compound **2**, the extract was found to contain also long chain fatty acids and different kinds of glycerides.

Compound **2**, $C_{16}H_{24}O_2$, m.p. 84° (after sublimation at 60°/0.1 mm Hg), $\alpha_D^{25°} + 58°$ (*c*, 0.1 $CHCl_3$), λ_{max} (MeOH) 290 nm (2400). ν_{max}^{neat} 3480, 2930, 2870, 1610, 1585, 1460, 1410, 1380, 1365, 1340, 1325, 1290, 1240, 1215, 1125, 1095, 1025, 1015, 985, 915, 885 and 835 cm^{-1}; *m/e* (%) 248 (M^+, 20), 233 ($M-CH_3^+$, 1), 219 (M-29, 1), 205 (M-iPr$^+$, 100), 190 (8), 175 (5), 169.5 (m*, 248→205), 105 (0.5) and 91 (2); (^1H and ^{13}C NMR (see Tables 1 and 2) shows an ^1H NMR (270 MHz) as follows: 6.50s (1H), 3.80s (OCH_3), 3.20 quin (H-1, $J = 6.3$ Hz), 2.73dt (H-4, $J = 2$ and 5 Hz), 2.21s (3H), 2.02 oct (H-11, $J \simeq 7$ Hz), 1.93m (H-2β), 1.81–1.77m (2H, H-3α and H-3β, $J_{gem} = 14$ Hz), 1.45dt (H-2α, $J_{gem} = 14$ and 3 Hz) Me's—Table 1.

Micro acetylation of **2** (5 mg) was carried out by leaving the sample overnight in the presence of one drop of Ac_2O and one drop of pyridine. Evaporation gave the mono acetate (**4**) as an oil: $C_{18}H_{26}O_3$ *m/e* (%) 290 (M^+, 50), 248 ($M-CH_2=C=O^+$, 80), 212.5 (m*, 290→248), 205 (M-42–43, 100), 189 (50), 170 (m*, 248→205), ^1H NMR: 6.50s (1H), 3.75s (OMe), 3.1m (1H), 2.25s (Me_{15}), 2.08 (OAc), 1.07s (Me_{14}), 0.82s and 0.86s (Me_{12} amd Me_{13}).

Acknowledgements—We wish to express our appreciation to Dr. Y. Loya and Mr. Y. Benayahu for collecting the coral. Thanks are due to Miss H. Ben-Yaacov for her technical assistance.

REFERENCES

[1]A. I. Scott, *Interpretation of the UV Spectra of Natural Products*. Pergamon Press, Oxford (1964).
[2]J. B. Stothers, *Carbon-13 NMR Spectroscopy*. Academic Press, New York (1972).
[3]F. W. Wehrli and T. Wirthlin, *Interpretation of Carbon-13 NMR Spectra*. Heyden, London (1978).
[4]N. K. Wilson and R. D. Zehr, *J. Org. Chem.* **43**, 1768 (1978).
[5]K. D. Croft, E. L. Ghisalberti, P. R. Jefferies, C. L. Raston and A. H. White, *Tetrahedron* **33**, 1476 (1977).
[6]H. Tsuruta and T. Mukai, *Bull. Chem. Soc. Jap.* **41**, 2489 (1968).
[7]N. Platzer and P. Demerseman, *Bull. Soc. Chim. Fr.* 192 (1972).
[8]P. V. Demarco, E. Farkas, D. Doddrell, B. L. Mylari and E. Wenkert, *J. Am. Chem. Soc.* **90**, 5480 (1968).
[9]N. H. Anderson, D. D. Syrdal and G. Graham, *Tetrahedron Letters* 905 (1972).
[10]N. H. Anderson and D. D. Syrdal, *Phytochem.* **9**, 1325 (1970).
[11]A. J. Weinheimer, F. J. Schmitz and L. S. Ciereszko, *Drugs from the Sea*. Trans. Marine Technol. Soc. 135 (1967).
[12]J. Alexander and G. S. Krishna Rao, *Tetrahedron* **27**, 645 (1971).
[13]D. Neville Jones, *MTP International Reviews of Science* Org. Chem. Series One, Vol. 1, p. 85. Butterworths, London (1973).