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The Capnellenes Revisited: New Structures and New Biological Activity

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Abstract: Three capnellanes have been isolated from the soft coral *Capnella imbricata*, one known (**1**) and two new structures (**9**, **10**). Their structures were determined by a mixture of spectroscopic methods and comparisons to the previous literature. Cytotoxicity against a panel of cancer cell lines was determined for all the compounds with compound **1** showing an IC_{50} of 0.7 μM against K562 leukaemia. In addition compound **9** was cytotoxic to promyelogenous leukaemia and **10** was active against renal leiomyoblastoma cells. © 1998 Elsevier Science Ltd. All rights reserved.

INTRODUCTION

The soft coral *Capnella imbricata* (Order Alcyonacea, Family Nephtheidae) is known for producing a range of sesquiterpene alcohols known as capnellenes which have a skeleton of three fused five membered rings with an exo double bond at position C9.[1,2,3,4,5,6] Those already isolated include several di- tri- and tetra substituted alcohols and a range of acetoxycapnellenes (**1-8**), as well as the unfunctionalised capnellene **11**. All previous extractions of this species have readily produced the compound capnellene-8β,10α-diol **1** as did the dichloromethane extract of this sample. However it has been found that most of the other alcohol and acetoxy compounds isolated are unique to the particular sample extracted. From our sample we have isolated two previously unrecorded compounds with the capnellene skeleton, the unique capnellene-8β-ol **9** and 3β-acetoxycapnellene-8β,10α,14β-triol **10**. The structure of these compounds was ascertained using 1D and 2D NMR data and by analogy with previously reported capnellenes.

RESULTS AND DISCUSSION

The sample of *Capnella imbricata* was collected from Mayu Island, Molucca Sea, Indonesia, in November 1996. It was identified as Capnella imbricata by Leen van Ofwegen at the National Natural History Museum of the Netherlands. The sample was preserved in EtOH and transported to the home laboratory where it was subjected to solvent extraction followed by solvent partitioning of the crude extract. The hexane and dichloromethane partition fractions were subjected to size exclusion chromatography followed by normal and reverse phase HPLC respectively. Capnellene-8β,10α-diol **1** was obtained as 60 mg of colourless crystals after reverse phase HPLC. Its ^1H NMR spectrum indicated the presence two methyl singlets and a methyl doublet suggesting a sesquiterpene type of structure. A combination of the DEPT-135 and ^{13}C NMR spectra of **1** revealed this compound to have fifteen carbon atoms, four quaternary, three methines, five methylenes and three methyls, making the number of carbons with attached protons $C_{15}H_{22}$. Low resolution mass spectrometry of this compound gave a molecular ion at 236 *m/z*. The presence of two oxygenated carbons in the ^{13}C NMR at δ 73.6 and δ 90.2 suggested a molecular formula of $C_{15}H_{24}O_2$, which was confirmed by high resolution mass spectroscopy (236.1775; Δ 0.2 mmu of calcd) This formula gave four double bond equivalents, one of which was ascribed to an exo double bond seen in the ^{13}C spectrum at δ 109.8 (s) and δ 162.4 (t), this left three rings in the compound. The formula and substructure information was then entered into the Marinlit database[7] and

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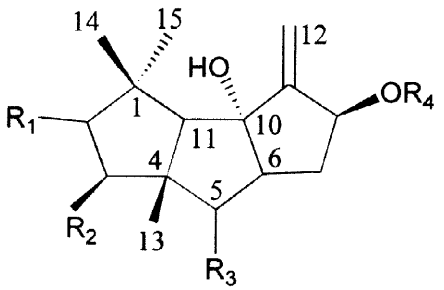
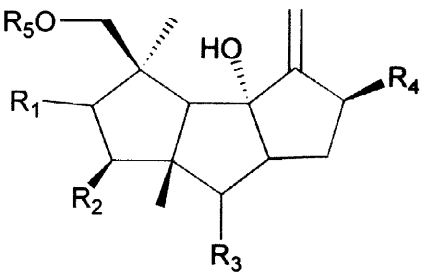
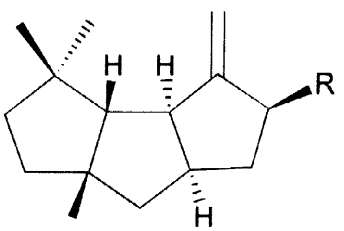
only one matching structure found, capnellene-8β,10α-diol **1**. The stereochemistry of **1** is the same as that reported by Sheikh *et al*[1] as the ^{13}C chemical shifts match closely (Table 1).



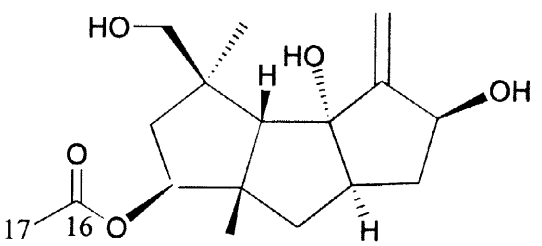


[**1**] R_1= R_2= R_3=R_4= H ref.1
[**2**] R_1= R_3=R_4= H R_2= OH ref.1
[**3**] R_1= R_2= R_4= H R_3= OH ref.1
[**4**] R_2= R_3= R_4= H R_1= OH ref.1
[**5**] R_2= R_4= H R_1= R_3= OH ref.2

[**6**] R_1= R_2= R_3= R_5= H R_4= OH ref.3
[**7**] R_1= R_3= R_5= H R_2= R_4= OH ref.4
[**8**] R_1= R_2= R_5=H R_3= OH R_4= OAc ref.4



[**9**] R=OH
[**11**] R=H ref. 6



10

Capnellene-8β-ol **9** was obtained as 30 mg of an amorphous yellow solid after normal phase HPLC. The ^1H NMR spectrum of **9** indicated that the structure of **9** was similar to that of **1**, with an exo double bond evident as well as two methyl singlets and one methyl doublet. Information from the ^{13}C and DEPT-135 NMR spectra revealed fifteen carbon atoms, three quaternary, four methines, five methylenes and three methyls, thus giving an attached proton formula of $C_{15}H_{23}$. Low resolution mass spectrometry of this compound gave a molecular ion at 220 *m/z*, and coupled with one oxygenated carbon at δ 75.6 in the ^{13}C NMR spectrum led to a proposed formula of $C_{15}H_{24}O$, which was confirmed by high resolution mass spectrometry (220.1827; Δ 0.0 mmu of calcd). This formula gave four double bond equivalents, characteristic of the capnellenes, with three rings and an exo double bond. The presence of an exo double bond was further confirmed by the carbon atoms with chemical shifts of δ 105.4 t and δ 160.6 s. The similarity of chemical shifts between this compound and compound **1** suggested a singly hydroxylated capnellene. Further evidence for this was the splitting of the signal for exo double bond protons in the ^1H NMR spectrum compared with that seen in compound **1**. As no other singly substituted hydroxy capnellenes have been isolated, ^1H-^1H COSY and HMBC correlations were used to position all the carbon atoms in the ring system (Table 1). Using the exo double bond protons as anchor point, on the capnellene framework, the HMBC correlation from δ_C 75.6 (d) to the exo double bond protons placed this CHOH at C8. Similarly, the ring junction CH10 was ascribed to δ_C 49.5 (d) by its HMBC correlation to the exo double bond protons. Having delineated this part of the structure, the process was continued by placing the CH_2 at δ_H 2.13 at $CH_2$7 using its HMBC correlation to C9. The other ring junction protons were placed using HMBC correlations C6-H7 and C9-H11. A listing of all the discernible ^1H-^1H COSY and HMBC correlations is given in Table 1. Capnellane **9** has the same relative stereochemistry as **1**, as 14 of the 15 ^{13}C NMR shifts are comparable (Table 1). The chemical shift for C10 has changed from 90.2 ppm in **1** to 49.5 ppm in **9**, and this is

Table 1. NMR data for compounds 1, 10 at 400/100 MHz and for 9 at 250/62.5 MHz in $CDCl_3$

	1	1	9	9	9	9	10	10	10	10
	$\delta^{13}C$ (mult) Obs	$\delta^{13}C$ (mult) Lit[1]	$\delta^{13}C$ (mult)	$\delta^{1}H$ (mult, *J* Hz)	^{1}H-^{1}H COSY correlations	$^{13}C \rightarrow {}^{1}H$ HMBC correlations	$\delta^{13}C$ (mult)	$\delta^{1}H$ (mult, *J* Hz)	^{1}H-^{1}H COSY correlations	$^{13}C \rightarrow {}^{1}H$ HMBC correlations
C1	44.0 s	43.3 s	42.5 s	-		H14, H15	43.4 s	-		H11, H14/14', H15
C2	43.2 t	42.7 t	41.5 t	1.51 m			43.8 t	1.68 dd 11.2, 5.6 1.55 t 11.2	H3, H15	
C3	42.0 t	41.4 t	40.5 t	2.09 m 1.45 m		H2, H13	82.1 d	5.08 dd 10.6, 5.6	H2/2', H3	H13
C4	49.9 s	49.3 s	53.3 s	-		H3, H11, H13	51.4 s	-		H11, H13
C5	46.2 t	45.6 t	48.9 t	1.79 dd 13.8, 8.4 1.51 dd 13.8, 4.8	H6	H13	45.6 t	2.25 m 1.20 m	H6, H7	H3, H11, H13
C6	49.6 d	48.7 t	42.1 d	2.22 ddt 7.6, 3.5, 4.2	H5, H7, H10	H5, H7	49.2 d	2.52 m	H5/5', H7	H11
C7	38.1 t	36.8 t	40.3 t	2.13 * dd 8.4, 4.2 1.39 m	H6, H8		38.3 t	2.35 m 1.42 dt 14.4, 4.0	H5, H6, H8	
C8	73.6 d	73.6 d	75.6 d	4.74 tt 8.0, 4.2	H7/7', H12/12'	H12/12'	73.5 t	4.75 m	H7', 12	H7, H12
C9	162.4 s	160.3 s	160.6 s	-		H7, H11	161.8 s	-		H7, H11, H12
C10	90.2 s	89.8 s	49.5 d	2.36 ddd 4.6, 2.8, 1.6	H6, H11, H12/12'	H5, H12/12'	87.9 s	-		H11, H12
C11	65.7 d	64.6 d	68.0 d	1.75 d 3.3	H10, H15	H13, H14, H15	64.9 d	2.30 s	H3, H12, H14/14'	H13, H14/14', H15
C12	109.8 t	107.5 t	105.4 t	5.05 t 2.5 4.96 t 2.4	H8, H10		109.7 t	5.30 t 2.5	H8, H11	
C13	32.8 q	31.5 q	32.1 q	1.24 s		H3	25.3 q	0.82 s		H3, H11
C14	31.4q	30.3 q	30.8 q	1.02 s		H15	74.1 t	3.55 d 9.4 3.42 d 9.4	H15	H11, H15
C15	24.1 q	23.2 q	26.1 q	0.82 s	H11	H14	20.8 q	1.31 s	H2', H14	H11, H14/14'
C16							171.1 s	-		H3, H17
C17							21.0 q	1.99 s		

* Not all *J*s could be determined due to severe overlap.

consistent with the expected α shift of +38 ± 4 ppm for such a modification.[8] This capnellene is closely related to the biogenetic precursor of this class of compounds, capnellene (**11**).[6]

14-Acetoxycapnellene-3β,8β,10α-triol **10** was obtained as 20 mg of a yellow oil after reverse phase HPLC. The NMR spectra of this compound was similar to those of the **1** and **9** indicating it had the same carbon skeleton, but had a further two carbon atoms, one quaternary and one primary giving an attached proton formula of $C_{17}H_{23}$. Low resolution mass spectrometry gave a molecular ion at 310 *m/z*. The ^{1}H NMR spectrum showed the presence of an acetate methyl at δ 1.99, and the presence of an acetate moiety was confirmed using the ^{13}C NMR spectrum. In addition to this the ^{13}C NMR spectrum showed the presence of four oxygenated carbons, suggesting a formula of $C_{17}H_{26}O_5$, which was confirmed by high resolution chemical ionisation mass spectrometry (328.2125; Δ 0.1 mmu $[M + NH_4]^+$). This information was entered onto the Marinlit database but no match was found, so the database was used to obtain all references to acetoxycapnellenes previously isolated. From the literature it was seen that various acetoxycapnellenes have been isolated, or synthesised by acetylation of other capnellenes, but the chemical shifts recorded for these did not match with this compound. It was therefore proposed that a novel acetoxycapnellene had been isolated. By further comparison with known compounds, the closest match was found to be capnellene-3β,8β,10α,14-tetrol **7**.[4] It was therefore proposed that this compound was an analogue of the tetrol with the OH group at C3 replaced by OAc. The positioning of the acetoxy group was performed by using the HMBC correlation from the acetate carbonyl C16 to the CHOH at δ_H 5.08. This CHOH was placed at CH3 by the HMBC correlation from C3 to Me13 and ^{1}H-^{1}H COSY correlations from Me15 to H2 and H2 to H3. The remaining ^{1}H-^{1}H COSY and HMBC correlations confirming the structure of **10** are given in Table 1. In order to determine the relative stereochemistry of capnellene-8β-ol **9**, a NOESY spectrum was acquired with a mixing time of 0.6 s. The data is summarised in Figure 1, and it can be seen that there are *cis* ring junctions at C4-C7 and C6-C10. The pivotal nOe's are from H11 to H7, H12, Me13 and CH14, confirming that all of these are on the same face of the molecule. In addition, further correlations from H6-H5, H5-Me15, Me15-H3 and H3-H2 indicate that all these must be on the opposite face of the molecule to H11. This stereochemistry is identical to that found previously.[4]

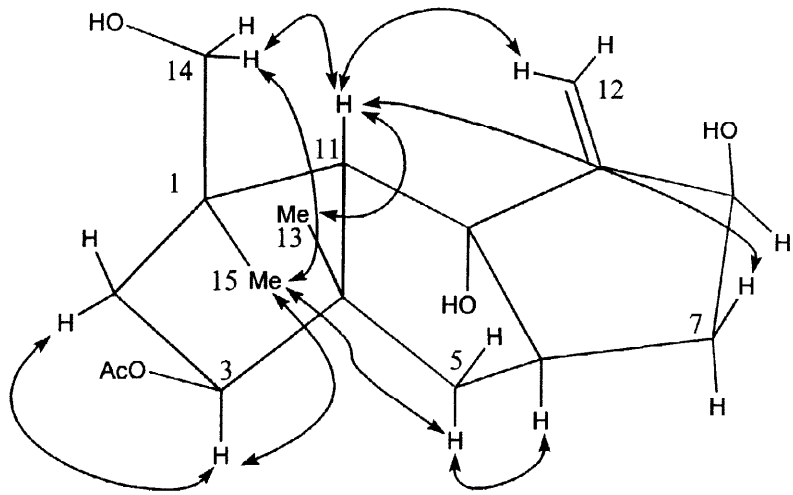


Figure 1. NOESY data for compound **10**.

BIOACTIVITY DATA

The compounds were tested in Aberdeen against human leukaemia, renal leiomyoblastoma, colon and breast cancer cell lines. All cells were exposed to the compounds for 24 h and cytotoxicity was measured by the MTT assay.[9] IC_{50} values were calculated from a minimum of 3 separate experiments with 6 replicates per experiment. Toxicity was compared with the known cytotoxic agent, cisplatin. Additional data for these compounds was obtained by workers at the Paterson Institute for Cancer Research in Manchester.

Compound **1** was cytotoxic in all cell lines tested with IC_{50} values in the range 0.7-93 μM (Table 2), with the greatest activity being displayed against K562 leukaemia. Compound **10,** which has an additional 3β-acetoxy group and 14-hydroxyl was also cytotoxic and it showed some selectivity for the renal leiomyoblastoma and ovarian cancer cell lines (Table 2). Compound **9** which lacks the 10α hydroxyl group of **1** was effective against the promyelogenous leukaemia cell line, HL-60 as well as K562 leukaemia.

Table 2. Cytotoxicity of capnellenes in human cancer cells (IC_{50}, μM)

Compound	Leukaemia		Renal Leiomyo-blastoma	Breast	Colon	Ovarian
	HL-60	K562	G402	MCF-7	HT115	A2780
1	51	0.7	42-51	93	63	9.7
9	68	4.6	>4500	>4500	>4500	6.6
10	713	24	52	1029	n.d.	32

EXPERIMENTAL SECTION

General Mass spectra were obtained on a Jeol JMS 700. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity INOVA 400 spectrometer at 400 MHz and 100 MHz respectively or on a Bruker AC 250 at 250/62.5 MHz in $CDCl_3$ solution. HPLC separations were carried out using a Spectra-Physics Spectrasystem P4000 pump, an Alltech Econosphere ODS (reverse phase) or SiO_2 (normal phase) 10μm particle/100Å pore size column and monitored using a Hewlett-Packard Series 1050 tunable single wavelength UV detector .

Collection and Identification. The sample of *Capnella imbricata* (Order Alcyonacea, Family Nephtheidae, coll no. 96309) was collected in November 1996 at a depth of 15 m from a sloping reef, Mayu Island, Molluca Sea, Indonesia (1°20.854' N; 126°23.482' E). A voucher specimen is preserved at the Marine Natural Products Laboratory, Department of Chemistry, University of Aberdeen, (voucher number 96309) and at the Nationaal Natuurhistorisch Museum in Leiden, The Netherlands (Voucher number RMNH Coel. 23950). The identification was carried out by Leen P. van Ofwegen at the Nationaal Natuurhistorisch Museum in Leiden, The Netherlands. The identification is based on the work of Quoy and Garmand.[10] A brief taxonomic identification is as follows: Stalked colony with polyps situated on small terminal twigs ('catkins'). Catkins are rounded, about 10 mm long. Polyp sclerites are leaf clubs with distinct leaves. Surface layer of coenenchyme with leaf capstans of about 0.11 mm high, with a bud-like head of leaves. The species is rather common in the Indo-Pacific.[11]

Extraction and isolation The sample was preserved by immersing in a 1:1 EtOH: seawater mixture. After 24 h the mixture was decanted and discarded, after which the organism was transported back to Aberdeen at ambient temperature. The organism was extracted with MeOH for 24 h (3×) and CH_2Cl_2 (3×) and the concentrated extracts combined. The crude oil was partitioned between water and CH_2Cl_2, after which the CH_2Cl_2 layer was stripped of solvent and the resulting oil partitioned between *n*-hexane and 10% aqueous MeOH. The MeOH layer was then phase adjusted to 50% aqueous MeOH and extracted with CH_2Cl_2. The CH_2Cl_2 fraction was then subjected to Sephadex LH20 size exclusion chromatography (1 : 1 CH_2Cl_2 : MeOH), the last fraction obtained from this then underwent reversed phase ODS HPLC (10% aqueous MeOH) to give 20 mg and 60 mg of the pure compounds **1** and **10** respectively. The hexane fraction obtained by solvent partitioning of the crude oil also underwent size exclusion chromatography and the last fraction obtained then underwent normal phase HPLC (1 : 9 EtOAc : Hex) to give 30 mg of pure compound **9**.

Capnellene-8β,10α-diol (**1**) Colourless crystals, $[\alpha]_D$ 90.9 (c 0.24, $CHCl_3$); IR ν (cm^{-1}) 3400 (s), 2930 (s), 2864 (s), 1709 (m), 1450 (m), 1170 (m); HREIMS 236.1775 Δ 0.2 mmu of calcd for $C_{15}H_{24}O_2$; LREIMS *m/z* (%) 236

(M^+, 3), 203 (25), 185 (7), 166 (12), 149 (8), 147 (6), 126 (50), 112 (92), 109 (100), 95 (30), 70 (36), 55 (21); For NMR data see Table 1.

Capnellene-8β-ol (**9**) Amorphous yellow solid, $[\alpha]_D$ 19.5 (c 0.20, $CHCl_3$); IR ν (cm^{-1}) 3441 (w), 2929 (s), 2864 (s), 1709 (s), 1459 (m), 1376 (m), 1170 (m), 889 (w); HREIMS 220.1827 Δ 0.0 mmu of calcd for $C_{15}H_{24}O$; LREIMS *m/z* (%), 220 (M^+, 26), 205 (12), 202 (6), 187 (14), 177 (3), 162 (25), 151 (32), 149 (15), 133 (8), 131 (11), 123 (17), 121 (12), 117 (10), 109 (100), 95 (35), 81 (58), 79 (22), 69 (18), 67 (18); For NMR data see Table 1.

3β-Acetoxycapnellene-8β,10α,14β-triol (**10**) Yellow oil, $[\alpha]_D$ 39.7 (c 0.10, $CHCl_3$); IR ν (cm^{-1}) 3383 (s), 2956 (s), 2925 (s), 2870 (s), 1734 (s), 1717 (s), 1458 (m), 1375 (m), 1247 (m), 1030 (m); HRCIMS (NH_3) 328.2125 Δ 0.1 mmu of calcd for $C_{17}H_{30}O_5N$ $[M + NH_4]^+$; LRCIMS (NH_3) *m/z* (%) 328 ($[M + NH_4]^+$, 78), 310 (100), 292 (81), 275 (24), 250 (36), 233 (15), 215 (12); For NMR data see Table 1.

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