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Tetraprenyltoluquinols from the brown alga *Cystophora fibrosa*

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Abstract

Six cyclised tetraprenyltoluquinols and five stereoisomers with the previously reported amentol skeleton have been isolated from the lipophilic extract of the South African brown alga *Cystophora fibrosa*. Structures and relative stereochemistry were determined using spectrometric techniques, particularly 1D and 2D NMR, and molecular modelling experiments. The compounds isolated appear to be enantiomeric to compounds with the same skeleton isolated from brown algae of the genus *Cystoseira* collected in northern Africa and the Mediterranean Sea. The isolation of tetraprenyltoluquinols with the amentol skeleton from this alga suggests that *C. fibrosa* should be moved from the genus *Cystophora* into the *Cystoseira*.
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1. Introduction

As part of an ongoing study of metabolites from brown algae of the genus *Cystophora* and their use as potential intra-generic taxonomic markers, a collection of *Cystophora fibrosa* (Simons) was made in January 2001. *C. fibrosa* (*Cystoseiracea*, Fucales, Phaeophyceae) occupies a unique position in the genus as it is the only species of the 26 described to date that occurs outside the cold temperate waters of Australasia (Womersley, 1987). It has only been recorded from Cape Province in South Africa where two disjunct populations, separated by over 100 km, are known (Simons, 1970; Stegenga et al., 1997).

Geographic overlap of South African and Australian flora at the generic level is relatively common, especially in terrestrial systems. However, it is unusual that only a single representative of the *Cystophora* genus has been described from South African waters. As a consequence the taxonomic status of *C. fibrosa* is still cause for debate and Australian phycologists believe that *C. fibrosa* should be classified in the closely related genus *Cystoseira* (Womersley, 1987).

As opposed to the restricted geographical range of *Cystophora*, *Cystoseira* has a worldwide distribution with a major radiation in the Mediterranean Sea and two species have been recorded from South African waters (Silva et al., 1996). A large number of structurally diverse terpenoid secondary metabolites have been isolated from *Cystoseira* species and have been used as taxonomic markers. In particular, linear and cyclic meroditerpenoids have proven to be very effective tools for discriminating between species of *Cystoseira* (Amico, 1995; Amico et al., 1990; Valls and Piovetti, 1995) and have even been utilised to identify natural hybrids (Amico et al., 1988a). In contrast to the complexity of secondary metabolites reported from *Cystoseira*, those produced by species of *Cystophora* are mostly simple isoprenoid derivatives, acetogenins and polyenes, none exhibiting the complex cyclisations found in the more advanced *Cystoseira* metabolites (van Altena, 1988 and references therein). Thus far, no compound common to both *Cystoseira* and *Cystophora* genera has been reported. In light of the significant structural differences reported for *Cystophora* and *Cystoseira* metabolites it was anticipated that investigation of the secondary metabolites present in *C. fibrosa* would provide some insight into its taxonomic position.

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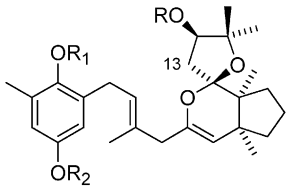
Investigation of the secondary metabolites of *C. fibrosa* resulted in the isolation of compound **1** which has been previously reported from a study of a *Cystoseira* species from Morocco (Valls et al., 1993). However, further exploration of the chemical literature revealed that this report was in error and we present a full structure elucidation of **1**. In addition, we report the isolation of a further five related meroditerpenoid derivatives with planar structures (**4**, **6**–**8**, **10**) and five more with reported planar structures (*epi*-14-**8**, **9**, *epi*-14-**10**, **11**, **13**) but different stereochemistries.



1 R & R_1 = H, R_2 = CH_3

4 R = H, R_1 & R_2 = CH_3

7 C-13,14 = ~CH=CH~, R_1 & R_2 = CH_3

8 R & R_2 = CH_3, R_1 = H

10 R, R_1 & R_2 = CH_3

13 C-13,14 = ~CH=CH~, R_1 = H, R_2 = CH_3

2. Results and discussion

Specimens of *C. fibrosa* were collected from rock pools at Koppie Alleen, De Hoop Nature Reserve, Cape Province, South Africa in January 2001, immediately frozen and then transported to Australia for chemical analysis. The frozen alga was extracted with acetone at room temperature and a 2 g portion of this crude extract was subjected twice to chromatography over Sephadex LH-20, first eluent MeOH–$CHCl_3$ (1:1), second eluent $CHCl_3$–petrol–EtOH (10:10:1). Further separation and purification was carried out using semi-preparative HPLC and resulted in the isolation of 3.5 mg of compound **1** as a pale yellow, optically active oil.

The molecular formula of **1** was determined as $C_{28}H_{40}O_5$ by HREIMS (456.2853 found; 456.2876 calculated) and its IR spectrum indicated the presence of a hydroxy moiety (3464 cm^{-1}). Carbon-13 and DEPT spectroscopy (Tables 1 and 2) showed the presence of ten quaternary (including 6 sp^2 hybridised) carbons, five methine (including 4 sp^2 hybridised), six methylene and seven methyl groups. As the molecular formula indicates the existence of 9 double bond equivalents and 5 are clearly due to double bonds, compound **1** contains 4 rings. There remains a single, significantly downfield, quaternary carbon whose chemical shift (δ_C 113.1) is consistent with an acetal carbon (C-12) (Silverstein and Webster, 1998).

Analysis of the ^1H, gHMQC, gHMBC and DQF-COSY NMR spectra (Fig. 1, Table 1) allowed the majority of the structure of compound **1** to be elucidated. Homo-nuclear correlations were observed for four spin systems and included four long range couplings. Two of these long range correlations permitted the aromatic methyl group (C-6′Me) to be placed adjacent to one of the aromatic methine groups (C-5′) and the remainder of the molecule to be extended from a carbon adjacent to the second aromatic methine (C-3′). The third long range coupling allowed the incorporation of the tri-substituted double bond (C-2,3) into the spin system containing C-3′ and the fourth resulted in the inclusion of the enol-ether moiety (δ_C 145.1, *s*, C-5; δ_C 109.7, *d*, δ_H 4.32, 1H, *s*, C-6), where this spin system terminates.

Correlations in the HMBC spectrum between C-4′ and the two aromatic protons H-3′ and H-5′ as well as an aromatic methoxy group (δ_H 3.71, 3H, *s*; δ_C 55.6, 4′-OMe) (Silverstein and Webster, 1998; Capon et al., 1981), in combination with correlations between a phenolic (δ_H 6.21, 1H, *s*, D_2O exchangeable) carbon (δ_C 146.3, *s*, C-1′) and aromatic methyl (H-6′Me) and methylene (H-1) groups allowed the first two spin systems to be joined via a 1,4-disubstituted benzene ring.

At the enol-ether end of the major spin system, the olefinic carbon at C-6 has a HMBC correlation to the protons of the 3H singlet at δ_H 0.99 that are, in turn, correlated to two quaternary carbons at δ_C 44.5 and 46.8 which, themselves, couple to another singlet methyl group at δ_H 0.90 as well as H-6. Of the two quaternary centres, only the carbon at δ_C 44.5 has a HMBC coupling to the very broad methylene multiplet at δ_H 1.57 (H-8), the carbon of which is correlated to the methyl group at δ_H 0.99, thus fixing the upfield quaternary carbon (δ_C 44.5) at C-7. Therefore, the upfield methyl group at δ_H 0.90 must be attached to the quaternary carbon at δ_C 46.8 and while this group must also be located near C-6 and C-7 it is not next to C-8, leading to their respective assignments as H-18 and C-11. Correlations observed between C-9 and C-8 as well as C-10, and the correlation of C-10 with the methyl group C-18 established a tetra-substituted cyclopentane ring as the second ring of the four required in the structure of compound **1**.

The HMBC spectrum indicates that the acetal carbon (C-12) is interposed between the quaternary carbon C-11 on the cyclopentane ring and the short spin system C-13, -14 in which C-14 is an oxygenated methine group (δ_C 77.7, *d*, δ_H 3.83, 1H, *d* (4.6 Hz)). Correlations between the singlet methyl groups H-16 and -17 and C-14, as well as the quaternary carbon C-15, completes the carbon chain of compound **1** but leaves the structure lacking two rings and carrying five non-terminating oxygen atoms, of which two pairs must be the same atom, attached to C-5, C-12, C-14 and C-15 that can only be satisfied by the presence of two cyclic ethers.

Unfortunately, there is no direct HMBC evidence that links any of the carbons on opposite sides of an ether moiety. As C-12 is an acetal carbon and a D_2O exchangeable

Table 1
NMR data for compound **1**[a,b]

C	^{13}C	^{1}H (*m*, Hz)	DQF-COSY	HMBC
1	29.9	3.18 *dd* (16.1, 4.8)	H-1b, H-2, H-4a, H-20, H-3′	H-2, H-3′
		3.42 *dd* (16.1, 6.8)	H-1a, H-2, H-4a, H-20	–
2	127.8	5.31 *bdd* (5.7, 5.7)	H-1a, H-1b, H-4a, H-4b, H-20	H-1a, H-1b, H-4a, H-4b, H-20
3	133.4	–	–	H-1b, H-4a, H-4b, H-20
4	44.8	2.56 *d* (13.6)	H-2, H-4b, H-1a, H-1b	H-20
		2.72 *d* (13.6)	H-2, H-4a, H-6	–
5	145.1	–	–	H-4a, H-4b, H-6
6	109.7	4.32 *s*	H-4b	H-4b, H-19
7	44.5	–	–	H-6, H-8, H-18, H-19
8	40.2	1.50–1.62 *bm*	–	H-10, H-19
9	20.2	1.45 *bm*	H-10b, H-18	H-8, H-10
10	34.0	1.37 *bm*	H-10b, H-18	H-18
		2.03 *bm*	H-10a, H-9	–
11	46.8	–	–	H-6, H-18, H-19
12	113.1	–	–	H-13a, H-18
13	38.3	1.96 *d* (13.9)	H-13b	–
		2.30 *dd* (13.9, 4.6)	H-13a, H-14	–
14	77.7	3.83 *d* (4.6)[c]	H-13b	H-13a, H-16, H-17
15	86.5	–	–	H-13a, H-16, H-17
16	27.1	1.11 *s*	H-17	H-17
17	23.5	1.27 *s*	H-16	H-16
18	18.9	0.90 *s*	H9, H10a	–
19	23.4	0.99 *s*	–	–
20	15.9	1.70 *s*	H-1a, H-1b, H-2	H-4b, H-2
1′	146.3	–	–	H-1b, H-3′, H-5′ 6′-Me, 1′-OH
2′	128.8	–	–	H-1b
3′	112.8	6.50 *d* (2.9)	H-1a	H-1b, H-5′
4′	153.2	–	–	H-3′, H-5′, 4′-OMe
5′	114.2	6.54 *d* (2.9)	6′-Me	H-3′, 6′-Me
6′	127.8	–	–	6′-Me
4′-OMe	55.6	3.71 *s*	–	–
6′-Me	16.8	2.20 *s*	H-5′	H-5′
14-OH[d]		3.81 *s*	–	–
1′-OH[d]		6.21 *s*	–	–

[a] Recorded at 300.13 MHz (^{1}H) and 75.47 MHz (^{13}C) in $CDCl_3$ which was also used as the reference for chemical shift (^{1}H: 7.24, ^{13}C: 77.0 ppm).
[b] For the diastereotopic methylene hydrogen signals the upfield signal is labelled 'a' and downfield 'b'.
[c] The individual signals of this doublet have $W_{1/2}$ ca. 2 Hz.
[d] D_2O exchangeable.

singlet is observed at δ_H 3.81 in the ^{1}H NMR spectrum, a structure with a hemi-acetal at C-12 and an epoxide linking C-14 and C-15, is a realistic possibility. Such a compound has been previously isolated (Amico et al., 1986) and the reported chemical shifts for C-14 and C-15 (δ_C 60.5 and 57.9), which are typical for an epoxide, differ markedly from those assigned for the current compound (δ_C 77.7 and 86.5, respectively) clearly indicating that **1** does not contain an epoxide moiety. Moreover, the chemical shift for a hemiacetal at C-12 is δ 102.5 (Amico et al., 1986), compared to δ 113.1 in the current compound, further suggesting that C-12 is not a hemi-acetal. Since neither C-12 nor C-5 can be hydroxylated they must participate in the third ring, placing the hydroxyl group at either C-14 or C-15.

Since the ^{13}C chemical shift for similar tertiary alcohols is typically in the range δ_C 70–74 (Amico et al., 1987a; Ayyad et al., 2003; Harding et al., 1995; Hashem et al., 1985; Verotta et al., 1998), whereas the chemical shift for C-15 is 86.5 ppm, it follows that the final ether bridge is between C-15 and C-12. This is consistent with the observation that compounds containing a 3-hydroxy-2,2-dimethyl-tetrahydrofuran moiety have ^{13}C chemical shifts for the hydroxy and dimethyl-substituted carbons which are typically ca. δ_C 78 and 84, respectively (Ahmad et al., 1998; Chang et al., 1989; Arantes and Hanson, 1999; Ujita et al., 1992).

The *E* stereochemistry for the acyclic double bond was assigned on the basis of the upfield chemical shift for C-20 (δ_C 15.9) (Coates et al., 1978). Finally, the stereochemistry of the spiroketal portion of **1** was assigned based upon a number of NOE difference experiments (Fig. 2). NOE interactions between H-18 and H-13b, and H-19 and H-13a places the two bridgehead methyl groups on the same face of the oxolane ring as the oxane methylene group (C-13) resulting in the stereochemistry being assigned as 7*R**, 11*R** and 12*R**. The series of NOE interactions between H-16 and H-13b, H-14 and H-13b, H-13b and H-18, and H-18 and H-10b intimates that all these hydrogens are on the same face of the molecule. These interactions, combined with observed NOEs between 14-OH and H-13a and 14-OH and H-17 led to the stereochemistry of C-14 being assigned as *S**. However, other NOEs were observed between H-14 and H-13a and H-16 and 14-OH, not entirely unexpectedly

Table 2
^{13}C NMR shifts of amentol derivatives in $CDCl_3$

C	1	2[a]	3[a]	4	6	7	8	10	11	13
1	29.9 *t*	27.9 *t*	30.1 *t*	28.6 *t*	27.3 *t*	28.3 *t*	31.2 *t*	28.4 *t*	22.8 *t* (22.6)[d]	30.9 *t*
2	127.8 *d*	125.0 *d*	123.9 *d*	126.8 *d*	119.8 *d*	124.6 *d*	123.8 *d*	124.8 *d*	31.4 *t* (31.6)[d]	123.6 *d*
3	133.4 *s*	135.9 *d*	135.6 *s*	132.7 *s*	137.1 *s*	133.5 *s*	136.2 *s*	133.2 *s*	75.7 *s*	136.0 *s*
4	44.8 *t*	45.2 *t*	45.3 *t*	44.8 *t*	44.9 *t*	44.9 *t*	45.3 *t*	44.9 *t*	44.1 *t* (44.4)[d]	45.1 *t*
5	145.1 *s*	145.9 *s*	145.1 *s*	145.6 *s*	145.1 *s*	146.8 *s*	146.2 *s*	147.4 *s*	145.3 *s*	146.2 *s*
6	109.7 *d*	109.1 *d*	109.5 *d*	109.2 *d*	109.3 *d*	109.0 *d*	109.7 *d*	108.9 *d*	111.1 *d*	109.4 *d*
7	44.5 *s*	43.3 *s*	43.3 *s*	44.7 *s*[b]	43.1 *s*[b]	43.1 *s*[b]	43.1 *s*[b]	43.2 *s*[b]	43.0 *s*[b]	43.2 *s*[b]
8	40.2 *t*	40.5 *t*	40.4 *t*	40.3 *t*	40.3 *t*	40.5 *t*	40.3 *t*	40.4 *t*	40.6 *t*	40.5 *t*
9	20.2 *t*	20.4 *t*	20.4 *t*	20.3 *t*	20.1 *t*	20.4 *t*	20.5 *t*	20.4 *t*	20.5 *t*	20.2 *t*
10	34.0 *t*	36.2 *t*	36.2 *t*	34.1 *t*	36.0 *t*	36.2 *t*	36.1 *t*	36.2 *t*	36.2 *t*	36.1 *t*
11	46.8 *s*	46.3 *s*	46.3 *s*	46.8 *s*[b]	46.0 *s*[b]	46.2 *s*[b]	47.3 *s*[b]	46.0 *s*[b]	46.0 *s*[b]	46.2 *s*[b]
12	113.1 *s*	108.9 *s*	109.1 *s*	113.0 *s*	109.0 *s*	115.0 *s*	109.2 *s*	113.3 *s*	114.6 *s*	115.0 *s*
13	38.3 *t*	41.7 *t*	41.7 *t*	38.9 *t*	38.3 *t*	126.6 *d*	38.6 *t*	38.8 *t*	126.5 *d*	126.5 *d*
14	77.7 *d*	77.4 *d*	77.3 *d*	77.6 *d*	86.2 *d*	140.1 *d*	86.3 *d*	86.4 *d*	140.0 *d*	140.0 *d*
15	86.5 *s*	83.1 *s*	83.4 *s*	86.4 *s*	82.9 *s*	87.9 *s*	83.0 *s*	82.8 *s*	88.1 *s*	88.0 *s*
16	27.1 *q*	28.2 *q*	28.2 *q*	26.4 *q*	29.5 *q*	28.7 *q*	29.7 *q*	29.5 *q*	26.4 *q* (26.5)[c,d]	28.8 *q*[c]
17	23.5 *q*	22.2 *q*	22.3 *q*	23.7 *q*	22.5 *q*	26.3 *q*	22.5 *q*	22.6 *q*	28.8 *q* (28.9)[c,d]	26.3 *q*[c]
18	18.9 *q*	19.3 *q*	19.5 *q*	19.0 *q*	19.2 *q*	20.1 *q*	19.5 *q*	19.3 *q*	20.3 *q* (20.6)[d]	20.2 *q*
19	23.4 *q*	23.0 *q*	22.9 *q*	23.4 *q*	22.6 *q*	22.8 *q*	22.4 *q*	22.9 *q*	22.8 *q*	22.7 *q*
20	15.9 *q*	15.4 *q*	15.4 *q*	15.6 *q*	15.2 *q*	15.7 *q*	15.5 *q*	15.7 *q*	25.0 *q*	15.7 *q*
1′	146.3 *s*	150.6 *s*	146.4 *s*	150.4 *s*	188 *s*	150.4 *s*	147.0 *s*	150.2 *s*	145.8 *s*	147.0 *s*
2′	128.8 *s*	132.1 *s*	127.8 *s*	134.9 *s*	148.4 *s*	135.3 *s*	127.0 *s*	135.7 *s*	120.4 *s*	127.4 *s*
3′	112.8 *d*	114.3 *d*	114.2 *d*	112.7 *d*	132.1 *d*	112.7 *d*	113.0 *d*	113.0 *d*	110.9 *d*	112.9 *d*
4′	153.2 *s*	151.8 *s*	148.9 *s*	155.5 *s*	188 *s*	155.0 *s*	153.2 *s*	155.1 *s*	151.8 *s*	153.0 *s*
5′	114.2 *d*	115.6 *d*	115.6 *d*	113.9 *d*	133.1 *d*	113.6 *d*	114.0 *d*	113.6 *d*	114.0 *d*	114.1 *d*
6′	127.8 *s*	133.9 *s*	125.8 *s*	131.8 *s*	145.8 *s*	131.6 *s*	125.9 *s*	131.6 *s*	127.4 *s*	126.1 *s*
6′-Me	16.8 *q*	16.2 *q*	16.1 *q*	16.4 *q*	15.8 *q*	16.4 *q*	16.6 *q*	16.4 *q*	16.3 *q*	16.3 *q*
14-OMe	–	–	–	–	57.7 *q*	–	57.9 *q*	57.9 *q*	–	–
1′-OMe	–	60.5 *q*	–	60.6 *q*	–	60.5 *q*	–	60.5 *q*	–	–
4′-OMe	55.6 *q*	–	–	55.4 *q*	–	55.4 *q*	55.6 *q*	55.3 *q*	55.6 *q*	55.6 *q*

[a] 62.5 MHz, chemical shifts are quoted relative to TMS (Amico et al., 1986).
[b,c] Shifts with the same letter may be interchanged within a column.
[d] Chemical shift of minor stereoisomer in brackets.

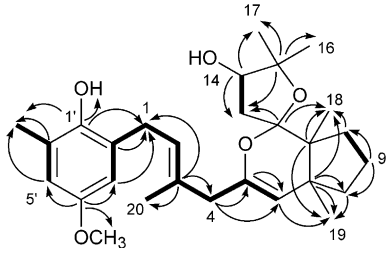


Fig. 1. Compound **1** showing COSY (bold bonds) and HMBC correlations (arrows).

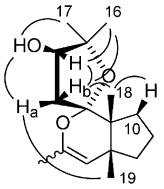
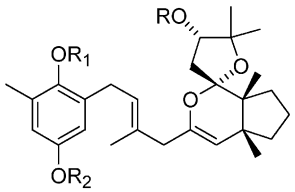


Fig. 2. Selected NOE interactions for the spiroketal portion of compound **1**.

considering the flexibility of 5-membered rings, and it is, in effect, only the *absence* of certain NOE interactions which favours the assignment of the S^* configuration at C-14 over R^*. Therefore, the relative stereochemistry of compound **1** was tentatively assigned as shown.

The recent publication of the structure elucidation of a triacetylated C-14 epimer (**5**) of amentol (**3**) (Amico et al., 1986), by Navarro and co-workers (Navarro et al., 2004) indicated to us that the magnitudes of the proton-proton coupling constants between C-13 and C-14 may be a way of securing the relative stereochemistry at C-14. Of particular note is the small size (<2 Hz), or absence, of a coupling constant between H-14 and H-13a in our compound (**1**) compared to a value of 7.5 Hz for amentol. The significant difference between these two values suggested that the stereochemistry at C-14 could be assigned if each epimer can be shown to have a sufficient bias towards a particular conformer.



2 R & R_2 = H, R_1 = CH_3

3 R, R_1 & R_2 = H (amentol)

5 R, R_1 & R_2 = $COCH_3$

Table 3
Most stable amentol derivative conformations[a] with their estimated $J_{13,14}$ values

Compound	Most stable conformer		Alternate conformer		ΔE (kJ/mol) between conformers	Weighted average J (Hz)
	ϕ (H-13,H-14)[b]	J (Hz)[c]	ϕ (H-13,H-14)[b]	J (Hz)[c]		
Amentol (**3**)	81°/−40°	1.6/4.7	150°/34°	8.2/8.0	20.8	1.6/4.7
1′-Methoxy (**2**)	83°/−37°	1.4/5.1	150°/34°	8.2/8.0	10.9	1.5/5.2
4′-Methoxy (**1**)	81°/−40°	1.6/4.7	154°/38°	8.8/7.4	30.4	1.6/4.7
1′,4′-Dimethoxy (**4**)	81°/−40°	1.6/4.7	154°/38°	8.8/7.4	12.0	1.6/4.7
Triacetoxy (**5**)	82°/−38°	1.5/5.0	156°/39°	9.2/7.3	2.8	3.4/5.6
4′,14-Dimethoxy (**8**)	150°/34°	8.2/8.0	86°/−35°	1.3/5.5	8.4	8.0/7.9
Trimethoxy (**10**)	156°/39°	9.2/7.3	86°/−35°	1.3/5.5	9.4	9.0/7.3
C-14 epimers						
Amentol	−44°/−163°	6.6/10.1	26°/−92°	6.9/1.2	33.0	6.6/10.1
1′-Methoxy	−44°/−163°	6.6/10.1	27°/−91°	6.7/1.2	22.0	6.6/10.1
4′-Methoxy	−45°/−163°	6.4/10.1	23°/−95°	7.3/1.2	30.3	6.4/10.1
1′,4′-Dimethoxy	−43°/−162°	6.7/10.0	25°/−93°	7.0/1.2	25.3	6.7/10.0
Triacetoxy	−43°/−161°	6.7/9.9	25°/−92°	7.0/1.2	13.6	6.7/9.8
4′,14-Dimethoxy	−45°/163°	6.4/10.1	22°/−96°	7.4/1.2	18.8	6.4/10.1
Trimethoxy	−44°/−163°	6.6/10.1	24°/−94°	7.1/1.2	16.2	6.6/10.1

[a] Determined using the Spartan '02 modelling package (Wavefunction, Inc., Irvine, CA, USA).
[b] Both dihedral angles between H_2-13 and H-14 given; *pro-R*-H-13,H-14 first, based on structure **3**.
[c] Estimated by the method of Navarro-Vázques et al., 2004.

To that end molecular modelling of 10 structures was undertaken: amentol (**3**), both aromatic mono-methoxy isomers (**1** and **2**), 1′,6′-dimethoxyamentol (**4**) and triacetoxyamentol (**5**), and their C-14 epimers using Spartan '02 (Wavefunction, Inc., Irvine, CA, USA). The initial structures were minimised by molecular mechanics (MMFF) and then the native Monte-Carlo search routine was employed to find the 100 lowest energy conformers for each structure, again using MMFF to determine the energy of each conformer.

Each resulting group of conformers, sorted in order of increasing energy, was inspected for the first occurrence of: (1) a H-13,H-14 dihedral angle of ca. 90°, as inferred from the near zero coupling constant in **1**, and (2) a conformation in which there are no H-13,H-14 dihedral angles near 90°. These conformations are given in Table 3 with their energy difference and estimated values for the coupling constants associated with the H-13,H-14 dihedral angles as determined by the method of Navarro-Vázques and co-workers (Navarro-Vázques et al., 2004).

As a final check, the energies of the two lowest energy conformations with different H-14,H-13 dihedral angles were recalculated for equilibrium geometry using MMFF as well as a restricted Hartree–Fock SCF procedure using the 6-31G* basis set. The outcome did not change substantially except for compound (**5**) where the lowest energy conformer and its alternate were transposed in the SCF calculation.

Inspection of the pairs of conformers generated in this manner indicated that they all had the same disposition of, and adjacent to, the fused 5,6-membered rings including the C-18 and C-19 methyl groups. In each case the lowest energy conformer is the one in which C-14 is puckered away from C-18, thus creating the dihedral angles found in the lowest energy conformations of both epimers. In the alternate conformer C-14 is puckered towards C-18. It is most likely that steric interactions between the protons on C-13 and the two bridgehead methyl groups and the relief of torsional strain around the penta-substituted furan ring are the origin of this conformational preference. There is no evidence that hydrogen bonding, either between C-14 OH and C-5 O or C-1′/4′ and C-14 OH, plays a role in determining the preferred conformation, although it may further stabilise the preferred conformation in the instances where it is possible.

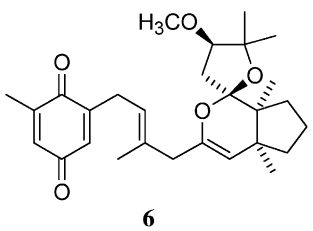
These modelling experiments indicate that the relative stereochemistry we suggest for **1** is correct as shown.[1] However, it now calls into question the relative stereochemistry assigned to amentol (**3**) and compound **2** in the original paper (Amico et al., 1986) and is further complicated by the assignment of both C-14 epimers of triacetoxyamentol (**5**) from the same alga by Navarro et al. (2004), based on the reported structure of amentol. The modelling experiments suggest that the configuration at C-14 of amentol should be reversed but the reported NOE data (Amico et al., 1986) does not: an X-ray study of a suitable derivative may be the only way of resolving this apparent conflict.

Compound **4** was isolated as an optically active pale yellow oil, $[\alpha]_D^{23}$ −58.6°, after normal phase HPLC of a slightly less polar fraction from the same Sephadex LH-20 separation that afforded compound **1**. Its IR spectrum showed the presence of a hydroxy group (3521 cm^{-1}) and HREIMS established its formula as $C_{29}H_{42}O_5$ (found 470.3026, calc. 470.3032), larger than compound **1** by the equivalent of a CH_2 group. Comparison of the chemical shifts of the sig-

[1] Compound **1** appears within the Scifinder Scholar database (American Chemical Society, 2003) and is attributed to Valls et al., 1993. However, this paper describes the structure elucidation of compound **2** and we believe that a typographical error in a figure within the article has led to this compound being mistakenly incorporated into the database. One of the authors, Professor Robert Valls, has confirmed this observation.

nals in the ^{13}C NMR spectrum of compound **4** with those of compound **1** showed that they were virtually identical with the exception of an extra methyl group signal at δ_C 60.6 and some slight downfield shifts of signals due to C-1′ and the carbons *ortho* and *para* to it. These observations suggested that compound **4** is simply the 1′-methoxy derivative of **1** which was confirmed by ^1H NMR (in particular the presence of an additional methyl singlet at δ 3.65) and 2D NMR correlations. Of particular interest was the nature of the ^1H spin system involving H-13 and H-14. Again, only one of the two protons on C-13 (2.01, *d*, $J = 13.9$ Hz and 2.32, *dd*, $J = 13.9$, 5.5 Hz) couple to H-14 which, on the basis of the foregoing modelling, indicates the same stereochemistry at C-14 as present in compound **1**. Remarkably, H-14 appears as a doublet of doublets ($J = 12.6$, 5.5 Hz), coupled to a signal at δ 3.18 (*d*, $J = 12.6$ Hz). When D_2O was added to the NMR sample its ^1H NMR spectrum showed the signal at δ 3.18 reduced to 30% of its original area with an accompanying *increase* in complexity of the signal for H-14 due to the presence of signals due to both the exchanged and unexchanged form of compound **4**. This behaviour of C-14 OH upon D_2O exchange in conjunction with the fact that coupling to the H-14 signal is observed, suggests that it is strongly hydrogen bonded (to C-5 oxygen), decreasing the rate of exchange below the NMR time scale. Inspection of the lowest energy conformer of this compound (Table 3) shows the C-5 and C-14 oxygens within hydrogen bonding distance (2.76 Å) and the dihedral angle between C-14 OH (pointing down toward C-5 oxygen) and H-14 at 180°, consistent with the large coupling constant observed between the two (12.6 Hz).

The chiral centres of compound **4** were assigned as 7*R**, 11*R**, 12*R** and 14*S**, the same as for compound **1**. Amico et al. (1986) have reported ^1H NMR data for a compound with the same relative stereochemistry as compound **4**, synthesised by the methylation of amentol (**3**). Comparison of ^1H NMR spectra finds that the data differs more than one would expect for compounds with identical relative stereochemistries; $\Delta\delta_H$ varies over a 0.5 ppm range. The largest differences appear around H-14 (suggesting again that the two compounds are epimers at C-14) and also at H-2.



6

Compound **6** was isolated after Sephadex LH-20 (light petroleum: $CHCl_3$: ethanol 10:10:1) and gradient HPLC chromatography of an earlier fraction from the initial Sephadex LH-20 column ($CHCl_3$: methanol 1:1) as an optically active yellow oil, $[\alpha]_D^{23}$ −18°, having the molecular formula $C_{28}H_{38}O_5$ (HREIMS 454.2705 found, 454.2719 calc.). The presence of a strong sharp absorption at 1655 cm^{-1} in its IR spectrum and absorbances at 240 (4.56) and 280 (3.42) nm in its UV spectrum suggested the presence of a *p*-quinone moiety in compound **6** (Capon et al., 1981; Valls et al., 1996). ^{13}C NMR spectroscopy showed signals for 27 carbons, the two quinone carbonyl carbons being coincident. HMBC spectroscopy showed that the carbonyl signal is correlated with a two proton singlet at δ_H 6.52 which is correlated with two carbon signals (δ_C 132.1 and 133.1) by HMQC. The ^{13}C signals at 132.1 and 133.1 ppm are further correlated with methylene (δ_H 3.12, 2H) and methyl (δ_H 2.04) groups, respectively, in the HMBC spectrum. Quaternary carbons at δ_C 148.4 and 145.8 are also correlated with these two proton signals providing all the atoms necessary for a 2,6-disubstituted *p*-quinone moiety, the same substitution pattern as present in compounds **1** and **4**. The fact that the bulk of the 1 and 2D NMR data gathered for compound **6** is very similar to **1** and **4** supports the proposition that **6** is an oxidised form of the amentol family of compounds. The additional carbon present in compound **6** compared to amentol (**3**) is due to the presence of a methoxyl group (δ_H 3.32; δ_C 57.7) that is correlated with C-14 in the HMBC spectrum, allowing the assignment of the complete planar structure of **6** as shown. Compound **6** decomposed before NOE experiments could be carried out so our assignment of the relative stereochemistry of **6** (7*R**, 11*R**, 12*R** and 14*S**) as identical to that of compound **1** is tentative. It is possible that **6** is an artefact of the isolation procedure, derived from the parent hydroquinone as such compounds are known to oxidise readily upon exposure to the atmosphere (Capon et al., 1981). The equivalent of the methanol elimination product of compound **6** (cystoquinone) has been reported from an extract from the brown alga *Cystoseira amentacea* collected on the western coast of the French Riviera (Valls et al., 1996).

Compound **7**, an optically active pale yellow oil, $[\alpha]_D^{23}$ −10.4°, was isolated from a less polar fraction of the same HPLC separation that afforded compound **1** and possesses the molecular formula $C_{29}H_{40}O_4$ as indicated by HREIMS (452.2924 found, 452.2927 calc.). All 1 and 2D NMR data obtained from compound **7** is consistent with a compound possessing the amentol (**3**) skeleton. However, some differences are apparent. The NMR data show the presence of two aromatic methoxy groups (δ_H 3.65, 3H; δ_C 60.5; δ_H 3.72, 3H; δ_C 55.4; correlated by HMBC spectroscopy with aromatic quaternary carbons at δ_C 150.4 and 155.0, respectively) and an isolated AX alkenyl spin system (δ_H 5.59, 1H, *d*, $J = 5.8$ Hz and δ_H 6.00, 1H, *d*, $J = 5.8$ Hz) located at C-13 and -14, respectively, by HMBC cross-peaks to C-12, and C-12 and -15, respectively. This allowed compound **7** to be assigned as 1′-methoxycystoketal, previously reported as a synthetic methylation product of cystoketal (**13**) (Amico et al., 1986) and possibly an artefact produced by elimination of compounds **4** and/or **8**. Only ^1H NMR data for **13** have been published and is consistent with our assignment of the structure of compound **7**.

Difference NOE spectroscopy places the two angular methyl groups and the disubstituted double bond (C-13, -14) on the same face of the 6,5-fused ring system as would be expected if the relative stereochemistry found in compound **1** is retained in **7**, thus 7*R**, 11*R**, 12*S**.

Gradient HPLC of an earlier fraction of the Sephadex LH-20 partition column chromatography that contained compound **6** resulted in purification of compound **8**. The purified fraction is a pale yellow oil and exhibits a molecular ion at 470.3033 in HREIMS, consistent with the molecular formula $C_{29}H_{42}O_5$ (470.3032 calc.). Cursory inspection of the 1D NMR data obtained from this fraction indicated the presence of two diastereomers as witnessed by 'doubling up' of many of the signals in a ca. 3:2 ratio. Fortuitously, the relative intensities of the cross-peaks in the 2D NMR spectra of this fraction was such that only the cross-peaks for the major isomer appeared in the plotted spectra and thus the structure of the major isomer (**8**) was elucidated and that of the minor isomer inferred from the initial ^{1}H NMR spectrum, in particular.

Once again, similarities in ^{1}H and ^{13}C NMR spectral data suggested that **8** is a derivative of compound **1** and all HMQC and HMBC data are consistent with this proposal. The observation of an additional two methoxy groups compared to the structure of amentol (**3**) indicated that two of the three hydroxyl groups present in amentol are methyl ethers in compound **8**. A signal at δ_H 5.06 in the proton spectrum was found to be D_2O exchangeable and also correlated to an aromatic quaternary carbon at δ_C 147.0 (C-1′) that is, in turn, coupled to an aromatic methyl group at δ_H 2.20 (3H, *s*, 6′-Me) and two mutually coupled aromatic doublets at δ_H 6.51 and 6.56 (1H each, *d*, $J = 3.4$ Hz, H-3′ and H-5′, respectively). This allowed the placement of the single hydroxyl group on C-1′ and, thus, the two methoxy groups at C-4′ and C-14. The ^{1}H and ^{13}C NMR chemical shifts of C-4′OMe (δ_H 3.72; δ_C 55.6) are typical of a methoxyl group at this position (Capon et al., 1981). The chemical shifts of the second methoxy group (δ_H 3.29; δ_C 57.9) are as one would expect for an aliphatic methyl ether at C-14. Complementary HMBC spectroscopy correlations were observed between the carbons and hydrogens at C-14 and C-14OMe, consistent with methylation of the C-14 hydroxyl group of amentol. Compound **8** is therefore a stereoisomer of 4′, 14-dimethoxyamentol.

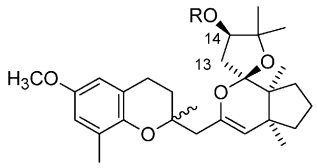
The minor isomer present with compound **8** has a ^{13}C NMR spectrum very similar to the major isomer and the only differences between their ^{1}H NMR spectra are observed around C-14 and these are significant - δ_H major/minor: δ 2.02 (*dd*, $J = 12.7$, 8.0 Hz)/2.18 (*dd*, $J = 13.7$, 9.0 Hz), H-13a; 2.32 (*dd*, $J = 12.7$, 6.9 Hz)/2.46 (*dd*, $J = 13.7$, 8.2 Hz), H-13b; 3.78 (*dd*, $J = 8.0$, 6.9 Hz)/3.42 (*dd*, $J = 9.0$, 8.2 Hz, H-14; 1.25 (*s*)/1.22 (*s*), 1.08 (*s*)/1.17 (*s*), H-16 and H-17). It seemed clear that the two stereoisomers are simply epimers at C-14 and it was anticipated that the stereochemistry at C-14 would be easily assigned based on coupling constant values and the results from the afore mentioned modelling experiments. It was therefore disconcerting to find that neither of the epimers exhibits a near zero coupling constant between H-14 and one of the H-13 protons, as found in compounds **1** and **4** (but not in compound **6** in which C-14 is also methoxylated). Assuming that the relative stereochemistry at C-7, -11 and -12 is identical to that of compound **1**, the two C-14 epimers of compound **8** (as well as the trimethoxy compound **10**) were modelled using Spartan '02 using the same protocol described above. This time none of the lowest energy conformers had H-13, H-14 dihedral angles near 90°, all having calculated coupling constants ca. 8 Hz (Table 3). If, as postulated above, the possibility of intramolecular hydrogen bonding involving substituents at C-14 is only of secondary importance, it appears that steric repulsion between a β-face methoxyl group and the portion of the molecule between C-2 and C-5 leads to the alternate oxane ring conformation being preferred. This conformation has no H-13,H-14 dihedral angle of ca. 90°.

Taking into consideration the results of these modelling experiments and careful analysis of the proton chemical shifts of all hydrogens possibly affected by a change in stereochemistry at C-14 for all relevant compounds isolated in this study and previously, no clear empirical way of distinguishing between the two epimers emerged. However, if one accepts that *C. fibrosa* produces, as the major compounds, a series of metabolites in which the configuration at C-14 is opposite to that isolated from algae collected from North Africa and the Mediterranean, their ^{1}H NMR data become more amenable to rationalisation. Thus it is proposed that the major stereoisomer of compound **8** has the same relative stereochemistry as compound **1** and the minor isomer is epimeric to it at C-14.

Compound **8** appeared to slowly decompose over a number of months as observed by TLC. Purification by HPLC led to the isolation of a very small amount of the decomposition product whose spectral data (HREIMS and ^{1}H NMR) suggested that the phenol had cyclised by addition to the double bond to form the chromane derivative, compound **9**.

Compound **10** was isolated from the Sephadex fraction preceding the one from which **8** was obtained and subsequently purified by HPLC. HREIMS shows an ion at *m*/*z* 484.3185 consistent with the molecular formula $C_{30}H_{44}O_5$ (calc. 484.3189), suggestive of a further methylation of a compound such as **8**. NMR spectra again indicated the presence a pair of stereoisomers, this time in the ratio 4:1. Only the signals for the major isomer had sufficient intensity to provide a complete set of NMR data. All data indicate that compound **10** is the C-1 methoxy derivative of compound **8** and that their relative stereochemistry is identical at all points. The only significant difference appears for one of the protons at C-13 (δ_H 2.17 (**10**) cf. δ_H 2.32 (**8**)) which appears to be consistent with the shifts observed for the C-1′ methoxylated compounds isolated by Amico et al. (1986). The similarity in difference in H-

13 chemical shifts may indicate that these C-1′ methoxylated compounds have a similar conformational orientation of the aromatic ring, distinct from the conformation in compounds in which C-1′ is a phenol and can form hydrogen bonds.



9 R = CH_3

11 C13-14 = ~CH=CH~

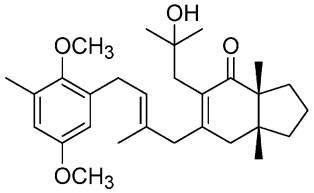
12 R = H

Compound **12** was isolated as an unstable yellow oil from the same HPLC separation which produced compound **8**. Unfortunately, it degraded before collection of sufficient spectral data for satisfactory structural elucidation. However, the structure of the major stereoisomer of the degradation product, compound **11** could be determined from 1D and 2D NMR data, assisted by comparison to published data. High resolution EIMS shows that compound **11** has the molecular formula $C_{28}H_{38}O_4$ (found 438.2774, calc. 438.2770) and provides UV, IR and NMR data indicating that it is another compound possessing the basic amentol skeleton. Significant clues to the specific structure of this compound were found in the observation that NMR signals attributable to the trisubstituted double bond typically found at C-2,3 are lacking, being replaced by a methylene group (δ_H 1.77, 2H, *m*; δ_C 31.4, *t*, C-2) and an oxygenated quaternary carbon (δ_C 75.7, *s*, C-3) carrying a methyl group (δ_H 1.31, 3H, *s*; δ_C 25.0, *q*, C-20). In addition, the NMR spectral data show the presence of a 1,2-disubstituted double bond in which the alkenyl protons belong to an isolated spin system (δ_H 5.55, 1H, *d*, $J = 5.8$ Hz; δ_C 126.5, *d*, C-13; δ_H 6.00, 1H, *d*, $J = 5.8$ Hz; δ_C 140.0, *d*, C-14). The absence of signals associated with oxygenation at C-14 suggests that **11** is the C-13,14 elimination product and is the compound previously identified as cystoketal chromane (Amico et al., 1984; Amico et al., 1987b; Valls et al., 1996). The results obtained from HMBC spectroscopy of compound **11** suggest that the previous tentative assignments of the ^{13}C NMR chemical shifts of C-13 and C-14 should be reversed, as should those for C-3′ and C-5′ (Amico et al., 1984). Compound **11** has always been isolated as an inseparable mixture of C-3 epimers and this has led to the speculation that the formation of the chromane may actually be the result of isolation and work-up procedures.

Only the ^1H NMR spectral data for compound **12** could be obtained. The alkenyl doublets observed in the spectrum of **11** are replaced by signals at δ_H 3.45 (1H), 3.47 (1H), 3.91 (1H) and a D_2O exchangeable signal at δ_H 5.28 (1H) consistent with the presence of a secondary alcohol at C-14 which appears to eliminate readily to form the chromane compound **11**. As indicated above, chromanes may be artefacts of isolation, in this case compound **12** arising from the major compound (**1**) isolated.

Finally, a later fraction from the same Sephadex column that provided compounds **1** and **7** was further purified by HPLC resulting in the isolation of compound **13**, also a possible artefact of compound (**1**), whose spectral data (HREIMS, IR, UV, 1D ^1H and ^{13}C NMR) corresponds to those published for cystoketal (Amico et al., 1986).

Being able to assign the stereochemistry of the compounds isolated from *C. fibrosa* proved to be the most difficult part of this work and, while we are reasonably confident or our assignments, objectively, further work is required before the assignments can be said to unambiguous. The major practical problems relate to the instability of the relatively small samples purified and the inability to separate some stereoisomeric mixtures into pure compounds. Our position on the stereochemistry of these compounds is as follows. Firstly, the *C. fibrosa* compounds are enantiomeric to those previously reported from northern Africa and the Mediterranean at C-7, -11 and -12. This conclusion was reached from the observation that, where measured, the specific rotation of the *C. fibrosa* compounds are all negative and opposite in sign to all previously reported compounds except for one case which apparently corresponds to the C-14 epimer of triacetoxyamentol (**5**) (Navarro et al., 2004). In particular, we point out the effective identity of the magnitude of specific rotation of compounds **7** and **13**, while having opposite signs i.e. $[\alpha]_D = -10.4°$ and $+11.5°$, respectively (Amico et al., 1984). The absence of the possibly confounding stereocentre at C-14 is notable. Work by Amico and co-workers (Amico et al., 1997) on a related compound (cystalgerone (**14**)) isolated from *Cystoseira algeriensis* established the absolute stereochemistry at C-7 and C-11 as 7*R*, 11*R* by Mosher ester analysis and this absolute stereochemistry has been attributed to all amentol derivatives reported to this point. The metabolites isolated from *C. fibrosa* are therefore shown as possessing 7*S*, 11*S* absolute stereochemistry and, consequently, 12*S*.



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Secondly, consideration of ^1H coupling constants and modelling experiments have led us to assign the configuration of C-14 in the major compounds where an oxygen substituent is present to have the same relative stereochemistry as assigned previously to amentol (**3**) while differences in coupling constant magnitudes suggest that they should be

epimeric at this point. It seems that a definitive X-ray structure determination of a compound closely related to amentol, or amentol itself, would be the best way to finally resolve these stereochemical uncertainties and it remains to point out that, if we are proven correct, assignments of all structures belonging to this series, including the chromanes, will need to be carefully re-evaluated stereochemically, even those proposed to be epimeric at C-12.

The compounds isolated are unlike any other previously isolated from the genus *Cystophora* but are typical of compounds reported from the closely related genus *Cystoseira* (Valls and Piovetti, 1995; Amico, 1995). As such they present an excellent chemotaxonomic case for the reclassification of the South African brown alga *C. fibrosa* to the genus *Cystoseira*, of which these compounds are typical In particular, *C. fibrosa* appears to be most closely related, chemotaxonomically, to *C. amentacea* collected from Sicily (Ruberto et al., 2001) and the environs of Marseille (Valls et al., 1996), *C. mediterranea* collected near Scilla on the Strait of Messina (Amico et al., 1988b; Guiry et al., 2005) and an unidentified species collected from the Canary Islands (Navarro et al., 2004). Removal of *C. fibrosa* from the *Cystophora* would result in this genus having a purely Australasian distribution.

In summary, we have reported herein the isolation and identification of isolation of six new amentol (**3**) derivatives from the brown alga *C. fibrosa* (compounds **1**, **4**, **6**–**8**, **10**) with a further five new amentol derivative stereoisomers. With the propensity of amentol and its derivatives to decompose via the formation of chromanes or elimination at C-13,14 it may be that compounds **7**, **12** and **13** are artefacts of the isolation procedure. It is also proposed that the isolation of compounds from *C. fibrosa* that are typical of the brown algal genus *Cystoseira* provides evidence for moving the species from *Cystophora* to *Cystoseira*.

3. Experimental

3.1. General

HREIMS analysis was conducted on a Kratos Concept ISQ mass spectrometer by Dr Noel Davies and Mr Marshall Hughes of the Central Science Laboratories, University of Tasmania, Hobart, Australia. 1D and 2D NMR were obtained with a Bruker Avance DPX300 instrument producing frequencies of 300.13 and 75.47 MHz for ^{1}H and ^{13}C, respectively. Chemical shifts are quoted in ppm (δ) referenced to the residual proton in $CDCl_3$ solvent (δ 7.24) and the carbon signal at 77.7 ppm. IR spectra were recorded neat on a Perkin Elmer Paragon 1000 FT-IR spectrometer and the UV spectrum was obtained from an ethanolic solution on a Hitachi U-2000 spectrophotometer. Optical rotation was recorded as solutions in $CHCl_3$ on a Perkin Elmer 241 Polarimeter. Semi-preparative HPLC utilised an Activon Goldpak Exsil 7 μm silica column (250 × 10 mm) at a flow rate of 3.5 mL/min and a PDA detector. Qualitative TLC was conducted using silica gel coated, aluminium backed plates (Merck).

3.2. Plant material

Specimens were collected by DWL from rock pools at Koppie Alleen, De Hoop Nature Reserve, Cape Province, South Africa in January 2001 and identified by Associate Professor John Bolton, University of Cape Town, South Africa. A voucher specimen has been lodged with the herbarium of the Royal Botanic Gardens, Sydney, Australia (Accession # NSW 717015).

3.3. Compound characterisation

Frozen alga was extracted with acetone (x4) at room temperature to give a dark brown, oily crude extract (8 g, 5% yield based on dry, extracted weight of alga). A 2 g portion of the crude extract was subjected to Sephadex LH-20, MeOH–$CHCl_3$ (1:1) gel permeation chromatography to provide four fractions. The second and third fractions were further separated by partition chromatography (Sephadex LH-20, light petroleum (60-80°)–$CHCl_3$–EtOH (10:10:1)) into six and five fractions, respectively, which were then chromatographed by normal phase HPLC, utilising gradients from 100% *iso*-octane or hexane to 100% EtOAc to afforded fractions suitable for structure elucidation. The second fraction from first partition column yielded compound **10** (R_f 0.60 (light petroleum–Et_2O (3:1)), 1.3 mg, 0.001%, based on dry, extracted weight of alga) while the third fraction gave compounds **12** (R_f 0.66 (hexane–EtOAc (17:3)), 1.5 mg, 0.001%) and **8** (R_f 0.44 (light petroleum–EtOAc (17:3)), 3.2 mg, 0.002%), respectively, and fraction five yielded compound **6** (R_f 0.54 (light petroleum–EtOAc (4:1)), 1.0 mg, 0.001%). The second fraction from the second partition column gave compound **4** (R_f 0.44 (light petroleum–EtOAc (4:1)), 5.6 mg, 0.004%) after HPLC, while the third fraction provided compounds **7** (R_f 0.64 (light petroleum–EtOAc (4:1)), 7.6 mg, 0.006%) and **1** (R_f 0.23 (light petroleum–EtOAc (9:1)), 3.5 mg, 0.003%) and HPLC of the fifth fraction resulted in the isolation of compound **13** (R_f 0.17 (light petroleum–EtOAc (99:1)), 1.0 mg, 0.001%).

Compound **1** ((7*S*,11*S*,12*S*,14*R*)-4′-methoxy amentol). Oil; $[\alpha]_D^{23}$ −28.1° ($CHCl_3$; *c* 0.032); UV λ_{max}^{EtOH} nm (log ε): 216 (4.9); IR ν_{max}^{neat} cm^{-1}: 3464 (OH), 2927, 2885, 1729, 1683, 1600, 1564, 1483, 1466, 1379, 1190, 1104, 1090, 1075, 955, 861, 802; HREIMS 70 eV, *m*/*z* (rel. int.): 456.2853 (calc. $C_{28}H_{40}O_5$ 456.2876) $[M]^+$ (7), 438 $[M-H_2O]$ (80), 420 $[M-2\times H_2O]$ (38), 404 $[M-2\times H_2O-Me]$ (16), 233 (14), 205 (6), 191 (12), 190 (19), 189 (100), 168 (33), 150 (51), 149 (26), 137 (14), 71 (20), 69 (11); ^{1}H and ^{13}C NMR: see Table 1; Difference ^{1}H,^{1}H NOE spectroscopy (300 MHz, $CDCl_3$), H-irradiated: H-enhanced (%): H-13a: H-13b (5.7), H-19 (0.25), H-13b: H-13a (8.3), H-18 (0.95), H-16 (0.36), H-14, H-14: H-13a (0.67), H-13b, H-16, H-16: H-13b (1.5), H-14 (3.8), 14-OH (3.8), H-18: H-13b (3.6), H-10a, H-19: H-6

(6.2), H-13a (1.8), H-14OH: H-13a (0.70), H-17 (0.25), H-16 (0.36).

Compound **4** ((7*S*,11*S*,12*S*,14*R*)-1′,4′-dimethoxyamentol). Pale yellow oil; $[\alpha]_D^{23}$ −58.6° ($CHCl_3$; *c* 0.28); UV λ_{max}^{EtOH} nm (log ε): 211 (4.21), 281 (3.19); IR ν_{max}^{neat} cm^{-1}: 3522 m (O–H), 2932 s, 1683 s, 1605 m, 1482 s, 1380 m, 1321 m, 1221 s, 1174 w, 1104 m, 1078, 1061, 1015, 954, 860, 668; ^{13}C NMR: see Table 2; ^1H NMR (300 MHz, $CDCl_3$): δ 0.91 (3H, *s*, H-18), 0.99 (3H, *s* H-19), 1.13 (3H, *s*, H-16), 1.33-1.43 (1H, *m*, H10a), 1.34 (3H, *s*, H-17), ∼1.56 (2H, *m*, H-9), ∼1.58 (2H, *m*, H-8), 1.70 (3H, *s*, H-20), ∼2.05 (1H, *m*, H-10b), 2.01 (1H, *d*, *J* = 13.9 Hz, H-13a), 2.25 (3H, *s*, H-6′Me), 2.32 (1H, *dd*, *J* = 13.9, 5.5 Hz, H-13b) 2.61 (1H, *d*, *J* = 14.1 Hz, H-4a), 2.69 (1H, *d*, *J* = 14.1 Hz, H-4a), 3.18 (1H, *d*, *J* = 12.6 Hz, H-14OH), 3.34 (1H, *dd*, *J* = 15.8, 6.4 Hz, H=1a), 3.38 (1H, *dd*, *J* = 15.8, 7.4 Hz, H-1b), 3.65 (3H, *s*, H-1′OMe), 3.72 (3H, *s*, H-4′OMe), 3.78 (1H, *dd*, *J* = 12.6, 5.5 Hz, H-14), 4.31 (1H, *s*, H-6), 5.41 (1H, *dd*, *J* = 7.4, 6.4 Hz, H-2), 6.53 (1H, *d*, *J* = 3.0 Hz, H-5′), 6.56 (1H, *d*, *J* = 3.0 Hz, H-3′); Difference ^1H,^1H NOE spectroscopy (300 MHz, $CDCl_3$), H-irradiated: H-enhanced (%): H-13b:H-13a (11.5), H-14 (3.7), H-16 (1.0),H-18 (1.4), H-16: H-13b (1.2), H-14 (5.0), H-17 (0.8), H-18: H-13b (4.4), H-19: H-6 (6.1), H-13a (1.4); HREIMS 70 eV, *m*/*z* (rel. int.): 470.3026 (calc. $C_{29}H_{42}O_5$ 470.3032) $[M]^+$ (3), 452 (7), 435 (1), 302 (5), 271 (3), 251 (7), 239 (11), 233 (27), 221 (8), 205 (11), 189 (12), 168 (100), 150 (65), 137 (20), 135 (18), 124 (10), 109 (18), 95 (20), 83 (12), 71 (18), 69 (11), 57 (4), 55 (5), 43 (17).

Compound **6** ((7*S*,11*S*,12*S*,14*R*)-14-methoxyamentol quinone). Yellow oil; $[\alpha]_D^{23}$ −18° (*c* 0.05); UV λ_{max}^{EtOH} nm (log ε): 205.0 (4.78), 250.0 (3.88); IR ν_{max}^{neat} cm^{-1}: 2928 s, 1727 w, 1655 s, 1463, 1368, 1292, 1120, 1043, 977, 668; ^{13}C NMR: see Table 2; ^1H NMR (300 MHz, $CDCl_3$): δ 0.96 (3H, *s*, H-18), 1.06 (3H, *s*, H-19), 1.08 (3H, *s*, H-17), 1.25 (3H, *s*, H-16), 1.27 (1H, *m*, H-10a), 1.44 (1H, *m*, H-8a), 1.50 (2H, *bm*, H-9), 1.58 (3H, *s*, H-20), 1.61 (1H, *m*, H-8b), 1.76 (1H, *m*, H-10b), 2.03 (1H, *dd*, *J* = 12.7, 8.2 Hz, H-13a), 2.04 (3H, *s*, H-6′Me), 2.23 (1H, *dd*, *J* = 12.7, 6.7 Hz, H-13b), 2.63 (2H, *s*, $W_{1/2}$ 6.4 Hz, H-4), 3.12 (2H, *d*, *J* = 7.4, H-1), 3.32 (3H, *s*, H-14OMe), 3.81 (1H, *dd*, *J* = 8.2, 6.7 Hz, H-14), 4.23 (1H, *s*, H-6), 5.23 (1H, *d*, *J* = 7.4 Hz, H-2), 6.52 (2H, *s*, H-3′ and -5′); HREIMS 70 eV, *m*/*z* (rel. int.): 454.2705 (calc. $C_{28}H_{38}O_5$ 454.2719) $[M]^+$ (3), 422 (7), 279 (14), 253 (10), 233 (13), 221 (23), 196 (14), 182 (75), 175 (71), 167 (33), 150 (87), 149 (100), 137 (36), 109 (26), 95 (29), 85 (26), 69 (32), 57 (29), 55 (13), 43 (23).

Compound **7** ((7*S*,11*S*,12*S*)-1′-methoxycystoketal). Yellow oil; $[\alpha]_D^{23}$ −10.4° (*c* 0.07); UV λ_{max}^{EtOH} nm (log ε): 240 (4.56), 280 (3.42); IR ν_{max}^{neat} (cm^{-1}): 3378 w, 2959, 2885, 1686, 1604, 1482, 1341, 1220, 1106, 1062, 981, 857, 757; ^{13}C NMR: see Table 2; ^1H NMR (300 MHz, $CDCl_3$): δ 0.86 (3H, *s*, H-18), 1.11 (3H, *s*, H-19), 1.26 (3H, *s*, H-17), 1.32 (3H, *s*, H-16), 1.35 (1H, *m*, H-10a), 1.55 (2H, *m*, H-8), 1.58 (2H, *m*, H-9), 1.68 (3H, *s*, H-20), 1.97 (1H, *m*, H-10b), 2.25 (3H, *s*, H-6′Me), 2.62 (1H, *d*, *J* = 14.6 Hz, H-4a), 2.69 (1H, *d*, *J* = 14.6 Hz, H-4b), 3.27 (1H, *dd*, *J* = 15.8, 6.8 Hz, H-1a), 3.37 (1H, *dd*, *J* = 15.8, 7.6 Hz, H-1b), 3.65 (3H, *s*, H-1′OMe), 3.72 (3H, *s*, H-4′OMe), 4.28 (1H, *s*, H-6), 5.36 (1H, *dd*, *J* = 7.6, 6.8 Hz, H-2), 5.59 (1H, *d*, *J* = 5.8 Hz, H-13), 6.00 (1H, *d*, *J* = 5.8 Hz, H-14), 6.52 (1H, *d*, *J* = 2.9 Hz, H-5′), 6.55 (1H, *d*, *J* = 2.9 Hz, H-3′); Difference ^1H,^1H NOE spectroscopy (300 MHz, $CDCl_3$), H-irradiated: H-enhanced (%): H-16:H-14 (1.0), H-18: H-13 (0.8), H-14 (0.2), H-19 (1.0), H-19: H-6 (1.6), H-18 (1.3); HREIMS 70 eV, *m*/*z* (rel. int.): 452.2924 (calc. $C_{29}H_{40}O_4$ 452.2927) $[M]^+$ (7), 288 (2), 233 (22), 206 (6), 190 (7), 150 (100), 137 (15), 135 (14), 109 (8), 95 (10), 69 (7), 55 (2), 43 (5).

Compound **8** ((7*S*,11*S*,12*S*,14*R*)-4′,14-dimethoxyamentol) and *epi*-14-Compound **8** ((7*S*,11*S*,12*S*,14*S*)-4′,14-dimethoxyamentol). Pale yellow oil; UV λ_{max}^{EtOH} nm (log ε): 203.5 (4.84), 288.5 (3.71); Compound **8**: ^{13}C NMR: see Table 2; ^1H NMR (300 MHz, $CDCl_3$): δ 0.94 (3H, *s*, H-18), 1.07 (3H, *s*, H-19), 1.08 (3H, *s*, H-17), 1.25 (3H, *s*, H-16), 1.28 (1H, *m*, H-10a), 1.42 (1H, *m*, C-8a), 1.58 (2H, *m*, H-9), 1.64 (1H, *m*, H-8b), 1.74 (3H, *s*, C-20), 1.80 (1H, *m*, H-10b), 2.02 (1H, *dd*, *J* = 12.7, 8.0 Hz, H-13a), 2.20 (3H, *s*, H-6′Me), 2.32 (1H, *dd*, *J* = 12.7, 6.9 Hz, H-13b), 2.68 (2H, *s*, H-4), 3.22 (1H, *dd*, *J* = 16.5, 6.0 Hz, H-1a), 3.29 (3H, *s*, H-14OMe), 3.46 (1H, *dd*, *J* = 16.5, 8.7 Hz, H-1b), 3.72 (3H, *s*, H-4′OMe), 3.78 (1H, *dd*, *J* = 8.0, 6.9 Hz, H-14), 4.30 (1H, *s*, H-6), 5.06 (1H, *s*, H-1′OH), 5.37 (1H, *dd*, *J* = 8.7, 6.0 Hz, H-2), 6.51 (1H, *d*, *J* = 3.4 Hz, H-3′), 6.56 (1H, *d*, *J* = 3.4 Hz, H-5′).

epi-14-Compound **8**: ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.5 (*q*, C-20), 16.6 (*q*, C-6′Me), 19.5 (*q*, C-18), 20.4 (*t*, C-9), 22.0 (*q*, C-17), 22.4 (*q*, C-19), 27.2 (*q*, C-16), 31.2 (*t*, C-1), 36.2 (*t*, C-10), 39.8 (*t*, C-13), 40.3 (*t*, C-8), 43.2 (*s*, C-11), 45.2 (*t*, C-4), 46.2 (*s*, C-7), 55.6 (*q*, C-4′OMe), 58.0 (*q*, C-14′OMe), 81.8 (*s*, C-15), 86.8 (*d*, C-14), 109.5 (*s*, C-12), 109.7 (*d*, C-6), 112.8 (*d*, C-3′), 114.2 (*d*, C-5′), 124.0 (*d*, C-2), 125.9 (*s*, C-6′), 127.0 (*s*, C-2′), 136.1 (*s*, C-3), 144.9 (*s*, C-5), 147.0 (*s*, C-1′), 152.9 (*s*, C-4′); ^1H NMR (300 MHz, $CDCl_3$): δ 0.97 (3H, *s*, H-18), 1.07 (3H, *s*, H-19), 1.17 (3H, *s*, H-17), 1.22 (3H, *s*, H-16), 1.27 (1H, *m*, H-10a), 1.41 (1H, *m*, H-8a), 1.58 (2H, *m*, H-9), 1.63 (1H, *m*, H-8b), 1.74 (3H, *s*, H-20), 1.79 (1H, *m*, H-10b), 2.18 (1H, *dd*, *J* = 13.7, 9.2 Hz, H-13a), 2.20 (3H, *s*, H-6′Me), 2.46 (1H, *dd*, *J* = 13.7, 8.2 Hz, H-13b), 2.66 (2H, s, H-4), 3.22 (1H, *dd*, *J* = 16.5, 6.0 Hz, H-1a), 3.31 (3H, *s*, H-14′OMe), 3.42 (1H, *dd*, *J* = 9.2, 8.2 Hz, H-14), 3.46 (1H, *dd*, *J* = 16.5, 8.7 Hz, H-1b), 3.71 (3H, *s*, H-4′OMe), 4.28 (1H, s, H-6), 5.09 (1H, *s*, H-1′OH), 5.37 (1H, *dd*, *J* = 8.7, 6.0 Hz, H-2), 6.51 (1H, *d*, *J* = 3.4 Hz, H-3′), 6.56 (1H, *d*, *J* = 3.4 Hz, H-5′); HREIMS 70 eV, *m*/*z* (rel. int.): 470.3033 (calc. $C_{29}H_{42}O_5$ 470.3032), $[M]^+$ (1), 438 (19), 420 (7), 288 (13), 270 (5), 233 (18), 205 (9), 191 (24), 189 (60), 182 (63), 150 (100), 137 (29), 135 (10), 109 (22), 95 (16), 82 (15), 69 (26), 57 (5), 55 (10), 43 (15).

Compound **10** ((7*S*,11*S*,12*S*,14*R*)-1′,4′,14-trimethoxyamentol). Pale yellow oil; UV λ_{max}^{EtOH} nm (log ε): 203.5

(4.83), 246.5 (3.19); ^{13}C NMR: see Table 2; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.96 (3H, *s*, H-18), 1.06 (3H, *s*, H-19), 1.08 (3H, *s*, H-17), 1.26 (3H, *s*, H-16), 1.27 (2H, *m*, H-10), 1.54 (4H, *m*, H-8 & -9), 1.67 (3H, *s*, H-20), 2.01 (1H, *dd*, $J = 12.6$, 8.2 Hz, H-13a), 2.17 (1H, *dd*, $J = 12.6$, 6.8 Hz, H-13b), 2.26 (3H, *s*, H-6′Me), 2.61 (1H, *d*, $J = 14.4$ Hz, H-4a), 2.62 (1H, *d*, $J = 14.4$ Hz, H-4b), 3.30 (3H, *s*, H-14OMe), 3.34 (1H, *d*, $J = 7.3$ Hz, H-1a), 3.67 (3H, *s*, H-1′OMe), 3.72 (3H, *s*, H-4′OMe), 3.80 (1H, *dd*,$J = 8.0$, 6.8 Hz, H-14), 4.22 (1H, *s*, H-6), 5.34 (1H, *t*, $J = 7.3$ Hz, H-2), 6.53 (1H, *d*, $J = 3.0$ Hz, H-3′), 6.58 (1H, *d*, $J = 3.0$ Hz, H-5′); HREIMS 70 eV, *m*/*z* (rel. int.): 484.3185 (calc. $C_{30}H_{44}O_5$ 484.3189) $[M]^+$ (1), 452 (6), 421 (3), 302 (7), 270 (4), 221 (15), 205 (13), 190 (14), 165 (26), 150 (100), 135 (38), 109 (13), 95 (18), 81 (8), 69 (14), 55 (6), 43 (21).

Compound **11** ((7*S*,11*S*,12*R*)-cystoketal chromane, C-3 mixture of epimers). Pale yellow oil; UV λ_{max}^{EtOH} nm ($\log\varepsilon$): 206.0 (4.59), 293.0 (3.60); ^{13}C NMR: see Table 2; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.86 (3H, *s*, H-18), 1.12 (3H, *s*, H-19), 1.28 (3H, *s*, H-16), 1.31 (3H, *m*, H-10a), 1.31 (3H, *s*, H-20), 1.34 (3H, *s*, H-17), 1.47 (3H, *m*, H-8a), 1.56 (2H, *m*, H-9), 1.61 (1H, *m*, H-8b), 1.77 (2H, *m*, H-2), 1.93 (1H, *m*, H-10b), 2.12 (3H, *s*, H-6′Me), 2.24 (2H, *s*, H-4), 2.69 (2H, *m*, H-1), 3.71 (3H, *s*, H-4′OMe), 4.32 (1H, *s*, H-6), 5.55/5.57 (1H, *d*, $J = 5.8$ Hz, H-13, major/minor epimer), 6.00/6.02 (1H, *d*, *J*, = 5.8 Hz, H-14), 6.41 (1H, *d*, $J = 2.8$ Hz, H-3′), 6.53 (1H, *d*, $J = 2.8$ Hz, H-5′); HREIMS 70 eV, *m*/*z* (rel. int.): 438.2774 (calc. $C_{28}H_{39}O_4$ 438.2770) $[M]^+$ (6), 288 (95), 270 (17), 253 (7), 233 (11), 206 (15), 191 (100), 182 (39), 150 (46), 137 (21), 109 (16), 95 (17), 83 (13), 69 (17), 55 (7), 43 (17).

Compound **12** (4′-methoxyamentol chromane). Unstable yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.97 (3H, *s*, H-18), 1.06 (3H, *s*, H-19), 1.09 (3H, *s*, H-17), 1.28 (3H, *s*, H-16), 1.32 (3H, *s*, H-20), 1.75 (2H, *m*, H-2), 2.13 (3H, *s*, H-6′Me), 2.22 (2H, *s*, H-4), 2.70 (2H, *m*, H-1), 3.45 (1H, *m*, H-13a), 3.47 (1H, *m*, H-13b), 3.70 (3H, *s*, H-4′OMe), 3.91 (1H, *m*, H-14), 4.27(1H, *s*, H-6), 5.28 (1H, *s*, H-14OH), 6.42 (1H, *s*, H-3′), 6.54 (1H, *s*, H-5′), signals due to H-8 to -10 could not be distinguished in the spectrum.

Compound **13** ((7*S*,11*S*,12*R*)-cystoketal). Yellow oil; UV λ_{max}^{EtOH} nm ($\log\varepsilon$): 205.0 (4.78), 250.0 (3.88); IR ν_{max}^{neat} (cm^{-1}): 3466 (O–H), 2929, 1684, 1654, 1466, 1342, 1197, 1107, 1081, 1048, 979; ^{13}C NMR: see Table 2; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.86 (3H, *s*, H-18), 1.12 (3H, *s*, H-19), 1.26 (3H, *s*, H-17), 1.29 (3H, *s*, H-16), 1.35 (1H, *m*, H-10a), 1.48 (2H, *m*, H-8), 1.58 (2H, *bm*, H-9), 1.62 (1H, *m*, H-8b), 1.74 (3H, *s*, H-20), 1.92 (1H, *m*, H-10b), 2.19 (3H, *s*, H-6′Me), 2.68 (2H, *s*, H-4), 3.21 (1H, *dd*,$J = 15.8$, 6.4 Hz, H-1a), 3.38 (1H, *dd*, $J = 15.8$, 7.6 Hz, H-1b), 3.72 (3H, *s*, H-4′OMe), 4.31 (1H, *s*, H-6), 4.85 (1H, *s*, H-1′OH), 5.36 (1H, *dd*, $J = 7.6$, 6.4 Hz, H-2), 5.62 (1H, *d*, $J = 5.7$ Hz, H-13), 5.99 (1H, *d*, $J = 5.7$ Hz, H-14), 6.49 (1H, *d*, $J = 3.0$ Hz, H-3′), 6.54 (1H, *d*, $J = 3.0$ Hz, H-5′); HREIMS 70 eV, *m*/*z* (rel. int.): 438.2770 (calc. $C_{28}H_{38}O_4$ 438.2770) $[M^+]$ (9), 420 (5), 288 (4), 233 (15), 221 (25), 205 (10), 189 (56), 150 (100), 137 (26), 109 (14), 95 (15), 81 (7), 69 (13), 55 (4), 43 (10).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phytochem.2006.03.011.

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