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Laurefurenynes A–F, new Cyclic Ether Acetogenins from a Marine Red Alga, *Laurencia* sp.

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ABSTRACT

We report here on the discovery and structure determination of three new diastereomeric pairs of cyclic ether acetogenins, laurefurenynes A–F, isolated from the aqueous extract of the alga *Laurencia* sp. collected in the Philippines. Extensive use was made of NMR spectroscopic data and high resolution MS to determine the structures of the pure compounds. The most stable and the lowest energy conformation was determined using molecular modelling, and their cytotoxic activity was tested against different tumour cells, a significant indication that laurefurenyne C and F are moderately cytotoxic, but non-selective whilst the others are inactive.

1. Introduction

Red algae of the genus *Laurencia* (class Rhodophyceae, order Ceramiales, family Rhodomelaceae) are unique for their ability to biosynthesize an astonishing variety of structurally unusual secondary metabolites and seem to be an endless source of new chemical constituents.[1–4] *Laurencia* is one of the most intensively chemically investigated of all marine genera.[5] The reasons for this are twofold: first, algae belonging to this genus are extremely widespread, being found in all oceans and seas as well as at almost all latitudes; and second because algae belonging to this genus, almost without exception, have a high secondary metabolite content. Both of these features make *Laurencia* species attractive sources for new and potentially biologically active novel natural products.[6] Most species of *Laurencia* biosynthesize a characteristic major metabolite or a class of compounds that are not commonly widely distributed within the genus.[7] The vast majority of *Laurencia* metabolites are C_{15}-acetogenins,[8,9] diterpenes,[10–16] sesquiterpenes and triterpenes.[17–20] Several other structural classes have also been reported,[21] with different biological activities such as antibacterial,[22] antimalarial,[23] ichtyotoxic,[24] antioxidant,[25] antifungal,[26] insecticidal activities,[27] as well as noteworthy cytotoxic activity against mammalian cancer cells.[28]

In the course of our ongoing research activities towards the isolation of biologically active compounds from marine organisms, and as a part of our collaboration with the U.S. National Cancer Institute Open Repository Program, which provides our laboratory with crude extracts of marine invertebrates that are screened for differential cytotoxicity at the Ford Cancer Centre, Detroit, USA, we had the opportunity to study a sample of *Laurencia* sp. collected from the Philippines. Here we report the structures of the new vinyl acetylenes from *Laurencia* sp. The structures of these compounds were elucidated using 1D and 2D NMR spectroscopy and MS techniques.

2. Results and discussion

2.1. Extraction and isolation

The U.S. National Cancer Institute's Open Repository Program provided our laboratory with crude extracts of marine organisms, which were screened for differential cytotoxicity at the Ford Cancer Center, Detroit, U.S.A. As part of this collaboration we had the opportunity to study a *Laurencia* sp. which was collected in 1991 from the Philippines by the National Cancer Institute (NCI). The aqueous extract was subjected to solid phase extraction using a modification of the method described by West et al.[29,30], followed by size exclusion and high pressure liquid column chromatography to afford three new diastereomeric pairs of cyclic ether acetogenins, laurefurenynes A–F (**1**–**6**).

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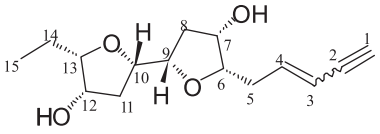
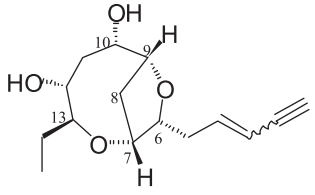
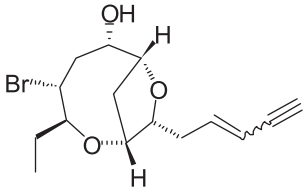


1 $\Delta_{3,4}$ *cis*
2 $\Delta_{3,4}$ *trans*



3 $\Delta_{3,4}$ *cis*
4 $\Delta_{3,4}$ *trans*



5 $\Delta_{3,4}$ *cis*
6 $\Delta_{3,4}$ *trans*

2.2. Structural analysis

The structures were elucidated on the basis of spectroscopic data (^{1}H NMR, ^{13}C NMR, ^{1}H–^{1}H COSY, TOCSY, HSQC and HMBC) in combination with mass spectrometric data. The relative stereochemistry was proposed on the basis of selective 1D NOE and 2D NOESY experiments in combination with molecular modelling and the cytotoxicity of the isolated metabolites was evaluated against different tumour cell lines.

Compound **1** was obtained as a colourless amorphous powder. ESIMS showed pseudomolecular ion peaks at *m*/*z* 267.2 $[M+H]^+$, *m*/*z* 289.2 $[M+Na]^+$ and *m*/*z* 305.2 $[M+K]^+$, consistent with a molecular weight of 266 amu. By accurate mass measurements, the molecular formula was established as $C_{15}H_{22}O_4$, thus implying five degrees of unsaturation. The IR spectrum showed bands attributed to a terminal acetylene function (3315 and 2107 cm^{-1}), intense hydroxyl absorptions at 3482 and 3435 cm^{-1}, and the absence of a carbonyl absorption.

The ^{1}H and ^{13}C NMR spectra in $CDCl_3$ (Tables 1 and 2) revealed **1** to be a C_{15}-acetogenin, comprising one methyl group (as part of an ethyl moiety), four methylenes, six oxygen-bearing sp^3 methines, two sp^2 methines, one sp. methine, and a quaternary carbon. On the basis of the coupling patterns, the presence of a *cis*-ene-yne function was readily established [H-4 (δ 6.05), H-3 (δ 5.57) and H-1 (δ_H 3.10)], which was confirmed by the respective ^{13}C NMR signals observed for C-4 (δ 139.9), C-3 (δ 111.3), C-2 (δ 80.0) and C-1 (δ 82.4).[8,31–33]

The combined analysis of the COSY, selective 1D TOCSY and HSQC spectra of **1** allowed for establishing a continuous chain of carbon atoms from C-1 through C-15, which was further corroborated by HMBC correlations, as shown in Figure 1. Re-analysis for **1** in DMSO-d_6 (Supplementary data, Table 3) was carried out to overcome the extensive overlapping of the proton signals, and to infer the positions of the two ether bridges required by the molecular formula. Signals attributable to two hydroxyl groups were observed (δ 4.93, OH-7, and δ 4.43, OH-12), which on the basis of the COSY spectrum could be unambiguously located at C-7 and C-12, respectively. Thus, to pinpoint the planar structure of **1**, three different combinations of ether linkages had to be considered, yielding three types of carbon frameworks, all of them with precedent in natural products obtained previously from *Laurencia* spp. (Fig. 1): Connection of C-6 and C-13 would yield a nine-

Table 1
^{1}H NMR spectroscopic data of Laurefurenynes A–F (**1**–**6**) (400 MHz, $CDCl_3$)

Proton(s) at carbon No.	**1** δ^1H/ppm, mult., *J*/Hz	**2** δ^1H/ppm, mult., *J*/Hz	**3** δ^1H/ppm, mult., *J*/Hz	**4** δ^1H/ppm, mult., *J*/Hz	**5** δ^1H/ppm, mult., *J*/Hz	**6** δ^1H/ppm, mult., *J*/Hz
1	3.10 (1H, d, 2.0)	2.83 (1H, d, 2.1)	3.09 (1H, d, 2.1)	2.76 (1H, d, 1.6)	3.07 (1H, d, 2.0)	2.79 (1H, d, 1.7)
2	–	–	–	–	–	–
3	5.57 (1H, dd, 10.9, 2.0)	5.58 (1H, dd, 15.5, 2.1)	5.55 (1H, dd, 11.2, 2.1)	5.59 (1H, dd, 16.0, 1.6)	5.51 (1H, dd, 10.9, 2.0)	5.55 (1H, dd, 15.9, 1.7)
4	6.05 (1H, dt, 10.9, 7.9)	6.22 (1H, dt, 15.5, 7.6)	6.08 (1H, dt, 11.2, 8.0)	6.26 (1H, dt, 16.0, 7.5)	6.01 (1H, dt, 10.9, 7.8)	6.18 (1H, dt, 15.9, 7.5)
5a	2.61 (2H, m)	2.44 (1H, m)	2.85 (1H, m)	2.54 (2H, m)	2.79 (1H, m)	2.56 (1H, m)
5b		2.37 (1H, m)	2.69 (1H, m)		2.68 (1H, m)	2.45 (1H, m)
6	3.90 (1H, ddd, 10.1, 6.5, 3.8)	3.90 (1H, ddd, 10.2, 6.6, 3.7)	3.78 (1H, m)	3.73 (1H, m)	3.72 (1H, ddd, 8.8, 7.1, 2.0)	3.67 (1H, ddd, 8.6, 7.3, 2.1)
7	4.12 (1H, m)	4.10 (1H, m)	4.02 (1H, m)	3.99 (1H, dd, 3.6, 2.0)	3.94 (1H, m)	3.94 (1H, m)
8a	1.86 (1H, m)	1.85 (1H, m)	2.98 (1H, d, 15.2)	3.02 (1H, d, 16.2)	2.24 (1H, d, 15.9)	2.24 (1H, d, 16.1)
8b	1.69 (1H, m)	1.68 (1H, m)	1.96 (1H, m)	1.93 (1H, m)	1.92 (1H, m)	1.92 (1H, m)
9	4.37 (1H, m)	4.40 (1H, m)	4.30 (1H, m)	4.29 (1H, dd, 3.9, 9.8)	4.10 (1H, dd, 9.6, 4.0)	4.10 (1H, dd, 9.6, 3.9)
10	4.14 (1H, m)	4.16 (1H, m)	4.09 (1H, m)	4.07 (1H, m)	3.96 (1H, m)	3.96 (1H, m)
OH-10	–		3.34 (1H, br s)	3.37 (1H, br s)		
11a	2.19 (1H, m)	2.20 (1H, m)	2.21 (2H, m)	2.20 (2H, m)	3.01 (1H, dddd, 15.3, 11.3, 9.7, 1.8)	2.98 (1H, dddd, 15.4, 11.2, 9.8, 1.8)
11b	1.81 (1H, dd, 3.1, 14.5)	1.79 (1H, dd, 3.0, 13.9)			2.28 (1H, dd, 15.2, 5.1)	2.28 (1H, dd, 15.1, 5.0)
12	3.98 (1H, dd, 2.3, 5.1)	4.01 (1H, dd, 2.3, 5.5)	4.29 (1H, m)	4.30 (1H, m)	4.55 (1H, t, 9.6)	4.54 (1H, t, 9.6)
OH-12	–		2.72 (1H, br s)	2.80 (1H, br s)		
13	3.49 (1H, ddd,13.6, 6.8, 2.4)	3.52 (1H, ddd, 9.4, 6.9, 2.4)	3.81(1H, m)	3.76 (1H, m)	3.86 (1H, ddd 11.8, 9.6, 2.8)	3.84 (1H, ddd,13.5, 9.6, 2.4)
14	1.66 (2H, m)	1.69 (2H, m)	1.74 (1H, m), 1.57 (1H, m)	1.77 (1H, m), 1.57 (1H, m)	1.93 (1H, m), 1.66 (1H, m)	1.96 (1H, m), 1.65 (1H, m)
15	0.94 (3H, t, 7.5)	0.97 (3H, t, 7.5)	1.01 (3H, t, 7.5)	0.98 (3H, t, 7.5)	1.02 (3H, t, 7.2)	1.01 (3H, t, 7.2)

Table 2
^{13}C NMR spectroscopic data of Laurefurenynes A–F (**1**–**6**) (100 MHz, $CDCl_3$)

Carbon No.	**1** δ^{13}C/ppm, mult	**2** δ^{13}C/ppm, mult	**3** δ^{13}C/ppm, mult	**4** δ^{13}C/ppm, mult	**5** δ^{13}C/ppm, mult	**6** δ^{13}C/ppm, mult
1	82.4, CH	76.7, CH	81.9, CH	76.2, CH	81.7, CH	76.6, CH
2	80.0, C	81.7, C	—	82.2, C	80.7, C	81.8, C
3	111.3, CH	112.0, CH	110.0, CH	110.8, CH	110.2, CH	111.3, CH
4	139.9, CH	140.7, CH	141.8, CH	142.5, CH	141.5, CH	142.1, CH
5	34.1, CH_2	37.1, CH_2	30.4, CH_2	32.8, CH_2	30.1, CH_2	32.8, CH_2
6	85.7, CH	85.4, CH	83.0, CH	82.9, CH	83.2, CH	83.4, CH
7	74.8, CH	74.9, CH	71.0, CH	70.2, CH	70.2, CH	70.1, CH
8	37.1, CH_2	37. 3, CH_2	32.0, CH_2	31.7, CH_2	33.1, CH_2	33.2, CH_2
9	79.1, CH	79.1, CH	79.5, CH	79.7, CH	78.6, CH	78.4, CH
10	78.2, CH	78.1, CH	73.3, CH	73.4, CH	70.5, CH	70.5, CH
11	34.5, CH_2	34.5, CH_2	30.8, CH_2	29.7, CH_2	38.8, CH_2	38.9, CH_2
12	70.8, CH	70.8, CH	72.8, CH	73.1, CH	51.8, CH	51.8, CH
13	85.6, CH	85.7, CH	78.9, CH	79.3, CH	83.2, CH	83.5, CH
14	21.8, CH_2	21.8, CH_2	23.1, CH_2	22.3, CH_2	23.1, CH_2	23.2, CH_2
15	10.5, CH_3	10.5, CH_3	11.3, CH_3	11.4, CH_3	11.8, CH_3	11.9, CH_3

– Not observed.

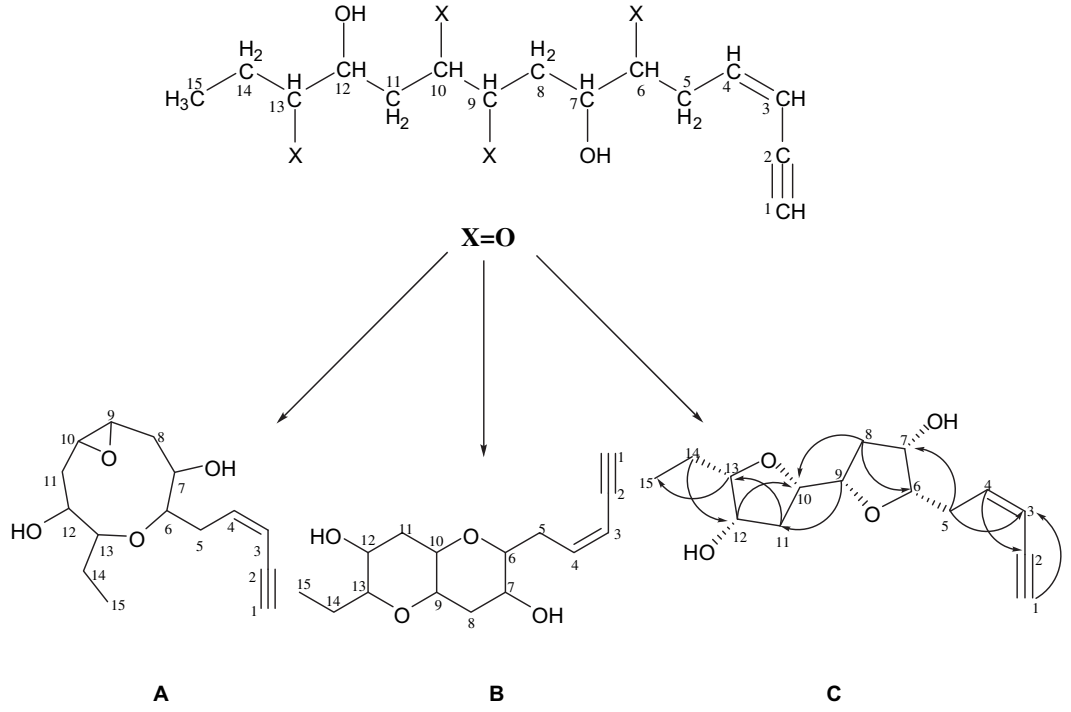


Figure 1. Partial structure and cyclisation possibilities of **1**, key ^{1}H–^{13}C HMBC correlations (H→C) ($CDCl_3$) of **C**.

membered ring with a concurrent epoxide between C-9 and C-10 (Fig. 1, A), the linkage between C-6 and C-10, and C-9 and C-13 would generate a pyrano[3,2-*b*]pyran system (Fig. 1, B), while joining C-6 and C-9, and C-10 and C-13 would result in a 2,2′-bifuran skeleton (Fig. 1, C). The first possibility was rejected because the ^{13}C NMR signals expected for the epoxide moiety would resonate considerably more upfield (ca. 51–55 ppm) than the observed ^{13}C shifts for C-9 (δ_C 79.2) and C-10 (δ_C 78.2).[32,33] Of the two remaining possibilities **B** and **C**, the 2,2′-bifuran system is clearly favoured based on the relative downfield chemical shifts for C-9 and C-10 (δ_C >76), while for a pyrano[3,2-*b*]pyran, the corresponding signals have been shown to suffer a distinct upfield shift (δ_C <76).[34–38]

The relative stereochemistry around each tetrahydrofuran ring of **1** was determined by a 2D NOESY experiment (Fig. 2), revealing that protons H-6, H-7 and H-9 were oriented *syn* with regard to H-8a based on mutual NOE correlations. In a similar manner, H-10, H-12 and H-13 were found to be oriented *syn* with regard to H11a, leaving the ethyl group in the *anti* position. The relative

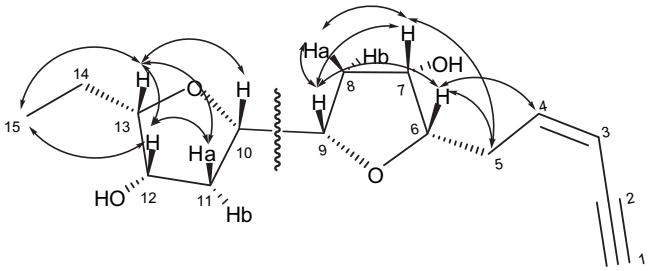


Figure 2. Observed ^{1}H–^{1}H NOESY (around each tetrahydrofuran ring) correlations for **1**.

stereochemistry across the two tetrahydrofuran rings was more difficult to define, and two possible diastereomers (Fig. 3, A and B) were modelled. The first diastereomer (Fig. 3A) was clearly favoured as indicated by the observation of strong NOE correlations of H-9 to both H-10 and H-11b, whereas the more stable conformer of second diastereomer (Fig. 3B) should not show any NOE between

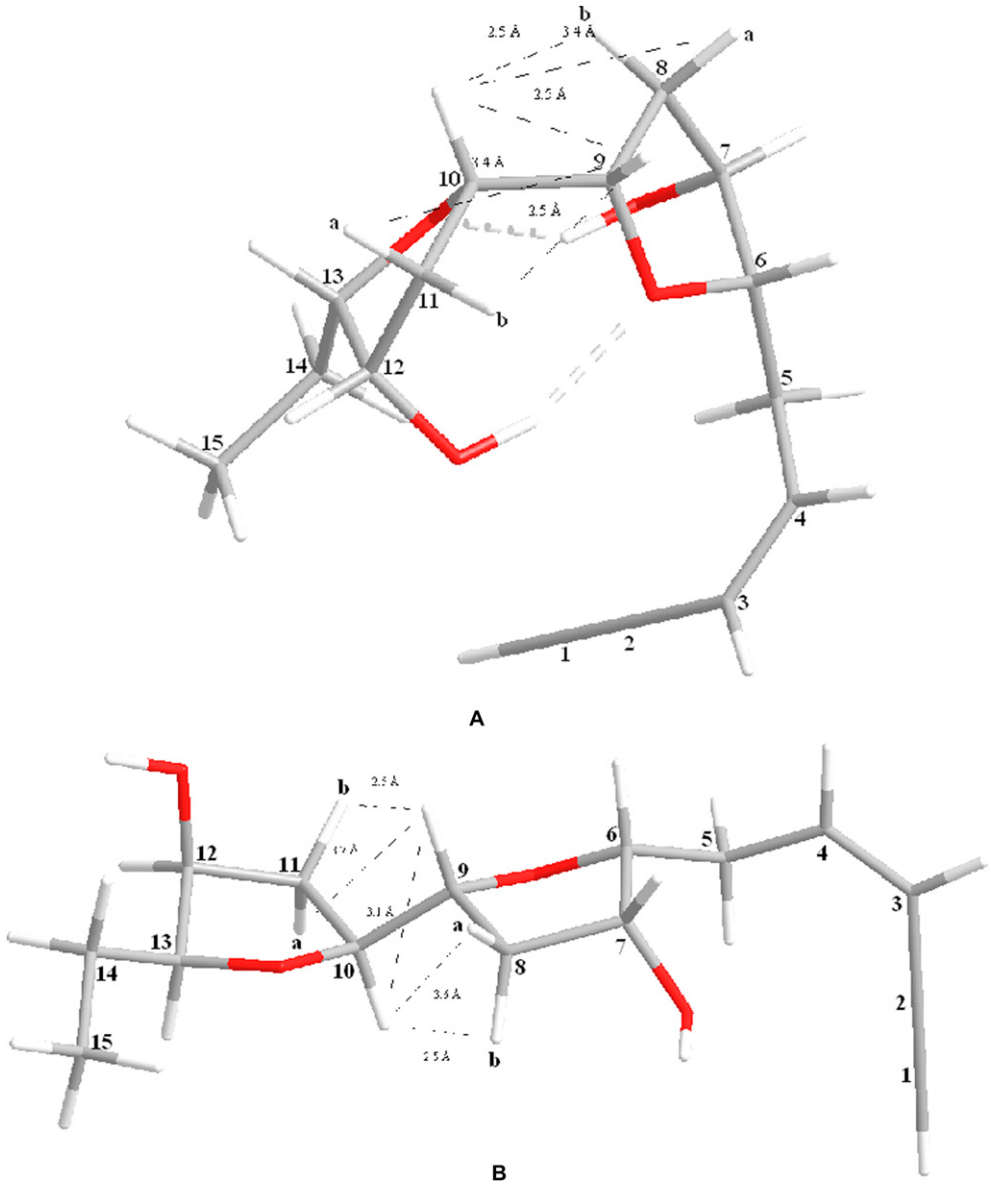


Figure 3. Possible conformers of compound **1**.

H-9 and H-10 due to their mutual *anti* relationship. Thus, **1** was identified as a new natural product for which the name laurefurenyne A is proposed, and its relative stereochemistry was assigned as (6*S**, 7*S**, 9*S**, 10*S**, 12*S**, 13*S**).

Compound **2** was obtained as colourless amorphous powder. By HRESIMS its molecular formula was determined as $C_{15}H_{22}O_4$, and consequently **2** was identified as an isomer of **1**. The IR as well as the ^{1}H and ^{13}C NMR data (Tables 1–3) of both compounds were very similar, except that **2** contained a *trans*-ene-yne function as evident from the coupling constant ($^3J_{H\text{-}3,H4}$=15.5 Hz, as opposed to 10.9 Hz for the *cis*-double bond in **1**). Consistent with this change in stereochemistry were the upfield shift of the acetylenic proton H-1 (δ 2.83) in **2** (compared to δ 3.12 in **1**), and the slight downfield shifts of H-3 and H-4 (δ 5.58 and 6.22, instead of δ 5.57 and 6.05 in the *cis* form).[33] Correspondingly, C-1 was found to resonate at δ 76.7 in **2**, whereas the corresponding signal appeared at δ 82.4 in **1**, which was in agreement with the reported shift differences for analogous *cis*- and *trans*-isomers in the literature.[39]

On the basis of the similarity in terms of the remaining coupling constants, ^{13}C NMR chemical shifts at all stereocentres, the comparable results of the 2D NOESY experiment and molecular modelling as described above, it was evident that **2** and **1** shared the same relative stereochemistry. As a result, **2** was identified as a new natural product for which we propose the name laurefurenyne B, and its relative stereochemistry was assigned as (3*E*, 6*S**, 7*S**, 9*S**, 10*S**, 12*S**, 13*S**).

Compound **3** was obtained as a colourless amorphous powder, displaying pseudomolecular ions peaks upon ESIMS at *m*/*z* 289.1 ($[M+Na]^+$) and *m*/*z* 305.1 ($[M+K]^+$). As in the case of laurefurenyne A (**1**) and B (**2**), the molecular formula of **3** was established as

Table 3
Selective cytotoxic activity of laurefurenynes A–F (**1**–**6**)

Sample Number	Murine						Human
	μg/disk	L1210	Colon 38	CFU-GM	H-116	H-125	CFU-GM
DMSO		0	0	0	0	0	0
1	60	0	0	0	0	0	0
2	60	0	0	0	0	0	0
3	60	200	200	0	200	100	0
4	60	0	50	0	0	0	0
5	60	0	100	0	0	0	0
6	60	250	450	400	200	100	0

$C_{15}H_{22}O_4$ by accurate mass measurement, while its IR spectrum was indicative of a terminal acetylene function. The presence of a *cis*-ene-yne function was readily detected from the ^1H and ^{13}C NMR spectra (Tables 1 and 2) as described above. Close inspection of the COSY and HSQC spectra of **3**, acquired in both $CDCl_3$ and DMSO-d_6 due to signal overlap, revealed a contiguous spin system comprising C-1–C-15 in a similar manner as depicted in Figure 1 for laurefurenyne A (**1**), but in the case of **3** the two hydroxyl groups were located at C-10 and C-12, instead of C-7 and C-12. This suggestion was confirmed by selective 1D TOCSY experiments as well as the HMBC spectrum (Supplementary data, S33 and S34). Moreover, the correlation from C-13 to H-7 as observed in the HMBC spectrum also established the ether linkage between the respective positions, leaving only C-6 and C-9 as the location of the second ether bridge required by the molecular formula. Thus, the core system in **3** was identified as a 2,8-dioxa-bicyclo[5.2.1]decane. In addition, the chemical shifts observed for C-6, C-9, C7 and C-13 were in agreement with reported data for structurally related compounds, and excluded other possible arrangements of the ether linkages, which would have required either an epoxide or a highly strained oxetane.[32,33,36,40]

The relative stereochemistry at centres C-6, C-7, C-9, C-10, C-12 and C-13 was established based on the results of a 2D NOESY experiment (Fig. 4A) and by comparing with previously reported compounds, and assigned as (3*Z*, 6*R**, 7*R**, 9*R**, 10*S**, 12*R**, 13*S**).[41,42] The global minimum energy conformation of compound **3** was calculated using Chem3D Ultra 10.0 (10,000 steps, global minimum shown in Fig. 4B). The molecular mechanics calculation was consistent with the outcome of the NOESY experiment, which showed H-6, H-7, H-9 oriented *syn* with regard to H-8b based on mutual NOE correlations. In a similar manner, NOE correlations were observed between H-7, H-10, H11b, H-12, H-14 and H-8a as well as correlations between H_3-15 and H-7. These correlations showed that H-7, H-10, H-11b, H-12, H_2-14 and H_3-15 were oriented *syn* with regard to H-8a, leaving H-13, OH-12 and OH-10 in *anti* positions. On this basis, **3** was identified as a new natural product, for which the name laurefurenyne C is suggested.

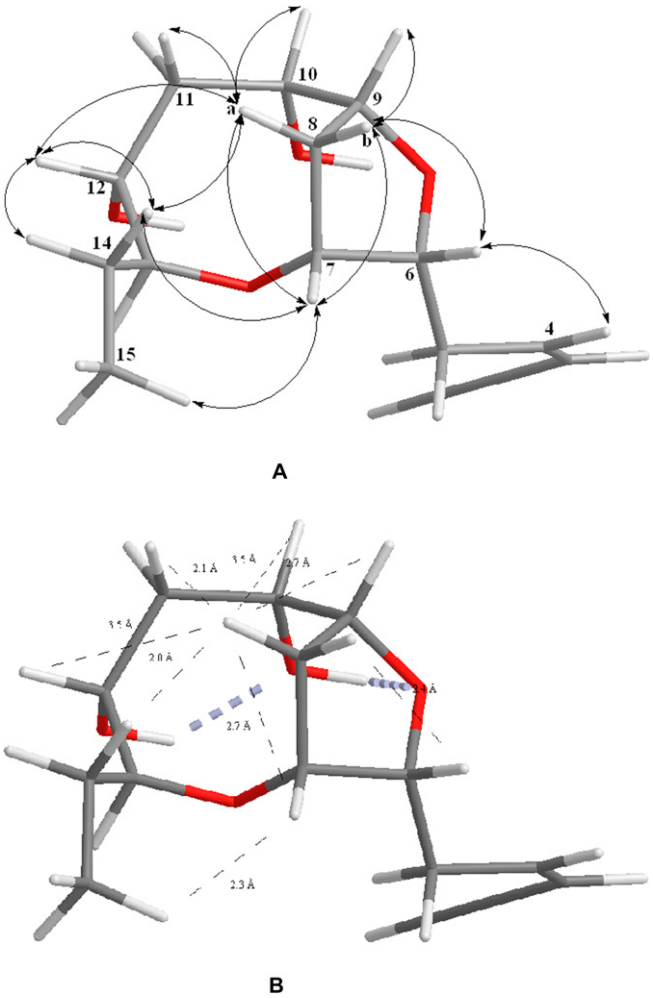


Figure 4. Key NOESY correlations (A) and global energy minimum (B) for compound **3**.

Compound **4** was obtained as colourless amorphous powder. By HRESIMS its molecular formula was determined as $C_{15}H_{22}O_4$, and thus **4** was identified as isomer of **3**. On the basis of the coupling constants ($^3J_{H\text{-}3,H4}$=16.0 Hz in **4**, as opposed to 11.2 Hz in **3**) and an analogous pattern of changes in ^1H and ^{13}C NMR chemical shifts (Tables 1 and 2) as described above for the isomeric pair **1** and **2**, **4** was identified as the 3-*trans*-isomer of **3**. Moreover, **4** and **3** were also assumed to share the relative stereochemistry as evident from virtually identical coupling constants, ^{13}C NMR chemical shifts at the stereocentres, and comparable results of the 2D NOESY experiment. Consequently, **4** was identified as laurefurenyne D, representing a new natural product.

Compound **5** was obtained as a colourless amorphous powder. LRESIMS showed a pseudomolecular ion peak at *m*/*z* 346.2/348.2 (1:1) for $[M+NH_4]^+$, indicating the presence of one bromine atom and a molecular weight of 328. By accurate mass measurement the molecular formula was established as $C_{15}H_{21}BrO_3$, thus requiring five degrees of unsaturation. As described above, the IR and ^1H as well as ^{13}C NMR data (Tables 1 and 2) suggested the presence of a *cis*-ene-yne function. Moreover, close inspection of the 1D and 2D NMR data spectra obtained for **5** with those of **3** suggested that the 12α-hydroxy function in **5** had been exchanged for a 12α-bromine substituent in **3**. This assumption was corroborated by the chemical shifts observed for position 12 (δ_H 4.55, δ_C 51.8), while the COSY, selective 1D TOCSY and HMBC spectra indicated an otherwise identical spin system as described above for **3**.

Mutual NOEs between H-6, H-7, H-8b and H-9 as well as between H-7, H-8a, H-10, H-11b, H-12, H_2-14 and H_3-15 (Fig. 5A) were analysed in conjunction with the global minimum energy conformation of compound **5**, calculated using Chem3D Ultra 10.0 (10,000 steps, global minimum shown in Fig. 5B). On this basis, the relative stereochemistry at centres C-6, C-7, C-9, C-10, C-12 and C-13 was assigned as (3*Z*, 6*R**, 7*R**, 9*R**, 10*S**, 12*R**, 13*S**).[42] Thus, **5** was identified as a new natural product, for which the name laurefurenyne E is suggested.

Compound **6** was obtained as colourless amorphous powder, with a molecular formula of $C_{15}H_{21}BrO_3$ as evident from accurate mass measurements, thus representing an isomer of **5**. Analogous differences in the NMR spectroscopic data (Tables 1 and 2) as described above for the pairs of *cis*- vs *trans*-enyne isomers **1** and **2**, or **3** and **4**, respectively, established that **6** was the *trans*-congener of **5** with otherwise identical relative stereochemistry, and consequently represented a new natural product, for which we propose the name laurefurenyne F.

Biogenetically, laurefurenynes A–F (**1**–**6**) are thought to be derived from a highly unsaturated straight chain C15 precursor (Fig. 6). In analogy to previous studies of related compounds in the genus *Laurencia*, we propose highly labile laurediols as putative intermediates,[43–45] which would undergo epoxide- or bromonium ion-mediated cyclisations to yield **1**–**6**.

2.3. Cytotoxic activity

The main reported biological activity of the reported acetogenins is antimicrobial activity[22,46] with no cytotoxic activity reported for the previously isolated vinyl acetylenic acetogenins. The total methanolic extract as well as individual compounds were

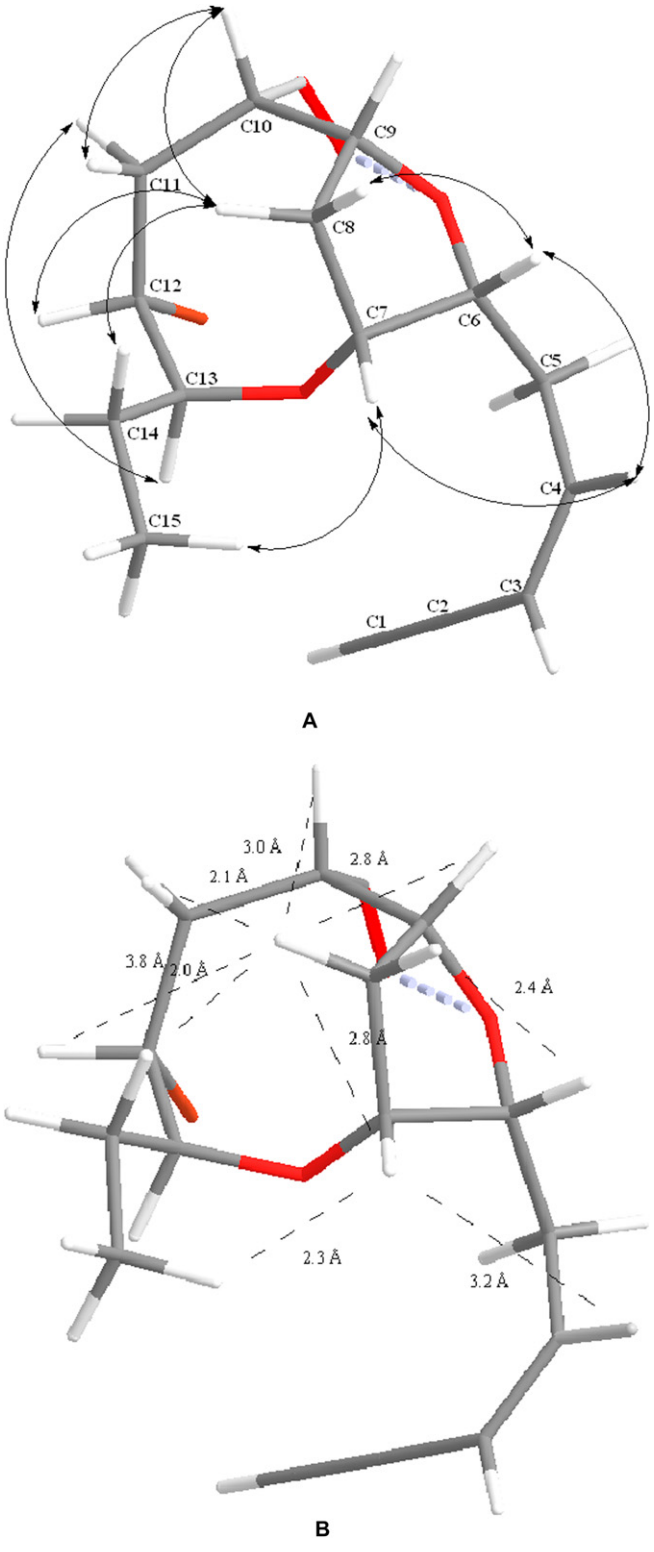


Figure 5. Key NOESY correlations (A) and global energy minimum (B) for compound **5**.

evaluated in vitro at the Ford Cancer Centre for their differential cytotoxicity in the soft agar assay.

The cytotoxic activities of isolated acetogenins were evaluated (Table 3) against leukaemia (murine L1210), and solid tumours (murine colon 38) as well as a murine and human normal cells (haematopoietic progenitor cell, CFU-GM). In this assay, those agents, which demonstrate a 250 or greater zone differential against one or more solid tumour cell lines and either the leukaemia or normal cells (CFU-GM) are considered solid tumour selective. Those that show a zone differential of between 150 and 250 units are considered to be moderately solid tumour selective.[47] Laurefurenyne A (**1**) and B (**2**) were found to be inactive against the murine cells. Laurefurenyne F (**6**) exhibited moderate non-selective cytotoxic activity against three solid tumours (murine colon 38, human colon H116 and human lung H125), leukaemia L1210 and human normal cells CFU-GM compared with its isomer, laurefurenyne E (**5**), which exhibited very weak activity against murine colon 38 only. Laurefurenyne C (**3**) showed moderate non-selective activity against leukaemia as well as solid tumours, but its isomer, laurefurenyne D (**4**), showed no significant activity against murine colon 38. However, none of them proved to be selective.

3. Conclusion

In the present study, six new acetogenins were isolated adding to the growing number of vinyl acetylenic acetogenins of this type isolated from the genus *Laurencia*. The difference in the oxygenation and halogenation pattern is of interest, but it is notable that the entire homologous series appears to be generated biosynthetically from the same precursor with the dominance of *cis*-ene-yne. The compounds exhibited non selective very weak cytotoxic activity.

4. Experimental

4.1. General experimental procedures

IR spectra were measured on an Ati Mattson Genesis Series FTIR machine. ^{1}H, ^{13}C and all 2D NMR experiments were recorded on a Varian Unity INOVA 400 MHz spectrometer, in $CDCl_3$ and DMSO-d_6. Low resolution electrospray mass spectra were obtained using a Perseptive Biosystems Mariner system, and high-resolution electrospray mass spectrum was obtained on a Finnigan MAT 900XLT at EPSRC National Mass Spectrometry Centre in Swansea, UK. HPLC separations were carried out using Phenomenex reversed-phase (C18, 10×250 mm) column and Agilent 1100 series gradient pump and monitored using a DAD G1315B variable-wavelength UV detector.

4.2. Biological material

A sample of *Laurencia* sp. (class Rhodophyceae, order Ceramiales, family Rhodomelaceae) was collected in 1991 from the Philippines and identified by Ernie Menez for the National Cancer Institute (NCI) where it was vouchered (J001460) and kept. Collected materials were stored at −20 °C. The aqueous extract (10 g) was shipped to our lab as a part of a collaborative screening program with the NCI.

4.3. Extraction and isolation

The frozen sample was ground in a coarse powder and extracted with H_2O. Solid materials were removed by centrifugation, and the resulting aqueous solution was freeze-dried to provide 10 g of the aqueous extract. The extract was stored at −20 °C until used. The crude aqueous extract was redissolved in the least amount of 50% aqueous methanol, filtered and desalting of the extract was carried out using a modification of the method described by West et al.[29,30] by passing it through DIAION HP20 column (25×1.5 cm) pre-equilibrated with MeOH/H_2O (1:1).

The eluent was diluted with H_2O (500 mL) and passed back through the column. Finally, the eluent was diluted with water (2 L) and passed back through the same column. The column was then washed with H_2O (1 L) and eluted with MeOH 100% (0.5 L). The methanolic eluent was concentrated under reduced pressure to

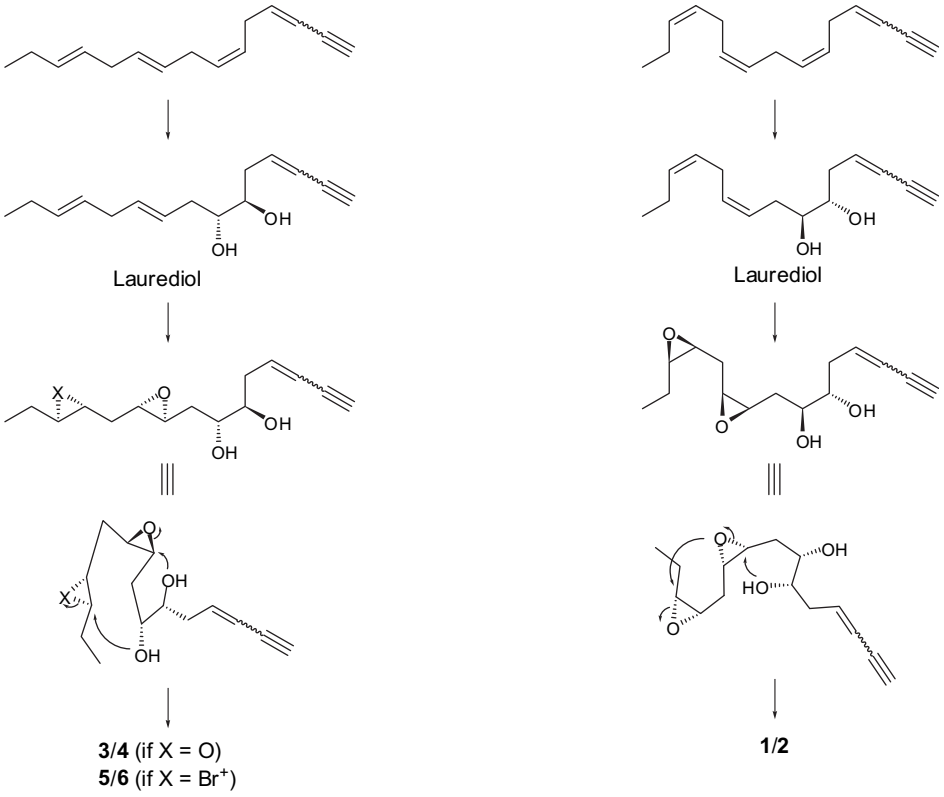


Figure 6. Proposed biogenetic scheme for Laurefurenynes A–F (**1**–**6**).

give 200 mg brown residue. The preliminary analysis by HPLC (C18) using a mixture of acetonitrile/water showed the presence of at least five compounds with promising ^{1}H NMR spectra. The extract was on Sephadex LH20 size exclusion chromatography using $CHCl_3$/MeOH (6:4) to get three promising fractions (A, B, C) that finally were purified using a reversed phase HPLC column using isocratic HPLC acetonitrile/water (30%, 40% and 60%) to yield laurefurenynes A (**1**) (11.3 mg), B (**2**) (2.5 mg), C (**3**) (8.2 mg), D (**4**) (4.4 mg), E (**5**) (4.8 mg) and F (**6**) (1.8 mg).

4.4. Selective cytotoxicity assay

Murine colon adenocarcinoma-38 (C-38) cells and the corresponding murine normal cells CFU (M) were inoculated on different Petri dishes. Circular-shaped filter disks (impregnated with test material at dosages from 50 to 100 μg/disk) were placed at the ends of different Petri dishes inoculated with the two cell types under investigation. 1 mg each of the laurefurenynes A–F (**1**–**6**) were solubilised in 0.25 mL 100% DMSO, from which 15 μL was impregnated into the filter disks and allowed to dry overnight before use. The plates were incubated for 7–10 days and examined by an inverted stereomicroscope (10×) for the measurement of 'zones of inhibition'. Zones of inhibition were defined by measuring the distance in millimetre from the edge of the filter disk to the beginning of normal-sized colony formation. The assay is designed to determine large differences in the relative sensitivity of leukaemias, solid tumours and normal cells for a given sample by comparison of the magnitude of inhibition zones. Generally, high values of inhibition zones are desirable. However, high values of inhibition zones for solid tumour cells were preferred over those for leukaemia cells (solid tumour selective). The diameter of the filter disk, 6.5 mm, is arbitrarily taken as 200 units therefore, 1 mm≡30.8 units. A zone of less than 100 units was taken as the extract was of insufficient activity to be of further interest. A difference in zones between solid tumour cells and either normal or leukaemia cells of 250 units defined solid tumour selective compounds.

*4.4.1. Laurefurenyne A (**1**).* Colourless amorphous substance; $[\alpha]_D^{25}$ −8 (*c* 0.1, MeOH); IR ν_{max} 3482, 3435, 3315, 2107, 1166, 1139, 1077, 911 cm^{-1}; NMR data (Tables 1–3); ESIMS *m*/*z* 267.2 $[M+H]^+$, 289.2 $[M+Na]^+$, 305.2 $[M+K]^+$; HRESIMS *m*/*z* 289.1410 ($[M+Na]^+$, calcd for $C_{15}H_{22}NaO_4$, 289.1410).

*4.4.2. Laurefurenyne B (**2**).* Colourless amorphous substance; $[\alpha]_D^{25}$ −13 (*c* 0.1, MeOH); IR ν_{max} 3477, 3440, 3312, 2115, 1158, 1124, 1086, 896 cm^{-1}; NMR data (Tables **1**–**3**); ESIMS *m*/*z* 267.2 $[M+H]^+$, 289.2 $[M+Na]^+$, 305.2 $[M+K]^+$; HRESIMS *m*/*z* 289.1411 ($[M+Na]^+$, calcd for $C_{15}H_{22}NaO_4$, 289.1410).

*4.4.3. Laurefurenyne **C** (**3**).* Colourless amorphous substance; $[\alpha]_D^{25}$ +20 (*c* 0.1, MeOH); IR ν_{max} 3463, 3430, 3300, 2100, 1135, 1085 cm^{-1}; NMR data (Tables 1–3); ESIMS *m*/*z* 289.1 $[M+Na]^+$, 305.1 $[M+K]^+$; HRESIMS *m*/*z* 289.1415 ($[M+Na]^+$, calcd for $C_{15}H_{22}NaO_4$, 289.1410).

*4.4.4. Laurefurenyne **D** (**4**).* Colourless amorphous substance; $[\alpha]_D^{25}$ +32 (*c* 0.1, MeOH); IR ν_{max} 3467, 3423, 3311, 2104,1141, 1052 cm^{-1}; NMR data (Tables **1**–**3**); ESIMS *m*/*z* 289.2 $[M+Na]^+$, 305.2 $[M+K]^+$; HRESIMS *m*/*z* 289.1412 ($[M+Na]^+$, calcd for $C_{15}H_{22}NaO_4$, 289.1410).

*4.4.5. Laurefurenyne **E** (**5**).* Colourless amorphous substance; $[\alpha]_D^{25}$ +11 (*c* 0.1, MeOH); IR ν_{max} 3429, 3292, 2110, 1129, 1063 cm^{-1}; NMR data (Tables **1**–**3**); ESIMS *m*/*z* 346.2/348.2 (1:1) $[M+NH_4]^+$; HRESIMS *m*/*z* 346.1014 ($[M+NH_4]^+$, calcd for $C_{15}H_{25}BrNO_3$, 346.1012).

*4.4.6. Laurefurenyne **F** (**6**).* Colourless amorphous substance; $[\alpha]_D^{25}$ +17 (*c* 0.1, MeOH); IR ν_{max} 3433, 3305, 2105, 1121, 1056 cm^{-1}; NMR data (Tables **1** and **2**); ESIMS *m*/*z* 351.1/353.1 (1:1) $[M+Na]^+$;

HRESIMS m/z 351.0568 ($[M+Na]^+$, calcd for $C_{15}H_{21}BrNaO_3$ 351.0566).

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2010.02.041.

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