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Bioactive constituents of *Artemisia monosperma*

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Abstract

During a study on the chemistry and biological activity of Kuwaiti plants, new metabolites including 4,6-dihydroxy-3-[3′-methyl-2′-butenyl]-5-[4″-hydroxy-3″-methyl-2″-butenyl]-cinnamic acid (**1**), the 3*R*,8*R* stereoisomer of the C_{17} polyacetylene dehydrofalcarindiol (**2**) and a C_{10} polyacetylene glucoside (**3**) were characterised by spectroscopic means. Additionally, the previously characterised natural products 1,3*R*,8*R*-trihydroxydec-9-en-4,6-yne (**4**), spathulenol (**5**) and eriodyctiol-7-methyl ether (**6**) were also isolated.

Compounds **2**, **3**, and **4** were evaluated for their ability to inhibit the enzyme 12-lipoxygenase and **3** and **4** showed moderate activity at 30 μg/ml. Compound **2** was evaluated against a panel of colorectal and breast cancer cell lines and IC_{50} values ranged from 5.8 to 37.6 μg/ml. Against a panel of fast-growing mycobacteria and a standard ATCC strain of *Staphylococcus aureus*, compound **6** exhibited minimum inhibitory concentrations in the range of 64–128 μg/ml.

Keywords: *Artemisia monosperma*; Kuwait; 12-Lipoxygenase; *Mycobacterium*; *Staphylococcus aureus*, cytotoxic; polyacetylene

1. Introduction

The native flora of Kuwait consists of approximately 400 vascular plants (Daoud and Al-Rawi, 1985; Al-Rawi, 1987), with the family Asteraceae being the second largest taxon, particularly noticeable in the spring with their colourful flowers covering the entire desert. As part of a continuing study into the chemistry and biological activity of Kuwaiti plants (Gibbons et al., 1999; Gibbons et al., 2000; Stavri et al., 2004), we have studied *Artemisia monosperma* Del. (Asteraceae), a plant that has restricted distribution in Kuwait, being found growing along the Wadi-Al-Batin, a dry river bed running in a north-westerly direction along the border with Iraq. Previous phytochemical studies on this species have yielded polyyne, sesquiterpene (Stavri et al., 2004) and acetophenone natural products (Bohlmann and Ehlers, 1977). In this paper we detail the characterisation of three new metabolites (**1**–**3**) and the isolation of known metabolites including the sesquiterpene spathulenol, an hydroxylated polyyne and the flavonoid eriodyctiol-7-methyl ether. Where sufficient material permitted, compounds were evaluated for their activity in 12-lipoxygenase, antibacterial and cytotoxicity assays.

2. Results and discussion

Vacuum liquid chromatography of the chloroform extract of the aerial parts of *A. monosperma* led to the

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isolation of a colourless oil (**1**). HREIMS of **1** suggested a molecular formula of $C_{19}H_{24}O_5$ $[M]^+$ (332.1623). The ^1H NMR spectrum (Table 1) provided signals for four olefinic protons and one aromatic proton (δ_H 7.20). Two of the olefins were doublets and coupled to each other with large coupling constants indicative of a *trans* double bond (δ_H 7.70 d, J = 16.0 Hz, δ_H 6.30 d, J = 16.0 Hz). The remaining two olefins appeared as triplets with fine splitting and were reminiscent of the olefinic protons of prenyl (dimethyl allyl) substituents (δ_H 5.61, δ_H 5.31) (Nayar and Bhan, 1972). Three methyl singlets and two protons of an oxymethylene group also appearing as a singlet were detected in the ^1H NMR spectrum, confirming the presence of two prenyl groups. The ^{13}C NMR spectrum provided signals for 19 carbons, including 8 quaternary carbons of which two were oxygen bearing, 5 were olefinic/aromatic carbons and one was a carbonyl group (δ_C 172.1, C-9). With the presence of two prenyl substituents (10 carbons), a *trans* double bond and aromatic quaternary carbons, the NMR data indicated the presence of a prenylated *trans*-cinnamate structure.

Assuming a cinnamate moiety, it was possible by COSY, HMQC and HMBC spectra to unambiguously assign all resonances in the molecule and show that **1** was a diprenylated-dihydroxycinnamic acid. In the COSY spectrum, the *trans* olefin (H-8), coupled to its olefinic partner (H-7) and in the HMBC spectrum gave a ^2J correlation to a carboxyl carbonyl at C-9. The attachment of this three carbon chain to the aromatic ring was achieved by a ^3J correlation in the HMBC spectrum between H-8 and an aromatic quaternary carbon (C-1, δ_C 126.5). A further ^3J signal between H-7 and C-2 placed an aromatic methine group here. The proton associated with this aromatic methine carbon then provided ^3J signals to two oxygen-bearing quaternary aromatic carbons positioned at C-4 and C-6 of the aromatic ring. The molecular formula and downfield nature of the ^{13}C resonances for these carbons confirmed that hydroxyl groups should be placed at these positions. In the HMBC spectrum, H-2 also gave a ^3J signal to a methylene group (C-1′) of a prenyl moiety. This confirmed that one of the prenyl groups should be attached at C-3 of the aromatic ring. From the ^1H NMR spectrum, H-2 appeared as a small doublet (J = 3.5) which coupled to H_2-1′ in the COSY spectrum. Therefore the coupling constant of 3.5 Hz can be attributed to allylic coupling between H-2 and H_2-1′. The methylene protons (H_2-1′) coupled to an olefinic proton (H-2′) in the COSY spectrum and in the HMBC spectrum H_2-1′ also gave a ^3J correlation to an olefinic quaternary carbon (δ_C 135.9) placing this at C-3′. Two methyl groups (both at δ_H 1.80 s) gave ^2J signals to C-3′ placing these groups on this quaternary carbon and completing the first prenyl substituent. Further signals in the HMBC spectrum included those for H_2-1′ to C-3, the aromatic carbon to which it is attached and a ^3J signal to the oxygen-bearing aromatic quaternary carbon, C-4. The second prenyl group was placed at C-5 based on a ^2J correlation to this carbon from protons of a further methylene (H_2-1″). In the COSY spectrum H_2-1″ coupled to an olefinic proton (H-2″) and in the HMBC spectrum H_2-1″ provided a ^3J correlation to an olefinic quaternary carbon (C-3″). A methyl singlet (δ_H 1.80, H_3-5″) and a downfield methylene singlet (δ_H 4.08, H_2-4″) both gave ^2J HMBC correlations to C-3″, therefore these groups must be directly attached to this carbon. An hydroxyl was placed on the H_2-4″ methylene carbon, which would account for the downfield shift of both the ^1H and ^{13}C signals for this group. This completed resonances for the second prenyl moiety.

A sharp singlet at δ_H 3.96, had no correlation in the HMQC spectrum and must therefore be an hydroxyl group. This signal in the HMBC spectrum gave a ^2J correlation to an aromatic quaternary carbon (C-6), fixing its position. Both prenyl groups must be *ortho* with respect to C-4 as both methylene protons at H_2-1′ and H_2-1″ gave ^3J HMBC correlations to this carbon.

The structure of **1** was confirmed by correlations detected in the NOESY spectrum (Fig. 1). A correlation between H-2 and H_2-1′ placed these protons in close spatial proximity. A second NOE between H-2 and H-7 also meant these protons were in close association. Two key NOEs determined the stereochemistry of the second prenyl group. Firstly, an interaction between H_2-1″ and H_3-5″ placed these protons on the same face of this group (*cis*). A second NOE between the olefinic proton H-2″ and the oxymethylene protons H_2-4″ further confirmed the proposed stereochemistry of this prenyl group. Natural product **1** is therefore assigned as

Table 1
^1H (500 MHz) and ^{13}C NMR (125 MHz) spectral data and ^1H–^{13}C long-range correlations of **1** recorded in $CDCl_3$

Position	^1H	^{13}C	^2J	^3J
1	–	126.5		
2	7.20 *d* (3.5)	128.5		C-4, C-6, C-7, C-1′
3	–	127.7		
4	–	155.3		
5	–	127.2		
6	–	146.7		
7	7.70 *d* (16.0)	147.1	C-8	C-2, C-9
8	6.30 *d* (16.0)	114.2	C-9	C-1
9	–	172.1		
1′	3.37 *d* (7.0)	30.0	C-2′, C-3	C-3′, C-4
2′	5.31 *t* (7.0)	121.0	C-1′	C-4′, C-5′
3′	–	135.9		
4′	1.80 *s*	17.9	C-3′	C-2′, C-5′
5′	1.80 *s*	25.8	C-3′	C-2′, C-4′
1″	3.40 *d* (7.5)	28.5	C-2″, C-5	C-3″, C-4
2″	5.61 *t* (7.0)	122.8	C-1″	C-4″, C-5″
3″	–	137.1		
4″	4.08 *s*	68.5	C-3″	C-2″, C-5″
5″	1.80 *s*	13.8	C-3″	C-2″, C-4″
6-OH	3.96 *s*	–	C-6	

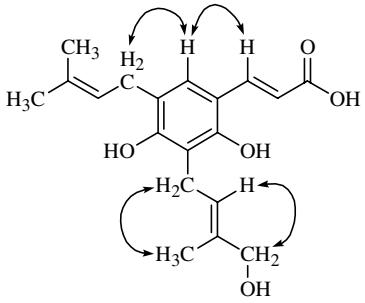


Fig. 1. Key NOE correlations for compound **1**.

4,6-dihydroxy-3-[3′-methyl-2′-butenyl]-5-[4″-hydroxy-3″-methyl-2″-butenyl]-cinnamic acid and is reported here for the first time.

Compound **2** was isolated as a pale yellow oil from the hexane extract. A molecular formula of $C_{17}H_{22}O_2$ was established by ESI-MS in the positive mode $[M + H]^+$ (259.0). The ^1H and ^{13}C NMR spectra were highly similar to those of falcarindiol, a widely occurring polyacetylenic natural product commonly found in the Apiaceae plant family and recently evaluated by us against multidrug-resistant strains of *Staphylococcus aureus* (Lechner et al., 2004). **2** differed from falcarindiol as it exhibited signals in the ^1H and ^{13}C NMR spectra (Table 2) indicating the presence of two vinyl groups. This accounted for the presence of an additional downfield methylene group at δ_H 2.05, (H_2-15). Four acetylenic carbons, two remaining olefins and two oxymethine groups were also present and similar to those found in falcarindiol, and by extensive 1 and 2-dimensional NMR studies (Table 2) we were able to show that compound **2** was the closely related metabolite, dehydrofalcarindiol. The data are in close agreement with those previously published (Bernart et al., 1996).

The absolute stereochemistry of **2** was determined by Mosher′s ester methodology (Seco et al., 2004), by esterifying the two hydroxyl groups attached to the chiral carbons with either *R*- or *S*-MPA (methoxyphenylacetic acid). The $\Delta\delta^{R,S}$ values ($\delta_R - \delta_S$) for H_2-1 (+0.19 and +0.15 ppm) and H-2 (+0.17 ppm) and also H-9 (+0.02 ppm) and H-10 (+0.10 ppm) were positive, indicating *R*-stereochemistry at both C-3 and C-8. This is the first report of the absolute stereochemistry of dehydrofalcarindiol as 3*R*,8*R*.

Natural product **3** was isolated as a pale yellow oil from the methanol extract by reverse-phase PTLC. A molecular formula of $C_{16}H_{22}O_8$ was assigned by ESI-MS $[M + Na]^+$ (365.1). The ^1H and ^{13}C NMR spectra (Table 2) provided resonances with similarity to **2** and almost identical to those found in 1,3*R*,8*R*-trihydroxy-dec-9-en-4,6-yne (**4**), a polyacetylenic natural product recently reported by us from this species (Stavri et al., 2004) but additional resonances were present for a hexose sugar. Signals for the aglycone moiety indicated the presence of an *exo*-methylene, an olefin, two oxymethine groups, a methylene and oxymethylene groups as well as four acetylenic quaternary carbons.

Table 2
^1H (500 MHz) and ^{13}C NMR (125 MHz) spectral data for **2** and **3** recorded in $CDCl_3$ and CD_3OD respectively

Position	^1H	^{13}C
2		
1	5.25 *dt* (10.5, 1.0)	117.3
	5.45 *dt* (17.0, 1.0)	
2	5.93 *ddd* (15.5, 10.5, 1.5)	135.8
3	4.93 *m*	63.5
4	–	78.3
5	–	70.3
6	–	68.7
7	–	79.8
8	5.20 *d* (8.5)	58.6
9	5.53 *ddt* (10.5, 8.5, 1.0)	127.8
10	5.61 *ddt* (10.5, 7.5, 1.0)	134.5
11	2.11 *m*	27.6
12	1.38 *m*	29.1
13	1.33 *m*	28.7
14	1.38 *m*	28.6
15	2.05 *m*	33.7
16	5.81 *ddt* (17.0, 10.0, 7.0)	139.0
17	4.95 *m*	114.3
	5.00 *dd* (17.5, 1.5)	
3		
1	3.73 *dt* (11.0, 6.0)	66.7
	3.99 *dt* (10.0, 6.0)	
2	1.97 *dd* (13.0, 7.0)	38.9
3	4.63 *t* (7.0)	60.0
4	–	82.0
5	–	68.9
6	–	70.1
7	–	79.5
8	4.88 *m*	63.8
9	5.91 *ddd* (17.0, 10.0, 5.5)	138.0
10	5.20 *dt* (10.0, 1.5)	116.6
	5.41 *dt* (17.0, 1.5)	
1′	4.27 *d* (7.5)	104.5
2′	3.17 *dd* (9.0, 8.0)	75.1
3′	3.35 *dd* (9.0, 8.0)	77.9
4′	3.28 *m*	71.6
5′	3.50 *m*	78.0
6′	3.68 *dd* (12.0, 5.0)	62.7
	3.87 *dd* (11.5, 1.5)	

In the COSY spectrum of **3**, the *exo*-cyclic methylene protons coupled to the olefin proton which also coupled to the first oxymethine signal (δ 4.88, C-8). This oxymethine then exhibited ^2J and ^3J correlations in the HMBC spectrum to two acetylenic carbons (C-7 and C-6). Further couplings in the COSY spectrum included those between the oxymethylene (C-1), methylene (C-2) and remaining oxymethine proton (δ 4.63, C-3) (Table 2), which resulted in a CH(O)-CH_2CH_2O system. In the HMBC spectrum the oxymethine resonance of this spin system also coupled to two acetylenic quaternary carbons (C-4 and C-5). The shielded nature of the two

triple bonds suggested that they must be conjugated and connected and this is a common feature in many acetylenes and was also seen with dehydrofalcarindiol (**2**). The deshielded nature of C-3 and C-8 and the ESIMS suggested that hydroxyl groups be placed at these positions. These data therefore confirmed that the aglycone is in fact 1,3,8-trihydroxydec-9-en-4,6-yne.

The hexose gave signals for four oxymethine groups, an oxymethylene and an anomeric carbon (δ_C 104.5) (Table 2). The COSY spectrum provided correlations between the anomeric proton (H-1′) and H-2′, H-2′ to H-3′, H-3′ to H-4′, H-4′ to H-5′ and H-5′ to the oxymethylene protons (H_2-6′). The coupling between the anomeric proton and H-2′ was large (7.5 Hz) as was the coupling between H-2′ and H-3′ (9.0 Hz). H-3′ appeared as a double doublet with a second coupling of 8.0 Hz indicating axial configuration for H-1′, H-2′, H-3′ and H-4′. An NOE between H-1′ and H-5′ indicated axial configuration for H-5′ and this was further confirmed by a second NOE between H-3′ and H-5′ and therefore the hexose was assigned as glucose. The point of attachment of the glucose moiety to the aglycone was shown to be at C-1 of the polyacetylene due to the presence of a ^3J correlation between the anomeric proton and the oxymethylene carbon (C-1) in the HMBC spectrum. Compound **3** is therefore assigned as 3(ζ),8(ζ)-dihydroxydec-9-en-4,6-yne-1-*O*-β-D-glucopyranoside. It is likely that the absolute stereochemistry of the hydroxyl groups at positions 3 and 8 is the same as that previously determined for the aglycone using Mosher's ester methodology, although the presence of glucose in the molecule has added to the difficulty in conducting the stereochemical analysis and therefore absolute configuration has not been assigned at these positions.

Compounds **4**, spathulenol (**5**) and eriodyctiol-7-methyl ether (**6**), were also isolated and characterised by direct comparison with the literature (Stavri et al., 2004; Inagaki and Abe, 1985; Wollenweber, 1981).

Acetylenic compounds are known to have activity against oxygenase enzymes (Resch et al., 2001; Liu et al., 1998) and compounds **2**, **3** and **4** were tested for their ability to inhibit 12-lipoxygenase (Table 3). This enzyme is implicated in many disorders including cancer, psoriasis, atherosclerosis, rheumatoid arthritis and epilepsy (Yoshimoto and Takahashi, 2002; Nie and Honn, 2002; Virmani et al., 2001; Müller, 1994). Compound **3** showed a moderate inhibitory dose dependent activity whereas **4** revealed an inverse dose activity relationship. The reason for this effect could not be elucidated during the present investigation. However, it has been shown previously that collagen or CRP (collagen-related peptide) mediated platelet 12-LOX product generation was potentiated by inhibition of PKC by the specific inhibitor chelerythrine (Coffey et al., 2004).

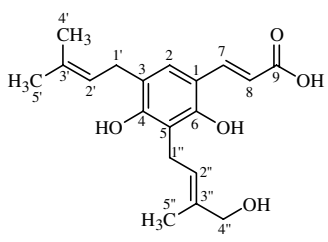
Table 3
12-Lipoxygenase inhibitory activity of **2**–**4**

Compound	Concentration (μg/ml)	% Inhibition[a]
2	10	0
2	30	0
3	10	8.11
3	30	25.81
4	10	32.17
4	30	18.15
Baicalein	10	56.23

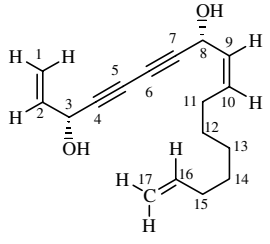
[a] % inhibition is calculated by comparing 12(*S*)-HETE contents of samples containing the test compound versus control (solvent instead of inhibitor) using the following equation: % inhibition = {[12(*S*)-HETE]$_{control}$−[12(*S*)-HETE]$_{sample}$}/[12(*S*)−HETE]$_{control}$.

The 3(ζ),8(ζ) isomer of compound **2** has been shown to have cytotoxicity against human hepatocellular and epidermoid carcinoma cell lines with IC_{50} values of 9.3 and 29.8 μg/ml, respectively (Setzer et al., 1995). Compound **2** was therefore tested against a panel of colorectal and breast cancer cell lines (Table 4) to ascertain its IC_{50}. The greatest efficacy (lowest IC_{50} value) was seen with the breast cancer line MCF7, and COLO320DM was the most sensitive of the colorectal cell lines. Interestingly, this was the opposite of how this cell line responds to doxorubicin where it is highly resistant in comparison to LS174T and SKCO1.

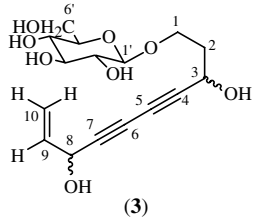
All compounds were also evaluated against fast-growing strains of *Mycobacterium* and a standard ATCC strain of *S. aureus* (Table 5). Only eriodyctiol-7-methyl ether (**6**) was active against the bacteria tested, with moderate minimum inhibitory concentrations ranging from 64 to 128 μg/ml.



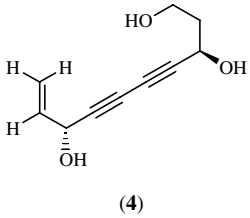
(**1**)



(**2**)



(**3**)



(**4**)

3. Experimental

3.1. General experimental procedures

NMR spectra were recorded on a Bruker AVANCE 500 MHz spectrometer. Chemical shift values (δ) were

Table 4
Cytotoxicity evaluation of compound **2**

Cell line	Type of cancer	Number of repeat assays	IC_{50} (μg/ml)[a]	Dilutions of DMSO causing toxicity	Doxorubicin IC_{50} (ng/ml)[c]
LS174T	Colorectal	3	14.8 (7.2)[b]	1/20–1/40	324 (100)[+]
SKCO1	Colorectal	3	13.3 (5.4)	1/20–1/40	28.5 (10)
COLO320DM	Colorectal	2	9.6	1/20–1/40	1163 (168)
WIDR	Colorectal	2	10.9	1/20–1/80	NT
MDA231	Breast	1	37.6	1/20–1/40	NT
MCF7	Breast	2	5.8	1/20–1/40	NT

NT, not tested.
[a] **2** was tested from 500 to 1.95 μg/ml and the DMSO control from 1/10 to 1/2560 dilutions to equate with the amount that would be present in the drug dilutions.
[b] Mean values with SDs in parentheses.
[c] Values for doxorubicin from previous studies (Ford et al., 2001).

Table 5
Antibacterial activity of **6**

Compound	*M. fortuitum* ATCC 6841	*M. phlei* ATCC 11758	*M. aurum* ATCC 23366	*M. smegmatis* ATCC 14468	*S. aureus* ATCC 25923
6	128	>128	64	128	128
Ethambutol	4	2	1	0.5	–
Isoniazid	0.5	2	2	2	–
Norfloxacin	–	–	–	–	0.5

–, Not tested

reported in parts per million (ppm) relative to appropriate internal solvent standard and coupling constants (*J* values) are given in Hertz. Mass spectra were recorded on VG ZAB-SE instrument (FAB-EIMS) and Finnigan navigator (ESIMS). IR spectra were recorded on a Nicolet 360 FT-IR spectrophotometer and UV spectra on a Perkin-Elmer Lambda 15 UV/Visible spectrophotometer.

3.2. Plant material

Artemisia monosperma was collected from the sandy gullies in north-western Kuwait that are bordered with sandstone ridges. These gullies open westwards into the plains of the Wadi Al-Batin that extend beyond the border into Iraq. The material was identified by K. T. M. A voucher specimen (KTM 4225, collected by K.T. Mathew and S. Gibbons on the 19th of February, 1999) is deposited at the Kuwait University Herbarium (KTUH).

3.3. Extraction and isolation

285 g of air-dried and powdered aerial parts were extracted in a Soxhlet apparatus using sequential extraction by hexane (3L), chloroform (3L) and finally methanol (3L). The chloroform extract (10 g) was subjected to vacuum liquid chromatography (VLC) on silica gel (15 g) eluting with hexane containing 10% increments of ethyl acetate to yield 12 fractions. The fraction eluted with 100% ethyl acetate was further purified by Sephadex LH-20 chromatography eluting with methanol. Final purification by multiple preparative TLC (2 times) (silica gel; toluene:EtOAc:AcOH, 30:68:2) afforded **1** (5.4 mg). Vacuum liquid chromatography of the hexane extract (10.0 g) on silica gel (15 g) eluting with hexane containing 10% increments of ethyl acetate yielded 12 fractions. The fraction eluted with 40% ethyl acetate was subjected to flash chromatography, eluting with hexane:ethyl acetate (8:2). Further purification by multiple PTLC (3 times) (silica gel; hexane: EtOAc; 8:2) afforded **2** (14 mg).

Vacuum liquid chromatography of the methanol extract (8.2 g) on silica gel (12 g) eluting with ethyl acetate containing 10% increments of methanol yielded 12 fractions. The fraction eluted with 70% ethyl acetate was further purified by C_{18} solid phase extraction (eluting with water:methanol; 8:2), followed by reverse phase PTLC (water:methanol; 7:3) afforded **3** (18 mg).

3.4. Preparation of Mosher's esters for compound 2

Compound **2** (5.26 mg) was dissolved in 525 μl of $CDCl_3$. 100 μl of this stock preparation was added to a vial containing 1.9 mg of *R*- or *S*-MPA, 16.3 mg of PS-carbodiimide resin (Argonaut Inc. Foster City CA, USA), 0.4 mg of DMAP and 0.75 mL of $CDCl_3$. The reaction mixtures were agitated overnight on a Turbula mixer. Each mixture was then applied to individual preconditioned silica solid phase extraction cartridges. The products were eluted from the cartridges using CH_2Cl_2 and evaporated to dryness under N_2.

3.5. 12-Lipoxygenase assay

The 12(*S*)-LOX inhibitory assay was conducted in vitro using human platelets as reported previously (Schneider et al., 2004). The platelets prepared from human blood were preincubated with reduced glutathione and the test compound or the positive control, baicalein. The suspensions were further incubated in the presence of arachidonic acid for 7 min and the reaction was terminated by adding 2 M HCl. 12(*S*)-HETE was quantified using a Correlate-EIA™-12(*S*)-HETE-kit (Assay Designs, Ann Arbor). The concentrations of 12(*S*)-HETE were calculated in relation to a standard 12(*S*)-HETE. The mean values of two measurements were taken and tests were conducted three times.

3.6. Cytotoxicity assay

The in vitro sensitivity of 6 human cancer cell lines (colorectal – LS174T, SKCO1, COLO320DM, WIDR: breast – MDA231, MCF7) to **2** was tested in an MTT assay, performed essentially as previously described (Ford et al., 2001). Cell lines were grown as monolayers in Eagle's MEM (MDA231, MCF7, WIDR, LS174T, SKCO1) or RPMI1640 (COLO320DM) containing 10% foetal calf serum and were maintained in culture as previously described (Ford et al., 1996). Briefly, cell lines were trypsinised and 10^4 cells plated into microplate wells, allowed a 24-h recovery, exposed to concentrations of **2** or equivalent dilutions of DMSO for 24 h followed by washing, a 24-h recovery period and then termination of the assay.

3.7. Antibacterial assay

Mycobacterium species were acquired from the NCTC. *S. aureus* strain ATCC25923 was the generous gift of E. Udo (Kuwait University, Kuwait). Mycobacterial strains were grown on Columbia Blood agar (Oxoid) supplemented with 7% defibrinated Horse blood (Oxoid) and incubated for 72 h at 37 °C prior to minimum inhibitory concentration (MIC) determination. *S. aureus* ATCC 25923 was cultured on nutrient agar and incubated for 24 h at 37 °C prior to MIC determination. Bacterial inocula equivalent to the 0.5 McFarland turbidity standard were prepared in normal saline and diluted to give a final inoculum density of 5×10^5 cfu/ml. The inoculum (125 μL) was added to all wells and the microtitre plate was incubated at 37 °C for 18 h for *S. aureus* and 72 h for *M. fortuitum*, *M. smegmatis* and *M. phlei*. For *M. aurum* the plate was incubated for 120 h. The MIC was recorded as the lowest concentration at which no bacterial growth was observed as previously described (Gibbons and Udo, 2000).

Ethambutol and isoniazid were used as positive controls for the mycobacterial strains and norfloxacin was used for *S. aureus*.

3.8. 4,6-Dihydroxy-3-[3′-methyl-2′-butenyl]-5-[4″-hydroxy-3″-methyl-2″-butenyl]-cinnamic acid (*1*)

Colourless oil; UV ($CHCl_3$) λ_{max} (log ε): 314 (8.15), 241 (7.86) nm; IR ν_{max} (thin film) cm^{-1}: 3328, 2915, 1684, 1635, 1473, 1270, 1199, 982; ^1H NMR and ^{13}C NMR ($CDCl_3$): see Table 1; HREI-MS (*m*/*z*): 332.1623 $[M]^+$ (calc. for $C_{19}H_{24}O_5$, 332.1623).

3.9. 3R,8R-Dehydrofalcarindiol (*2*)

Pale yellow oil; $[\alpha]_D^{25}$ + 39.8° (*c* 2.66, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε): 284 (8.27), 268 (8.52), 254 (8.36), 244 (8.25) nm; IR ν_{max} (thin film) cm^{-1}: 3351, 2929, 2856, 2232, 2146, 1641, 1457, 1285, 993; ^1H NMR and ^{13}C NMR ($CDCl_3$): see Table 2; ESI-MS (*m*/*z*): 259.0 $[M + H]^+$.

3.10. 3(ζ),8(ζ)-Dihydroxydec-9-en-4,6-yne-1-O-β-D-glucopyranoside (*3*)

Pale yellow oil; $[\alpha]_D^{25}$ − 45.3° (*c* 0.75, CH_3OH); UV (CH_3OH) λ_{max} (log ε): 265 (7.97), 212 (8.06); IR ν_{max} (thin film) cm^{-1}: 3376, 2889, 1654, 1244, 1077, 1035; ^1H NMR and ^{13}C NMR (CD_3OD): see Table 2; HRESI-MS (*m*/*z*): 365.1190 $[M + Na]^+$ (calc. for $C_{16}H_{22}O_8Na$, 365.1212).

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References

Al-Rawi, A., 1987. Flora of Kuwait, vol. 2. Alden Press, Oxford.

Bernart, M.W., Cardellina II, J.H., Balaschak, M.S., Alexander, M.R., Shoemaker, R.H., Boyd, M.R., 1996. Cytotoxic falcarinol oxylipins from *Dendropanax arboreus*. Journal of Natural Products 59, 748–753.

Bohlmann, F., Ehlers, D., 1977. Ein neues *p*-hydroxyacetophenonderivat aus *Artemisia monosperma*. Phytochemistry 16, 1450–1451.

Coffey, M.J., Jarvis, G.E., Gibbins, J.M., Coles, B., Barrett, N.E., Wylie, O.R.E., O'Donnell, V.B., 2004. Platelet 12-lipoxygenase activation via glycoprotein VI Involvement of multiple signaling pathways in agonist control of H(P)ETE synthesis. Circulation Research 94, 1598–1605.

Daoud, H.S., Al-Rawi, A., 1985. The Flora of Kuwait, vol. 1. KPI Publishers, London.

Ford, C.H.J., Tsaltas, G.C., Osborne, P.A., Addetia, K., 1996. Novel flow cytometric analysis of the progress and route of internalization of a monoclonal anti-carcinoembryonic antigen (CEA) antibody. Cytometry 23, 228–240.

Ford, C.H.J., Osborne, P.A., Rego, B.G., Mathew, A., 2001. Bispecific antibody targeting of doxorubicin to carcinoembryonic antigen-expressing colon cancer cell lines in vitro and in vivo. International Journal of Cancer 92, 851–855.

Gibbons, S., Mathew, K.T., Gray, A.I., 1999. A caffeic acid ester from *Halocnemum strobilaceum*. Phytochemistry 51, 465–467.

Gibbons, S., Denny, B.J., Ali-Amine, S., Mathew, K.T., Skelton, B.W., White, A.H., Gray, A.I., 2000. NMR spectroscopy, X-ray crystallographic, and molecular modeling studies on a new pyranone from *Haloxylon salicornicum*. Journal of Natural Products 63, 839–840.

Gibbons, S., Udo, E.E., 2000. The effect of reserpine, a modulator of multidrug efflux pumps, on the in vitro activity of tetracycline against clinical isolates of methicillin resistant *Staphylococcus aureus* (MRSA) possessing the tet(K) determinant. Phytotherapy Research 14, 139–140.

Inagaki, F., Abe, A., 1985. Analysis of ^{1}H and ^{13}C nuclear magnetic resonance spectra of spathulenol by two-dimensional methods. Journal of Chemical Society Perkin Transactions II, 1773–1778.

Lechner, D., Stavri, M., Oluwatuyi, M., Pereda-Miranda, R., Gibbons, S., 2004. The anti-staphylococcal activity of *Angelica dahurica* (Bai Zhi). Phytochemistry 65, 331–335.

Liu, J.H., Zschocke, S., Reininger, E., Bauer, R., 1998. Inhibitory effects of *Angelica pubescens biserrata* on 5-lipoxygenase and cyclooxygenase. Planta Medica 64, 525–529.

Müller, K., 1994. 5-Lipoxygenase and 12-lipoxygenase: attractive targets for the development of novel antipsoriatic drugs. Archiv der Pharmazie (Weinheim) 327, 3–19.

Nayar, M.N.S., Bhan, M.K., 1972. Coumarins and other constituents of *Hesperethusa crenulata*. Phytochemistry 11, 3331–3333.

Nie, D., Honn, K.V., 2002. Cyclooxygenase, lipoxygenase and tumor angiogenesis. Cellular and Molecular Life Sciences 59, 799–807.

Resch, M., Heilmann, J., Steigel, A., Bauer, R., 2001. Further phenols and polyacetylenes from the rhizomes of *Atractylodes lancea* and their anti-inflammatory activity. Planta Medica 67, 437–442.

Schneider, I., Gibbons, S., Bucar, F., 2004. Inhibitory activity of *Juniperus communis* on 12(*S*)-HETE production in human platelets. Planta Medica 70, 471–474.

Seco, J.M., Quinoa, E., Riguera, R., 2004. The assignment of absolute configuration by NMR. Chemical Reviews 104, 17–118.

Setzer, W.N., Green, T.J., Whitaker, K.W., Moriarity, D.M., Yancey, C.A., Lawton, R.O., Bates, R.B., 1995. A cytotoxic diacetylene from *Dendropanax arboreus*. Planta Medica 61, 470–471.

Stavri, M., Mathew, K.T., Gibson, T., Williamson, R.T., Gibbons, S., 2004. New constituents of *Artemisia monosperma*. Journal of Natural Products 67, 892–894.

Virmani, J., Johnson, E.N., Klein-Szanto, A.J.P., Funk, C.D., 2001. Role of platelet-type12-lipoxygenase in skin carcinogenesis. Cancer Letters 162, 161–165.

Wollenweber, E., 1981. Unusual flavanones from a rare American fern. Zeitschrift Fur Naturforschung 36, 604–606.

Yoshimoto, T., Takahashi, Y., 2002. Arachidonate 12-lipoxygenases. Prostaglandins and Other Lipid Mediators 68–69, 245–262.