Novel Drimane Sesquiterpene Esters from *Aspergillus ustus* var. *pseudodeflectus* with Endothelin Receptor Binding Activity

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A series of novel drimane sesquiterpene esters (**1**~**6**) was isolated from fermentations of *Aspergillus ustus* var. *pseudodeflectus* and their structures elucidated by spectroscopic methods including the HMQC, HMBC and INADEQUATE NMR experiments. The major component of the fermentation, **1**, was (2′*E*,4′*E*,6′*E*)-6-(1′-carboxy-2′,4′,6′-trien)-9-hydroxydrim-7-ene-11,12-olide. Compounds **1**, **2**, **3** and **5** exhibited endothelin receptor binding inhibitory activity against rabbit endothelin-A and rat endothelin-B receptors with IC_{50} values in the range 20~150 μM. These compounds had similar levels of activity in assays for binding to human endothelin A and endothelin B receptors. The isolation of 9,11-dihydroxy-6-oxodrim-7-ene, **7**, a probable biosynthetic precursor to the drimane esters is also reported.

The drimane sesquiterpene group of natural products includes compounds with a wide spectrum of biological activities. Warburganal, polygodial and related enedials have been subjected to detailed structure-activity relationship studies because of their insect antifeedant properties.[1,2] Compounds such as the muzagadial series from *Canella winterana*[3] and the pereniporins A and B from the basidiomycete *Perenniporia medulla-panis* were discovered as result of their phytotoxic and plant growth regulatory properties. The pereniporins also have weak antibacterial and cytotoxic activity.[4] Drimenin and aristolone were recently isolated from *Porella cordeana* after detection of their activity in an assay based on cytotoxicity to DNA-repair deficient *Saccharomyces cerevisiae*.[5]

As part of an ongoing programme for the discovery of endothelin antagonists we have recently discovered a series of novel drimane sesquiterpene esters produced by the fungus *Aspergillus ustus* var. *pseudodeflectus*. The endothelins (endothelin-1, -2, -3: ET-1, ET-2, ET-3) are a family of potent vasoconstricting peptides with a variety of biological functions including bronchoconstriction, positive inotropic and chronotropic effects, mitogenesis and potent renal effects.[6] Their effects are mediated by binding to two receptor sub-types, the endothelin-A (ET_A) and endothelin-B (ET_B) receptors. Endothelins are implicated in several human disease states including hypertension, congestive heart failure, renal failure, pulmonary hypertension, ischemia and cerebral vasospasm.[6] Our programme for the discovery of natural products with endothelin-binding inhibitory properties focused on fungal sources and has been successful in identifying several new series of inhibitors including a family of azaphilones from *Penicillium sclerotiorum*.[7]

The fermentation, purification, structure elucidation and endothelin receptor binding inhibitory properties of the drimane sesquiterpene esters produced by *A. ustus* var. *pseudodeflectus* are reported herein. The structures of compounds **1**~**7** are shown in Figure 1.

Materials and Methods

Chemicals

Chemicals were obtained as follows: Endothelin-1 (ET-1) from Novabiochem, Nottingham, England (Catalogue No. 05-23-3800) or from American Peptide Co., Sunnyville, CA; [^{125}I]-ET-1 from Amersham International, Amersham, England (Catalogue No. IM223) or from New England Nuclear, Beverly, MA; Endothelin-3 (ET-3) from Peptides International, Louisville, KY.

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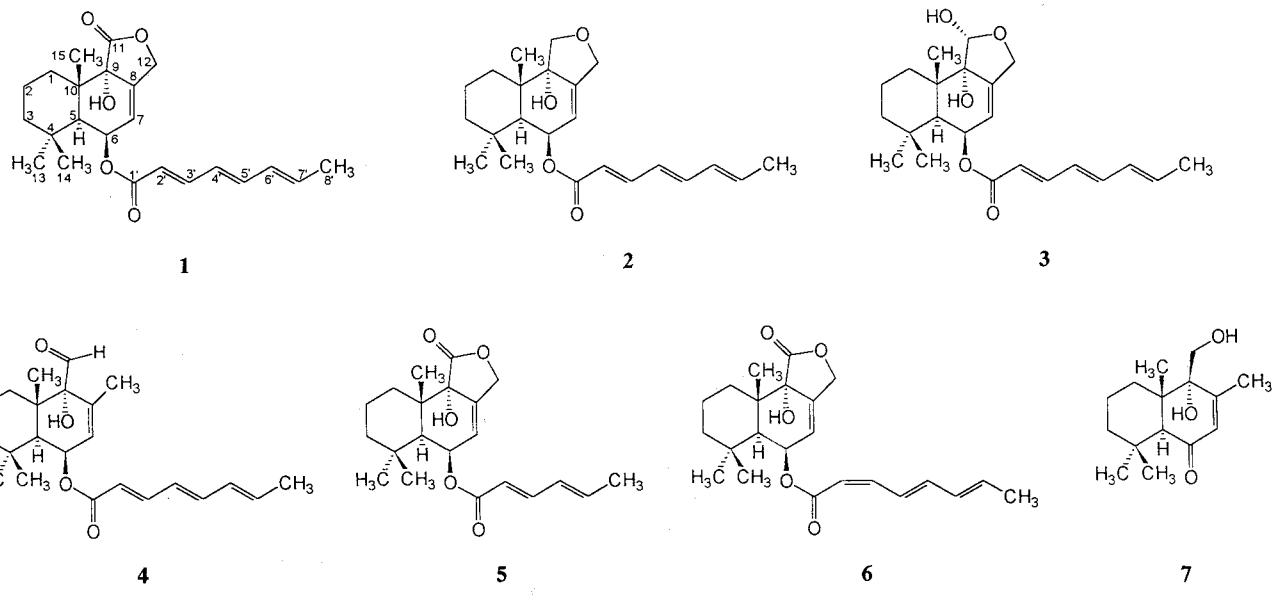
Fig. 1. The structures of the drimane sesquiterpene esters **1**~**6** and 9,11-dihydroxy-6-oxodrim-7-ene, **7**.



Source of Organism

The microfungus designated Xenova culture collection X3811 was isolated from Egyptian desert soil.

Fermentation of *A. ustus* var. *pseudodeflectus* X3811

A conidial suspension obtained from a mature slant culture, grown on malt extract agar (2% malt extract, 1.5% agar) was transferred into a 2-liter Erlenmeyer flask containing 250 ml of seed medium. The seed medium consisted of glycerol, D-glucose, malt extract, soybean peptone, NaCl, $CaCO_3$, and Tween 80 adjusted to pH 6.0 with sulphuric acid before sterilisation. The flask was shaken at 240 rpm, 25°C on a rotary shaker for 3 days. The contents of the flask were transferred to a 14-liter fermenter containing 10 liters of the following medium: beet molasses, casein, phytic acid, $CaCO_3$, Tween 80, and antifoam A (Sigma), adjusted to pH 6.0 with sulphuric acid before sterilisation. The vessel was agitated at 400 rpm and aerated at 0.5 vvm. The temperature was controlled at 25°C. After 72 hours the mycelium was collected by centrifugation. Dissolved oxygen tension (DOT) and pH were monitored *via* Ingold probes and dry cell weight (DCW) was determined by conventional methods. Production of compounds of interest was monitored by extracting biomass from 10 ml aliquots of culture with methanol (3 × 10 ml). The extracts were analysed by reversed phase HPLC on a Waters Novapak C-18 column with photodiode array (PDA) detection, eluting with a water-acetonitrile gradient (0→100% over 13 minutes).

Isolation of Drimane Sesquiterpene Esters

The biomass from an 11 liters fermentation of X3811 was harvested after 72 hours. The lyophilised cells were extracted with MeOH (3 × 3 liters). The organic extracts were combined and the solvent removed *in vacuo* yielding a brown oil (~240 g). A portion of this extract (10.0 g) was purified by flash column chromatography on silica gel eluting with an ethyl acetate-hexane gradient increasing from 10% to 100% ethyl acetate. Flash column fractions were then analysed by HPLC using PDA detection. Further purification was achieved by semi-preparative reversed phase HPLC on two Prep Nova-Pak HR C_{18} radial compression cartridge columns (40 mm × 10 cm, 6 μm particle size, 60 Å pore size, Waters, WAT037704) connected in series in a PrepPak Holder Assembly with Extension (Waters) along with a Prep Nova-Pak HR C_{18} Guard-Pak Insert (Waters, WAT037854). Compounds **1**, **2**, **3**, **4**, **5**, **6** and **7** were purified by eluting the system isocratically with acetonitrile - water (7 : 3) at 50 ml/minute. Compound **4** was further purified by preparative TLC (Merck 5717 plates, 20 cm × 20 cm, 2 mm thickness, Kieselgel 60F_{254}) eluting with ethyl acetate - hexane (7 : 3, 2 elutions). Compounds were isolated in the following quantities **1**, 72 mg; **2**, 25 mg; **3**, 20 mg; **4**, 22 mg; **5**, 3 mg; **6**, 20 mg and **7**, 10 mg.

Determination of Physico-chemical Properties

UV/visible spectra were measured on a Perkin-Elmer Lambda 17 UV/visible spectrometer. IR spectra were recorded on a Nicolet 5PC FTIR spectrometer using a Spectra Tech "Collector" diffuse reflectance accessory. Optical rotations were measured on a Bellingham and Stanley P-20 polarimeter. Low resolution EI-MS and DCI-MS were obtained on a VG Trio 3 triple quadrupole mass spectrometer. Electrospray MS (ESI-MS) were obtained on a Finnigan SSQ710C instrument interfaced to a Waters 600-MS HPLC system. High resolution EI-MS and ESI-MS were obtained on a Finnigan Mat 95 mass spectrometer. ^1H and ^{13}C NMR spectra were

Table 1. Morphological characteristics of fungus X3811 on Czapek Dox agar (CZ), 2% malt extract agar (MEA) and glycerol nitrate agar (G25N) following incubation at 25°C.

	CZ	MEA	G25N
7-Day mycelial diameter (cm)	2~2.5	3.7~3.9	1.0~1.2
Main colony colours* (above)	Clay-buff, sepia	Grey-olivaceous, olivaceous	Grey-olivaceous, mouse-grey
Main colony colours* (below)	Fulvous, sepia	Saffron, drab	Fuscous-black
Conidial head form	Radiate, biseriate	Radiate, biseriate	Radiate, biseriate
Conidiophore:			
Length (μm)	40~85	35~85	—
Width (μm)	2.5~3.5	2.0~3.0	—
Vesicle diameter (μm)	4.5~5.5	4.5~5.5	—
Metula:			
Length (μm)	4.0~5.0	4.0~6.0	—
Width (μm)	3.5~4.5	3.0~4.0	—
Phialide:			
Whorl No.	Usually 2	2~3	—
Length (μm)	4.5~7.0	5.0~9.0	—
Width (μm)	3.5~4.5	3.5~4.0	—
Warty conidial diameter (μm)	3.0~4.0	3.5~4.0	—

* Colours according to ANON.[10]

recorded at 308 K on a Bruker ACF400 spectrometer at 400 MHz and 100 MHz respectively. All chemical shifts (δ) are quoted in ppm and are referenced to external TMS (0 ppm). Standard techniques were used to obtain the DEPT, COSY-45, HMQC, HMBC and NOESY spectra. In HMQC experiments the $^1J_{CH}$ was optimised for 145 Hz. In HMBC experiments the long range coupling constant $^{3\sim5}J_{CH}$ was optimised for 5 Hz. Mixing times from 600 to 1600 ms were used in the NOESY experiments. A 2D INADEQUATE experiment was performed using a data matrix of 4 K points by 96 points, optimized for $^1J_{CC}$ of 45 Hz, with an experiment time of 28.8 hours. The concentration of **1** used in the experiment was approximately 1 M in 0.5 ml d_6-DMSO.

Receptor Binding Assays

The rabbit ET_A, rat ET_B, human ET_A and human ET_B receptor binding assays were performed as reported in PAIRET *et al.*[7] The concentration of the isolated compounds **1**~**7** which resulted in a 50% inhibition of the endothelin binding (IC_{50}) to the relevant receptor membrane preparation was determined. Functional activity for the ET_A receptor was investigated using an assay based on blockade of ET-1 stimulated arachidonic acid release from rabbit renal artery smooth muscle cells.[7]

Results

Taxonomy of Fungus X3811

The fungus *Aspergillus pseudodeflectus* was first described by SAMSON and MOUCHACCA.[8] A study of its conidial ultrastructure prompted KOZAKIEWICZ to reduce the taxon to varietal rank, *i.e. Aspergillus ustus* var. *pseudodeflectus* (Samson & Mouchacca) Kozakiewicz.[9]

Fig. 2. Changes in pH, dissolved oxygen tension (DOT), mycelial dry cell weight (DCW) and production of compound **3** observed during a typical batch fermentation of *A. ustus* var. *pseudodeflectus*.

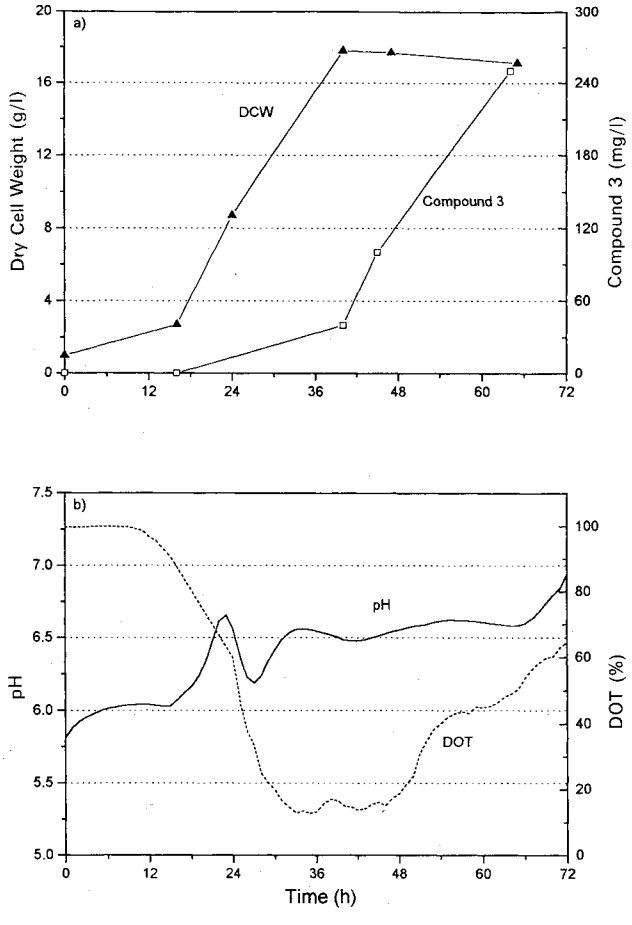


Table 2. Physico-chemical properties of compounds **1**~**7**.

Compound No.	**1**	**2**	**3**	**4**
Appearance	White powder	White powder	White powder	Colourless oil
Molecular formula	$C_{23}H_{30}O_5$	$C_{23}H_{32}O_4$	$C_{23}H_{32}O_5$	$C_{23}H_{32}O_4$
MS (*m/z*)	FAB (glycerol), 773 $(2M+H)^+$, 387 $(MH)^+$	FAB (glycerol), 373 $(MH)^+$	EI 388 $(M)^+$	CI (NH_3) 373 $(MH)^+$
High resolution MS (*m/z*)				
Found:	—	—	EI 388.2262	EI 372.2308
Calculated:	—	—	388.2249	372.2300
UV* λ_{max} (MeOH) nm (ε)	304 (36,600)	303 (37,000)	303 (32,000), 205 (6,000)	294 (34,500), 202 (8,400)
IR ν_{max} KBr cm^{-1}	3395 (br), 2947 (s), 1772 (s), 1709 (s), 1616 (s), 1464 (s), 1149 (s), 1032 (s), 916 (s), 773 (s), 663 (s)	3389 (br), 2947 (s), 1701 (s), 1616 (s), 1354 (s), 1269 (s), 1132 (s), 1005 (s)	3600~3200 (br), 2947 (s), 1703 (s), 1616 (s), 1269 (s), 1132 (s), 1032 (s)	3480 (s), 2947 (s), 1703 (s), 1616 (s), 1444 (w), 1267 (s), 1128 (s)
$[\alpha]_D$ (MeOH) ($c=0.1$)	−358°	−204°	−266°	−211°
Solubility Soluble:	MeOH, $CHCl_3$, CH_2Cl_2	MeOH, $CHCl_3$, CH_2Cl_2	MeOH, $CHCl_3$, CH_2Cl_2	MeOH, $CHCl_3$, CH_2Cl_2
Insoluble:	C_6H_{14}	C_6H_{14}	C_6H_{14}	C_6H_{14}

Compound No.	**5**	**6**	**7**
Appearance	White powder	White powder	Colourless oil
Molecular formula	$C_{21}H_{28}O_5$	$C_{23}H_{30}O_5$	$C_{15}H_{24}O_3$
MS (*m/z*)	ESI 383 $(M+Na)^+$	EI 386 (M^+)	EI 252 (M^+)
High resolution MS (*m/z*)			
Found:	ESI 383.1820	EI 386.2078	EI 252.1722
Calculated:	383.1834	386.2093	252.1726
UV* λ_{max} (MeOH) nm (ε)	255 (24,100)	300 (36,200)	232 (1,800)
IR ν_{max} KBr cm^{-1}	3431 (br), 2949 (br), 1765 (s), 1703 (s), 1685 (s), 1640 (s), 1246 (s), 1140 (s), 736 (s)	3433 (br), 2947 (br), 1772 (s), 1705 (s), 1612 (s), 1462 (s), 1369 (s), 1174 (s), 1006 (s), 914 (s), 742 (s), 665 (s)	3426 (br), 2926 (s), 1658 (s) 1383 (s), 1022 (s), 914 (s) 742 (s)
$[\alpha]_D$ (MeOH) ($c=0.1$)	−366°	−76°	−40°
Solubility Soluble:	MeOH, $CHCl_3$, CH_2Cl_2	MeOH, $CHCl_3$, CH_2Cl_2	MeOH, $(CH_3)_2CO$, $CHCl_3$, CH_2Cl_2
Insoluble:	C_6H_{14}	C_6H_{14}	C_6H_{14}

Fungus X3811 was confirmed as a strain of *A. ustus* var. *pseudodeflectus* on the basis of the morphological data shown in Table 1.[10] Petri dishes containing Czapek Dox agar (CZ), 2% malt extract agar (MEA) and glycerol nitrate agar (G25N) were centrally inoculated in triplicate with conidia transferred on a sterile agar coated needle. Media ingredients were as listed by KLICH and PITT.[11] The most striking microscopical structures were the short, cernuous ('nodding') brown conidiophores observed on all three media.

Fermentation

The changes in pH, DOT, DCW and concentration of **3**, that occurred during the fermentation are shown in Figure 2. The DCW reached a maximum of 18 g/liter after approximately 40 hours and remained constant. The compounds of interest were first detected after *ca.* 43 hours.

Structure Elucidation

The major metabolite present in fermentations of X3811 was **1**. Its physico-chemical properties are summarised Table 2. The compound is soluble in organic solvents such as methanol, DMSO and chloroform but insoluble in water and very non-polar solvents such as hexane. The ^{13}C and ^{1}H NMR spectra of **1** exhibited 22 carbon and 30 proton signals respectively and are summarized in Table 3. DEPT spectra revealed the presence of three CH_3, four CH_2, nine CH and six quaternary carbons. An HMQC (^{1}H-^{13}C correlation) experiment established the one bond connectivities of the proton and carbon atoms (see Table 3). The ^{1}H methyl signals at δ 1.05 and δ 1.78 both correlated to the ^{13}C signal at δ 18.2, indicating that the latter represented two coincident methyl carbon signals. All the other proton resonances correlated to carbon signals except the singlet at δ 6.2, suggesting that this was due to a hydroxyl proton. Consideration of the chemical shift data indicated that the 68.1 ppm CH_2, the 65.7 ppm CH and the quaternary carbons at 73.0, 165.2 and 174.2 ppm were attached to oxygen. The presence of six double bonds was deduced from the presence of eight olefinic carbons (119.5, 121.3, 127.3, 131.1, 135.5, 136.5, 141.7 and 145.4 ppm) and two carbonyl carbons (165.2 and 174.2 ppm). Mass spectrometric data suggested a molecular weight of 386 Da. A likely molecular formula of $C_{23}H_{30}O_5$ was deduced from this molecular weight and on the basis of the NMR data. Inspection of the ^{1}H-^{1}H 2D COSY data and HMBC ^{1}H-^{13}C data led to the

Table 3-1. ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectral data for compounds **1**~**4** in d_6-DMSO (**1**), and $CDCl_3$ (**2**~**4**).

Compound No.	**1**		**2**		**3**		**4**	
Position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	29.4	1.97 (1H, dd, J=13.7, 4.2 Hz) 1.84 (1H, d, J=13.7 Hz)	31.8	1.94 (1H, dd, J=13.2, 4.5 Hz), 1.42 (1H, d, J=13.2 Hz)	33.3	2.08 (1H, m), 1.35~1.50 (1H, m), overlap with H-3	33.1	1.83 (1H, m), 0.95 (1H, m)
2	17.3	1.60 (1H, m), 1.47 (1H, m)	18.4	1.64 (1H, dt, J=13.4, 3.2 Hz), 1.55 (1H, m)	19.4	1.80 (1H, m), 1.59 (1H, m)	17.8	1.50~1.70 (2H, m)
3	44.3	1.34 (1H, d, J=12.7 Hz), 1.20 (1H, m)	44.1	1.33 (1H, d, J=12.8 Hz), 1.27 (1H, dd, J=12.8, 3.1 Hz)	46.3	1.35~1.50 (2H, m), overlap with H-1	44.2	1.20~1.50 (2H, m)
4	33.2		33.6		34.9		33.8	
5	44.1	2.02 (1H, d, J=4.8 Hz)	45.1	2.00 (1H, d, J=4.4 Hz)	47.1	2.24 (1H, d, J=4.6 Hz)	44.6	1.91 (1H, d, J=4.2 Hz)
6	65.7	5.57 (1H, m)	66.4	5.65 (1H, d, J=4.7 Hz)	68.7	5.79 (1H, m)	66.6	5.70 (1H, dd, J=4.2, 1.0 Hz)
7	121.3	5.77 (1H, t, J=1.7 Hz)	127.0	5.89 (1H, m)	121.2	5.70 (1H, m)	126.3	5.96 (2H, m), overlapping with H-7′
8	136.5		141.0		143.9		133.8	
9	73.0	6.20 (1H, br s, 9-OH)	74.7		78.5		82.0	3.82 (1H, br s, 9-OH)
10	37.2		40.5		39.9		41.7	
11	174.2		62.3	3.76 (2H, m)	99.5	5.35 (1H, s)	205.4	9.88 (1H, s)
12	68.1	4.88 (1H, dt, J=12.6, 2.2 Hz), 4.75 (1H, d, J=12.6 Hz)	66.1	4.35 (1H, d, J=12.7 Hz), 4.12 (1H, d, J=12.7 Hz)	67.9	4.64 (1H, dt, J=12.8, 2.1 Hz), 4.62 (1H, dt, J=12.8, 1.1 Hz)	18.8	1.62 (3H, d, J=1.3 Hz)
13	32.0	0.9 (3H, s)	32.6	1.08 (3H, s)	33.6	1.25 (3H, s)	32.6	1.00 (3H, s)
14	24.1	1.06 (3H, s)	24.6	0.98 (3H, s)	25.5	1.07 (3H, s)	24.6	1.13 (3H, s)
15	18.2	1.05 (3H, s)	18.3	1.10 (3H, s)	19.7	1.33 (3H, s)	20.6	1.54 (3H, s)
1′	165.2		166.3		168.4		166.2	
2′	119.5	5.90 (1H, d, J=15.2 Hz)	119.9	5.78 (1H, d, J=15.2 Hz)	120.0	5.95 (1H, d, J=15.2 Hz)	120.3	5.85 (1H, d, J=15.2 Hz)
3′	145.4	7.23 (1H, dd, J=14.8, 11.3 Hz)	145.3	7.21 (1H, dd, J=15.3, 11.4 Hz)	147.3	7.36 (1H, dd, J=15.2, 11.2 Hz)	145.0	7.27 (1H, dd, J=15.2, 11.1 Hz)
4′	127.3	6.63 (1H, dd, J=15.1, 11.3 Hz)	127.4	6.17 (1H, dd, J=14.8, 11.4 Hz)	128.9	6.37 (1H, dd, J=14.8, 11.3 Hz)	127.4	6.21 (1H, dd, J=14.9, 11.3 Hz)
5′	141.7	6.68 (1H, dd, J=14.9, 10.7 Hz)	141.3	6.50 (1H, dd, J=14.9, 10.6 Hz)	143.4	6.69 (1H, dd, J=14.8, 10.5 Hz)	141.1	6.53 (1H, dd, J=14.8, 10.7 Hz)
6′	131.1	6.17 (1H, m)	131.1	6.12 (1H, ddd, J=14.7, 10.6, 1.0 Hz)	132.8	6.30 (1H, ddd, J=12.2, 10.8, 1 Hz)	131.1	6.15 (1H, ddd, J=12.2, 10.7, 1.3 Hz)
7′	135.5	6.00 (1H, dq, J=14.9, 6.9 Hz)	135.2	5.95 (1H, dq, J=14.7, 6.9 Hz)	136.8	6.10 (1H, dq, J=12.2, 6.8 Hz)	135.0	5.96 (2H, m), overlapping with H-7
8′	18.2	1.78 (3H, d, J=6.9 z)	18.1	1.81 (3H, dd, J=6.9, 1.0 Hz)	18.9	1.91 (3H, dd, J=6.8, 1 Hz)	18.3	1.84 (3H, d, J=7.3 Hz)

Spectra were measured at 25°C. TMS was used as an internal reference (δ 0.00). Chemical shifts are expressed in ppm and coupling constants in Hz.

identification of a 2*E*,4*E*,6*E*-octatrienoyl fragment and the drim-7-en-11,12-olide fragment shown in Figure 3. The lactone linkage between the CH_2 at 68.1 ppm and the carbonyl at 174.2 ppm was deduced from the $^3J_{CH}$ couplings between the ^{13}C signals at 174.2 and 73.0 ppm and the protons at 4.88 and 4.75 ppm. The $^3J_{CH}$ coupling from the proton at 2.02 ppm and the ^{13}C resonance at 73.0 ppm was also useful in establishing this carbon skeleton. The drim-7-en-11,12-olide and octatrienoyl fragments were linked by a further $^3J_{CH}$ coupling between the ester carbonyl at 165.2 ppm and the proton at 5.57 ppm. The carbon skeleton of **1**, particularly for the contiguous quaternary centres around C-10, was confirmed by an INADEQUATE experiment.[12]

The structures of **2**, **3**, **4**, **5**, **6** and **7** were determined by comparison with spectroscopic data from (2′*E*,4′*E*,6′*E*)-6-(1′-carboxyocta-2′,4′,6′-triene)-9-hydroxydrim-7-ene-11,12-olide **1**. The significant differences in the data are outlined herein:—.

(2′*E*,4′*E*,6′*E*)-6-(1′-Carboxyocta-2′,4′,6′-triene)-11,12-epoxy-9-hydroxydrim-7-ene **2**

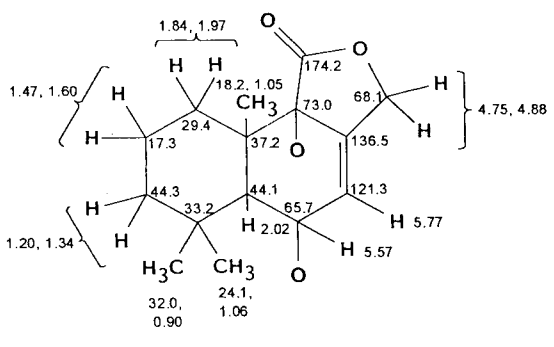
Twenty-three resonances were observed in the ^{13}C NMR spectrum including a single carbonyl carbon at δ 166.3. The lactone resonance (δ 174.2) of **1** was replaced by a methylene carbon at δ 62.3. A multiplet at δ 3.76 in the ^1H NMR spectrum reinforced these data. The identity of **2** was further confirmed by mass spectrometry (FAB) which showed an MH^+ of 373 Da again indicating reduction of C=O in **1** to CH_2 in **2**. Remaining spectroscopic data indicated an oxygenated drimane skeleton with a 2*E*,4*E*,6*E*-octatrienoate side chain. An HMBC correlation between H-6 at δ 5.65 and carbon 1′ (δ 166.3) identified the point of attachment of the side

Table 3-2. ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectral data for compounds **5**~**7** in $CDCl_3$.

Compound No.	5		6		7	
Position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	30.2	2.10 (1H, m), 1.74 (m, 1H), overlap with 2-H	30.2	2.12 (1H, m), 1.70 (1H, m)	31.5	1.95 (1H, dd, J=11, 5 Hz), 1.40 (1H, m)
2	17.7	1.60 (1H, m), 1.74 (m, 1H), overlap with 1-H	17.6	1.60 (2H, m)	17.8	1.60~1.50 (2H, m)
3	44.7	1.42 (1H, m), 1.31 (1H, m)	44.8	1.42 (1H, m), 1.30 (1H, m)	42.5	1.30~1.20 (2H, m)
4	33.7		33.7		32.1	
5	44.7	2.05 (1H, d, J=4.8 Hz)	44.7	2.02 (1H, d, J=4.9 Hz)	55.6	2.75 (1H, s)
6	66.1	5.71 (1H, m)	65.6	5.71 (1H, m)	199.9	
7	123.8	5.90 (1H, m)	124.1	5.93 (1H, m)	129.1	5.71 (1H, d, J=1 Hz)
8	134.7		134.5		154.6	
9	74.6	2.62 (1H, br s, 9-OH)	74.6		74.7	3.10 (1H, br s, 9-OH)
10	37.7		37.7		44.8	
11	174.7		174.4		61.7	3.85 (1H, d, H=11 Hz), 3.72 (1H, d, H=11Hz)
12	68.8	4.96 (1H, dt, J=12.3, 2.4 Hz), 4.94 (1H, dt, J=12.3, 1.2 Hz)	68.8	4.96 (1H, dt, J=12.3, 2.4 Hz), 4.74 (1H, dt, J=12.3, 1.1 Hz)	19.8	2.05 (3H, d, J=1 Hz)
13	32.3	1.00 (3H, s)	30.7	0.90 (3H, s)	33.6	1.18 (3H, s)
14	24.5	1.19 (3H, s)	24.4	1.15 (3H, s)	21.7	1.15 (3H, s)
15	18.5	1.12 (3H, s)	18.4	1.10 (3H, s)	17.6	0.94 (3H, s)
1′	166.2		165.4			
2′	118.6	5.75 (1H, d, J=15.4 Hz)	115.6	5.57 (1H, d, J=11.2 Hz)		
3′	145.8	7.23 (1H, dd, J=15.4, 9.9 Hz)	146.0	6.62 (1H, t, J=11.4 Hz)		
4′	129.6	6.19 (1H, m), overlap with H-5′	126.1	7.43 (1H, dd, J=15.0, 11.7 Hz)		
5′	140.1	6.19 (1H, m), overlap with H-4′	142.6	6.49 (1H, dd, J=15.0, 10.7 Hz)		
6′	18.3	1.85 (3H, d, J=6.7 Hz)	131.8	6.22 (1H, ddd, J=12.2, 10.7, 1.5 Hz)		
7′			135.5	5.97 (1H, dq, J=12.0, 6.8 Hz)		
8′			18.3	1.84 (3H, d, J=6.7 Hz)		

Spectra were measured at 25°C. TMS was used as an internal reference (δ 0.00). Chemical shifts are expressed in ppm and coupling constants in Hz.

Fig. 3. Drim-7-en-11,12-olide fragment of **1** indicating chemical shift assignments (δ_H, δ_C).



chain.

(2′*E*,4′*E*,6′*E*)-6-(1′-Carboxyocta-2′,4′,6′-triene)-11,12-epoxy-9,11-dihydroxy-drim-7-ene **3**

Mass spectral data (EI) indicated a molecular weight of 388 Da, accurate mass measurements indicating a likely molecular formula of $C_{23}H_{32}O_5$. The ^{13}C NMR spectrum again showed 23 resonances, with one ester carbonyl signal at δ 168.4. The lactone carbonyl of **1** was replaced with a methine signal at δ 99.5, which showed a correlation to one proton at δ 5.35 in the HMQC spectrum. The relative stereochemistry at C-11 was assigned on the basis of a NOESY experiment, a correlation being observed between H-11 and the methyl group at C-15. The remaining spectroscopic data indicated an oxygenated drimane skeleton with 2*E*,4*E*,6*E*-octatrienoate side chain.

(2′*E*,4′*E*,6′*E*)-6-(1′-Carboxyocta-2′,4′,6′-triene)-9-hydroxydrim-7-ene-11-al **4**

Accurate mass measurements (EI) indicated a molecular formula of $C_{23}H_{32}O_4$. Distinctive features of the ^1H and ^{13}C NMR data included the presence of a carbonyl carbon at δ 205.4 which showed a one bond correlation to a proton singlet at δ 9.88 in the HMQC spectrum. This was indicative of an aldehyde moiety at C-11. The methylene group at C-12 in compounds **1**, **2**, and **3** (δ 66.1~68.1) was replaced by a methyl group at δ 18.8 in the ^{13}C NMR spectrum, δ 1.62 in the ^1H spectrum. Allylic coupling (1.3 Hz) between 12-CH_3 and

H-7 was observed, confirming the position of the new methyl group.

(2′*E*,4′*E*)-6-(1′-Carboxyhexa-2′,4′,-diene)-9-hydroxydrim-7-ene-11,12-olide **5**

UV data (λ_{max} 262 nm) showed that **5** was less conjugated than other members of this series. Electrospray mass spectra of **5** showed an $[M+Na]^+$ at 383, indicating a molecular weight of 360 Da. Accurate mass measurements on the sodium adduct of **5** confirmed a molecular formula of $C_{21}H_{28}O_5Na$. ^1H and ^{13}C NMR data showed that the drimane skeleton was identical to that of **1** and that structural differences were to be found in the unsaturated side chain. Examination of the ^1H NMR data, particularly coupling constants indicated the presence of a 2*E*,4*E*-hexadienoate system.

(2′*Z*,4′*E*,6′*E*)-6-(1′-Carboxyocta-2′,4′,6′-triene)-9-hydroxydrim-7-ene-11,12-olide **6**

Accurate mass measurements yielded a molecular formula of $C_{23}H_{30}O_5$ indicating that **6** was a structural isomer of **1**. ^1H and ^{13}C NMR data showed that the drimane skeleton was identical to that of **1** and that structural differences were in the unsaturated side chain. Examination of the coupling constants from the ^1H NMR data revealed a coupling of 11.2 Hz between H-2′ and H-3′ indicating *Z* double bond stereochemistry. Further examination of the coupling constants within the side chain confirmed the side chain to be 2*Z*,4*E*,6*E*-octatrienoate.

9,11-Dihydroxy-6-oxodrim-7-ene **7**

UV data (λ_{max} 232 nm) were indicative of an α,β-unsaturated ketone. Accurate mass measurements on the parent molecular ion at 252 Da indicated a molecular formula of $C_{15}H_{24}O_3$ and suggested sesquiterpenoid origin. When compared with **1** the ^1H and ^{13}C NMR data showed the absence of C-6 or C-8 unsaturated side chain and also the absence of a proton at H-6. The possible presence of a ketone at C-6 was reinforced by a ^{13}C resonance at δ 199.9. Long-range coupling was observed between a methyl carbon at C-12 and H-7 (*cf.* compound **4**). A pair of diastereotopic protons (ABq) at δ 3.78 was assigned to a methylene group at C-11 (these occurred in a similar environment to a compound prepared by the $NaBH_4$ reduction of **4**. These data will be reported elsewhere).

Table 4. "Endothelin receptor binding results" IC_{50} values for compounds **1**~**7**.

Compound	Rabbit ET_A (μM)	Rat ET_B (μM)	Human ET_A (μM)	Human ET_B (μM)
1	155	50	135	73
2	80	55	112	61
3	65	21	62	41
4	>200	>200	nt	nt
5	50	70	>250	148
6	>200	nt	nt	nt
7	>200	>200	nt	nt

Binding data was computer-analysed by non-linear least squares analysis giving the best fit for a one site model.
nt = Not tested.

Biological Activity

The IC_{50} values of compounds **1**~**7** for the inhibition of ET-1 binding in the rabbit and human ET_A receptor assays and ET-3 binding in the rat and human ET_B assays are shown in Table 4. Compounds **1** and **2** also showed activity in an ET_A functional assay, inhibiting ET-1 stimulated release of arachidonic acid from rabbit renal artery smooth muscle cells when present at a concentration of 30 μM. The remaining compounds were not tested in the arachidonic acid release assay.

Discussion

Compounds **1** to **6** are members of a novel series of fungal metabolites consisting of esters of drimane sesquiterpenes with unsaturated fatty acids. These compounds were discovered when extracts from fermentations of *A. ustus* var. *psuedodeflectus* (X3811) were investigated as a result of their significant inhibitory activity in a rabbit ETA receptor binding assay. This activity was, however, found to be due to a number of molecules which individually exhibited endothelin receptor binding properties with only moderate potencies (IC_{50} values of 20~150 μM).

The drimane sesquiterpene portions of some members of this series are closely related to known drimanes. Thus compounds **1**, **5** and **6** can be regarded as derivatives of (6β,9α)-6,9-dihydroxydrimenin esterified at C-6. Compound **3** is a 6-*O*-ester of pereniporin A.[4] Compound **2**, where the C-11, 12 carbons are linked by an oxygen bridge to form a furan moeity, and the monoaldehyde compound **4** are more unusual and represent new drimane structures. The octatrienoic acid moieties present in compounds **1**~**5** are also unusual and a (2*E*,4*E*,6*E*)-octatrienoyl fragment has been previously reported to be present in only one other natural product, Mer-WF3010, an antibiotic of the papulacandin family produced by *Phialophora cyclaminis*.[13] Esters of drimanes with complex fatty acid side chains are rare although a series of fatty acid esters of uvidin A and drimenol, esterified at C-11, was recently reported from *Lactarius uvidus*.[14] Compound **7** (9,11-dihydroxy-6-oxodrim-7-ene) has been previously reported as a syn-

thetic intermediate and not as an isolated natural product.[15] It did not inhibit endothelin binding significantly but may be a biosynthetic precursor to compounds **1** to **6**.

Compounds **3** and **5** were the most active in the rabbit ET_A receptor binding assay, while compound **3** was the most active in the rat ET_B assay. These compounds had broadly similar IC_{50}'s in the human ET_A and ET_B receptor binding assays. Levels of activity observed in all assays were found not to be dependent on a pre-incubation step with the receptor, as was the case for the recently reported azaphilone series of ET binding inhibitors.[7] The presence of a 5-membered ring incorporating the carbon atoms at positions 11 and 12 appears to be important for endothelin receptor binding activity in this series of drimane derivatives. This receptor binding inhibitory activity is the first biological activity of this type to be reported for this class of compounds.

Addendum in Proof

Compound **1** appears to be identical to the recently published RES-1149-2, also isolated as an endothelin binding inhibitor (OGAWA, T.; K. ANDO, T. TANAKA, Y. UOSAKA & Y. MATSUDA: RES-1149-1 and -2, novel non-peptidic endothelin type B receptor antagonists produced by *Aspergillus* sp. I. Taxonomy of producing strain, fermentation, isolation, and physico-chemical and biological properties. J. Antibiotics 49: 1~5, 1996; UOSAKA, Y.; M. YOSHIDA, T. OGAWA & Y. SAITOH: RES-1149-1 and -2, novel non-peptidic endothelin type B receptor antagonists produced by *Aspergillus* sp. II. Structure determination and derivatisation. J. Antibiotics 49: 6~12, 1996).

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