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Novel Inhibitors of Fungal Protein Synthesis Produced by a Strain of *Graphium putredinis*

Isolation, Characterisation and Biological Properties

TRACY C. KENNEDY, GRAHAM WEBB, RICHARD J. P. CANNELL, OONAGH S. KINSMAN, ROBERT F. MIDDLETON, PHILIP J. SIDEBOTTOM, NICHOLAS L. TAYLOR, MICHAEL J. DAWSON* and ANTONY D. BUSS

Glaxo Wellcome Medicines Research Centre, Gunnels Wood Road, Stevenage, Herts SG1 2NY, UK

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The isolation and structure determination of 6 analogues of the fungal protein synthesis inhibitor GR135402, from *Graphium putredinis*, is described. The relative potencies of the compounds as protein synthesis inhibitors and as *in vitro* antifungal agents provide interesting insights into the structure-activity relationships in this series.

The novel acylated diterpene glycoside GR135402 (**1**) was discovered during a screening programme for inhibitors of fungal protein synthesis[1]. GR135402 is a 3′-(2-methyl-2,4-hexadienoyl) derivative of the antifungal antibiotic sordarin discovered by workers at Sandoz in the 1960s[2]. Compounds with the same sordaricin skeleton have been reported from *Zofiella marina* (zofimarin, **5**)[3], *Penicillium* sp. F31405 (BE-31405)[4], *Xylaria* sp. A19-91[5] and an unidentified fungal strain SCF1082A (SCH57404)[6]. GR135402 is highly selective for inhibition of fungal as opposed to mammalian protein synthesis[1].

This paper describes the isolation, characterisation and antifungal activity of minor components, related to **1**, isolated from *Graphium putredinis* F13302.

Materials and Methods

Fermentation

Graphium putredinis F13302 was maintained on malt extract agar plates. Agar plugs (6 mm dia.) were taken from the plates and used directly or stored in distilled water at ambient temperature. Seed cultures were inoculated with two agar plugs added to a 250 ml Erlenmeyer flask containing 50 ml of seed medium of the following composition: peptone (Oxoid L34) 10 g/litre, malt extract (Oxoid L39) 21 g/litre, glycerol 40 g/litre, Junlon PW110 (Honeywell and Stein Ltd., Wallington, Surrey, UK) 1 g/liter, in distilled water. The pH of the medium was adjusted to 6.5 by the addition of NaOH before autoclaving. The flasks of inoculated seed medium were incubated on an orbital shaker rotating at 250 rpm with a 50 mm throw for 3～4 days. Growth was at 25°C throughout. Optionally the seed cultures were aliquoted and stored at −20°C.

45 Litre Fermentation

Further flasks (400 ml seed medium per 2 litre flask) were inoculated using 0.5 ml of seed culture and incubated as above. The contents of two of these seed flasks were pooled and used to inoculate a fermenter containing 45 litres of production medium of the following composition: arkasoy soy flour 15 g, glycerol trioleate (Estol 1434) 10 g, KH_2PO_4 1 g, coarse heat treated wheat bran (Jas. Bowman & Sons Ltd., Hitchin, UK) 60 g in 1 litre of tap water. The contents of the fermenter were aerated at 22.5 lpm and agitated at 500 rpm. The culture was harvested after 5 days.

500 Litre Fermentation

Two fermenters each containing 5 litres of seed medium were inoculated with 100 ml of seed culture per fermenter. The fermenter cultures were aerated at 2 lpm and agitated at 400 rpm. After 4 days the contents of these fermenters

were used to inoculate another fermenter containing 500 litres of production medium. The production fermenter was aerated at 200 lpm and agitated at 350 rpm. The culture was harvested after 4 days.

Preparation of Compounds **1**, **2**, **3**, **4**, and **7**

Extraction

The fermentation broth (500 litres) was adjusted to pH 2.5 using H_2SO_4 and filtered through a bed of Dicalite 488L (Redland Minerals Ltd., UK) on a rotary vacuum filter. The solids were extracted with methanol (2 × 200 litres) and removed by filtration on a pan filter. The combined methanol extracts were concentrated using a wiped film evaporator to 17.2 litres then further concentrated on a rotary evaporator to 10 litres. On storage at 4°C, a flocculent precipitate formed containing most of the **1** and related materials. The precipitate was collected by filtration on a Dicalite bed and extracted with methanol (10 litres). The extract was concentrated to 6 litres and stirred with Partisil BioPrep P40 ODS-3 (Whatman Biosystems, UK; 500 g) whilst water (8 litres) was added. The P40 was collected by filtration and eluted sequentially with CH_3CN-H_2O (1 : 1; 6 litres), CH_3CN-H_2O (3 : 2; 4 litres) and CH_3CN-H_2O (7 : 3; 2 litres). The eluates were combined and readsorbed to P40 (500 g) by stirring again with the addition of water (8 litres). The P40 was eluted with CH_3CN (3 litres) and the eluate evaporated under reduced pressure to yield a red oil. The oil was dissolved in CH_3CN-water-MeOH (4 : 1 : 1, 300 ml) and subjected to preparative HPLC on a column (23.5 cm × 5 cm i.d.) of 7 μm Kromasil C8 (Eka Chemicals AB, Bohus, Sweden); mobile phase CH_3CN-0.05 M $NH_4H_2PO_4$ (3 : 2); flow rate 120 ml/minute; detection wavelength 266 nm. A total of 21 injections were performed and appropriate fractions bulked.

Isolation of **1**

The fraction eluting at 22.8 ~ 24.4 minutes was collected, diluted with an equal volume of water and pumped back onto the same column at 120 ml/minute. The column was washed with water and eluted with CH_3CN-H_2O (9 : 1). The eluate was evaporated to an aqueous residue and then lyophilised to yield 2.6 g of a crude solid. The crude solid was crystallised by addition of 0.05 M $NH_4H_2PO_4$ to a CH_3CN solution held at 60°C on a water bath (CH_3CN : 0.05 M $NH_4H_2PO_4$, 1 : 3). After holding overnight at 4°C, crystals were recovered by filtration, washed with cold CH_3CN-H_2O (3 : 7), then water, and dried *in vacuo* over P_2O_5 to yield **1**, 1.99 g.

Isolation of **2**

The fraction eluting at 21.8 ~ 22.6 minutes was collected, desalted and evaporated to an aqueous residue as for **1** above. The residue was subjected to a second HPLC separation on a column (25 cm × 2.1 cm i.d.) of 5 μm Spherisorb ODS-2 (Phase Separations Ltd., UK); mobile phase CH_3CN-0.05 M $NH_4H_2PO_4$ (3 : 2); flow rate 25 ml/minute; detection wavelength 266 nm. A total of 5 injections were performed and appropriate fractions bulked. The fraction eluting at 16.6 ~ 17.4 minutes was collected, diluted with an equal volume of water and returned to the column at 25 ml/minute. The column was washed with water and then eluted with CH_3CN-H_2O (9 : 1). The eluate was evaporated to an aqueous residue and lyophilised to yield **2**, 19 mg.

Isolation of **3**

The fraction eluting at 19.4 ~ 20.0 minutes was processed and subjected to a second HPLC separation as for **2** above (4 injections). The fraction eluting at 14.6 ~ 16.2 minutes was collected, desalted as above, and evaporated to an aqueous residue which was lyophilised to yield **3**, 58 mg.

Isolation of **4**

The fraction eluting at 20.0 ~ 20.6 minutes was processed and subjected to a second HPLC separation as for **2** above (3 injections). The fraction eluting at 15.5 ~ 16.4 minutes was collected, desalted as above, and evaporated to an aqueous residue which was lyophilised to yield **4**, 3.5 mg.

Isolation of **7**

The fraction eluting at 6.2 ~ 10.4 minutes (5 litres) was collected, diluted with water (7.5 litres) and pumped onto a column (25 cm × 2.1 cm i.d.) of Partisil P40. The column was washed with water and eluted with CH_3CN. The eluate was evaporated to a concentrate which was subjected to a second HPLC separation on the same column; mobile phase CH_3CN-0.05 M $NH_4H_2PO_4$ (1 : 1); flow rate 120 ml/minute; detection wavelength 266 nm. A total of 9 injections were performed and fractions corresponding to **7** were bulked, diluted with an equal volume of water and pumped onto a column (25 cm × 2.1 cm i.d.) of Spherisorb ODS-2 at 25 ml/minute. The column was washed with water and eluted with CH_3CN-H_2O (9 : 1). The eluate was evaporated to an aqueous residue and lyophilised to yield **7**, 160 mg.

Preparation of Compounds 5 and 6

Extraction

The fermentation broth (50 litres) was filtered and the mycelium extracted with methanol as above to yield a methanol extract which was evaporated to a largely aqueous concentrate (2.75 litres). The concentrate was diluted with CH_3CN (3.75 litres) to dissolve precipitated material and stored at 4°C. On standing the mixture separated into two phases. The upper phase (3.12 litres) was taken and stirred with P40 (200 g) whilst water (6 litres) was added. The P40 was recovered by filtration and slurried in CH_3CN-H_2O (1:3; 300 ml). This slurry was placed onto the top of a column (29 cm × 5.7 cm dia) of P40 equilibrated in CH_3CN-H_2O (1:3). The column was eluted sequentially with CH_3CN-H_2O (1:3; 1.3 litres), CH_3CN-H_2O (7:13; 1.25 litres), CH_3CN-H_2O (9:11; 1.0 litre), CH_3CN-H_2O (11:9; 6.2 litres), CH_3CN-H_2O (3:2; 2.7 litres) and CH_3CN-H_2O (7:3; 2.0 litres).

Isolation of 5

The fraction eluting between 9.6~10.6 litres was evaporated to a crude residue which was subjected to preparative HPLC on a column (23.5 cm × 5 cm i.d.) of 7 μm Kromasil C8; mobile phase CH_3CN-0.05 M $NH_4H_2PO_4$ (3:2); flow rate 120 ml/minute; detection wavelength 266 nm. Two injections were performed and the fractions eluting at 17.0~17.8 minutes were bulked, diluted with an equal volume of water and pumped onto a column (25 cm × 2.1 cm i.d.) of 5 μm Spherisorb ODS-2. The column was eluted with CH_3CN-0.05 M $NH_4H_2PO_4$ (3:2) at 25 ml/minute. The fraction eluting at 14.6~15.7 minutes was collected and evaporated to dryness. The residue was extracted with CH_3CN (2 × 10 ml). The extracts were combined, filtered (Whatman 54 paper) and evaporated to dryness to yield **5**, 1 mg.

Isolation of 6

The fraction eluting between 5.1~6.1 litres was evaporated to a crude residue which was subjected to preparative HPLC on a column (23.5 cm × 5 cm i.d.) of 7 μm Kromasil C8; mobile phase CH_3CN-0.05 M $NH_4H_2PO_4$ (1:1); flow rate 110 ml/minute; detection wavelength 266 nm. Four injections were performed and the fractions eluting at 16.5~17.1 minutes were bulked, diluted with an equal volume of water and pumped onto a column (25 cm × 2.1 cm i.d.) of 5 μm Spherisorb ODS-2. The column was eluted with CH_3CN-0.05 M $NH_4H_2PO_4$ (2:3) at 25 ml/minute. The fraction eluting at 30~35 minutes was collected, diluted with an equal volume of water and returned to the column. The column was washed with water and eluted with CH_3CN-H_2O (9:1). The eluate was evaporated to an aqueous residue and lyophilised to yield **6**, 22.3 mg.

Chemical Derivatisation

Preparation of 8

To **1** (50 mg) dissolved in CH_3CN (3 ml) was added 0.1 M NaOH (7 ml). The solution was incubated at 50°C for 2 hours then neutralised by addition of 1 M-HCl. Compound **8** was purified by preparative HPLC on a column (25 × 2.1 cm i.d.) of 7 μm Kromasil C8 with a mobile phase of CH_3CN-0.05 M $NH_4H_2PO_4$ (1:1); flow rate 25 ml/minute; detection wavelength 210 nm. Appropriate fractions were bulked and desalted by diluting with an equal volume of water, and returning to the column. The column was washed with several volumes of water then eluted with CH_3CN-H_2O (9:1). The eluate was evaporated to an aqueous residue and lyophilised to yield **8** (28 mg). MS *m/z* 493 (M+H), 492 (M). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.80 (3H, d, J=7 Hz, H-17), 0.98 (3H, d, J=6.5 Hz, 15-H), 1.03 (4H, m), 1.23 (1H, m, 1/2 6-H), 1.29 (3H, d, J=6 Hz, 6'-H), 1.30 (1H, d, J=12.5 Hz, 1/2 18-H), 1.77~2.14 (8H, m), 2.34 (1H, m, 14-H), 2.66 (1H, m, 11-H), 3.19 (1H, dd, J=3 and 9 Hz, 4'-H), 3.42 (3H, s, 7'-H), 3.62 (1H, d, J=9Hz, 1/2 19-H), 3.70 (1H, dq, J=9 and 6 Hz, 5'-H), 3.90 (1H, dd, J=1.5 and 4 Hz, 2'-H), 4.13 (1H, d, J=9 Hz, 1/2 19-H), 4.20 (1H, dd, J=3 and 4 Hz, H-3'), 4.68 (1H, d, J=1.5 Hz, 1'-H), 6.07 (1H, dd, J=1.5 and 3.5 Hz, 12-H), 9.73 (1H, s, 16-H).

Preparation of 9

To **1** (49 mg) dissolved in MeOH (5 ml) was added $NaBH_4$ (approx. 3-fold excess). The solution was incubated with mixing at ambient temperature for 24 hours. The reaction mixture was diluted with 7 volumes of CH_3CN-0.05 M $NH_4H_2PO_4$ (7:3) and 2 volumes of methanol containing 15 μl acetic acid per ml. After filtering, **9** was purified by preparative HPLC as described for **8** above except that the mobile phase was CH_3CN-$NH_4H_2PO_4$ (7:3). Appropriate fractions were bulked, desalted and lyophilised as above to yield **9** (18 mg). MS *m/z* 603 (M+H), 602 (M). ^{1}H NMR (500 MHz, CD_3OD) δ 0.22 (1H, d, J=12.5 Hz, 1/2 18-H), 0.80 (3H, d, J=7 Hz, 17-H), 1.02 (3H, d, J=6.5 Hz, 15-H), 1.13 (4H, m), 1.20 (1H, m, 1/2 6-H), 1.29 (3H, d, J=6.5 Hz, 6'-H), 1.71 (1H, dd, J=13.5 and 12.5 Hz, 1/2

9-H), 1.75~1.88 (5H, m), 1.96 (3H, brs, 7″-H), 1.99 (1H, dd, J=12.5 and 5 Hz, 1/2 18-H), 2.03 (1H, dd, J=13.5 and 5 Hz, 1/2 9-H), 2.05~2.13 (2H, m), 2.45 (1H, m, 14-H), 2.66 (1H, m, 11-H), 3.30 (1H, m, 4′-H), 3.33 (2H, brs, 16-H), 3.35 (3H, s, 7′-H), 3.69~3.78 (3H, m), 3.99 (1H, d, J=10 Hz, 1/2 19-H), 4.53 (1H, d, J=1.5 Hz, 1′-H), 5.47 (1H, dd, J=3 and 4 Hz, 3′-H), 5.94 (1H, dd, J=1 and 3.5 Hz, 12-H), 5.99 (1H, dq, J=15 and 7 Hz, 5″-H), 6.51 (1H, brd, J=11.5, 3″-H), 7.15 (1H, ddq, J=15, 11.5 and 1.5 Hz, 4″-H). ^{13}C NMR (125 MHz, CD_3OD) δ 17.9 (q, C-17), 18.3 (q, C-6′), 18.7 (q, C-6″), 20.7 (q, C-7″), 21.5 (q, C-20), 23.1 (q, C-15), 26.9 (t, C-5), 29.2 (d, C-14), 30.7 (t, C-9), 32.5 (t, C-18), 32.9 (t, C-6), 33.2 (d, C-7), 42.9 (d), 43.5 (d), 46.2 (d, C-11), 49.9 (s, C-3), 57.6 (q, C-7′), 67.4 (t, C-16), 67.9 (s, C-10), 69.4 (d, C-3′), 70.0 (d, C-2′), 71.5 (d, C-5′), 73.9 (s, C-2), 77.4 (t, C-19), 79.6 (d, C-4′), 100.6 (d, C-1′), 124.2 (s, C-2″), 130.3 (d, C-4″), 130.5 (d, C-12), 137.8 (d, C-5″), 142.6 (d, C-3″), 150.4 (s, C-13), 167.5 (s, C-1″), 178.5 (s, C-1).

Structure Determination

NMR spectra were recorded on a Bruker AM500 using standard pulse sequences. Mass spectra were recorded on a Finnigan MAT TSQ700 mass spectrometer using a thermospray interface.

Protein Synthesis Inhibition

Inhibition of protein synthesis in cell-free preparations of *Candida albicans* was determined by assaying poly-uridine directed incorpration of L-[U-^{14}C] phenylalanine into trichloroacetic acid precipitatable material as described previously[1].

In Vitro Antifungal Activity

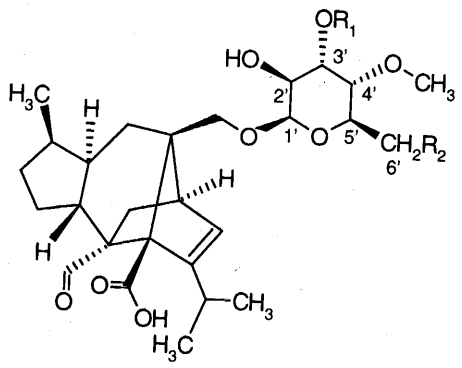
In vitro antifungal activity was determined by the agar dilution method with Iso-Sensitest agar (pH 7.4; Oxoid Ltd.). The agar was inoculated with 10 μl of microbial suspension (10^7 conidia/ml for *Aspergillus fumigatus*, 10^7 cfu/ml for *Cryptococcus neoformans*, 10^5 cfu/ml otherwise) and incubated at 37°C. MIC's were determined after 18 hours except for *Aspergillus fumigatus* and *Cryptococcus neoformans* (48 hours).

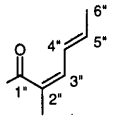
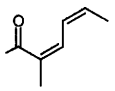
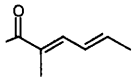
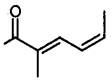
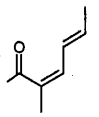
Results

Isolation of Antifungal Metabolites

Bioassay of fractions from analytical HPLC of extracts from the fermentation of *Graphium putredinis* F13302 revealed several components which were active in the assay for inhibition of protein synthesis in *C. albicans*.

Table 1. Compounds isolated from *Graphium putredinis*.



Compound	R_1	R_2
1 (GR135402)		–H
2		–H
3		–H
4		–H
5 (Zofimarin)		–H
6		–H
7		–OH

The stereochemistry indicated is relative within the sordaricin moiety and within the sugar moiety (see text).

The GR135402-related metabolites were extracted from the mycelium of *Graphium putredinis* with methanol and absorbed onto Partisil P40. After elution, components were purified by preparative HPLC. Where appropriate, the fractionation was guided by using the *Candida* protein synthesis inhibition assay to locate the compounds of interest.

Structure Elucidation

The structures of the novel metabolites (Table 1) were determined by comparison of their NMR (Table 2) and MS data (Table 3) with that for the known compounds **1**[1] and zofimarin[3]. No attempt was made to determine

Table 2. Proton NMR data in $CDCl_3$[a].

Protons[b]	Compounds						
	1	2	3	4	5	6	7
1′	4.60 (d, 1.5)	4.60 (d, 1.5)	4.62 (d, 1.5)	4.62 (d, 1.5)	4.61 (d, 1.5)	4.60 (d, 1.5)	4.65 (d, 1.5)
2′	3.85 (dd, 4.5, 1.5)	3.84 (dd, 4.5, 1.5)	3.84 (dd, 4.5, 1.5)	3.86 (dd, 4.5, 1.5)	3.85 (dd, 4.5, 1.5)	3.80 (dd, 4.5, 1.5)	3.85 (dd, 4.5, 1.5)
3′	5.56 (dd, 4.5, 3.0)	5.58 (dd, 4.5, 3.0)	5.55 (dd, 4.5, 3.0)	5.58 (dd, 4.5, 3.0)	5.51 (dd, 4.5, 3.0)	5.47 (dd, 4.5, 3.0)	5.62 (dd, 4.5, 3.0)
4′	3.32 (dd, 9.0, 3.0)	3.30 (dd, 9.0, 3.0)	3.31 (dd, 9.0, 3.0)	3.32 (dd, 9.0, 3.0)	3.31 (dd, 9.0, 3.0)	3.27 (dd, 9.0, 3.0)	3.67 (dd, 9.0, 3.0)
5′	3.77 (dq, 9.0, 6.5)	3.75 (dq, 9.0, 6.5)	3.79 (dq, 9.0, 6.5)	3.78 (dq, 9.0, 6.5)	3.78 (dq, 9.0, 6.5)	3.75 (dq, 9.0, 6.5)	3.72~3.80 (m)
6′	1.32 (d, 6.5)	1.31 (d, 6.5)	1.31 (d, 6.5)	1.32 (d, 6.5)	1.32 (d, 6.5)	1.30 (d, 6.5)	3.88 (m); 3.72~3.80 (m)
7′	3.36 (s)	3.36 (s)	3.35 (s)	3.37 (s)	3.37 (s)	3.36 (s)	3.37 (s)
2″	—	—	—	—	5.60 (brd, 11.5)	2.12 (s)	—
3″	6.44 (brd, 11.5)	6.81 (dm, 11.5)	7.17 (brd, 11.5)	7.57 (dm, 12.0)	6.61 (t, 11.5)	—	6.45 (brd, 11.5)
4″	7.16 (ddq, 15.0, 11.5, 2.0)	7.00 (tq, 11.5, 2.0)	6.37 (ddq, 15.0, 11.5, 1.5)	6.33 (ddq, 12.0, 11.0, 2.0)	7.38 (ddm, 15.0, 11.5)	—	7.15 (ddq, 15.0, 11.5, 1.5)
5″	5.96 (dq, 15.0, 7.5)	5.83 (dq, 11.5, 7.5)	6.14 (dq, 15.0, 7.0)	5.96 (dq, 11.0, 7.0)	6.13 (dq, 15.0, 7.0)	—	5.97 (dq, 15.0, 7.0)
6″	1.84 (brd, 7.5)	1.83 (dd, 7.5, 2.0)	1.89 (brd, 7.0)	1.88 (dd, 7.0, 2.0)	1.90 (dd, 7.0, 2.0)	—	1.85 (brd, 7.0)
7″	1.97 (brs)	2.03 (brs)	1.95 (brs)	1.96 (brs)	—	—	1.97 (brs)

[a] Referenced to residual $CHCl_3$ at δ 7.26 ppm.
[b] Data for protons not given in the table are essentially the same for all compounds.

Table 3. Mass spectral data.

Compound	Positive ion mode (M+H)	Negative ion mode (M)
1	601	600
2	601	600
3	601	600
4	601	600
5	587	586
6	535	534
7	617	616

Table 4. Inhibition of protein synthesis in *Candida albicans*.

Compound	IC_{50} (μg/ml)
1	0.028
5	0.069
6	0.031
7	0.047
8	0.036
9	>40

the absolute stereochemistry but, especially considering the very similar biological properties, it is likely to be the same as for the known compound sordarin (**8**)[7], *ie* as drawn.

Compounds **2**, **3**, and **4** were clearly isomers of **1**. The geometry of the 2″-3″ double bond was established in each case from the chemical shift of the 3″ proton. Chemical shift additivity rules for alkenes[8] indicate that *E* isomers would have values $>\delta$ 7.0 ppm and *Z* isomers would have values $<\delta$ 7.0 ppm. The 4″-5″ double bond geometry followed from the magnitude of the 4″-5″ coupling constant.

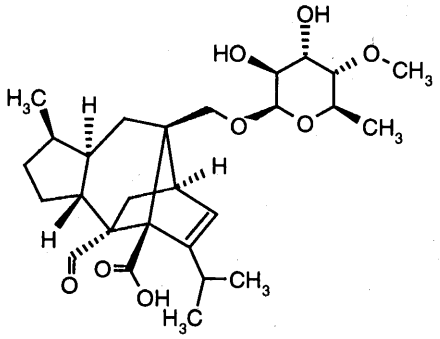
Compound **5** was apparently identical with zofimarin[3].

The NMR and MS data of compound **6** were in accord with replacement of the 2-methyl-2-4-hexadienoyl side chain by an acetyl group.

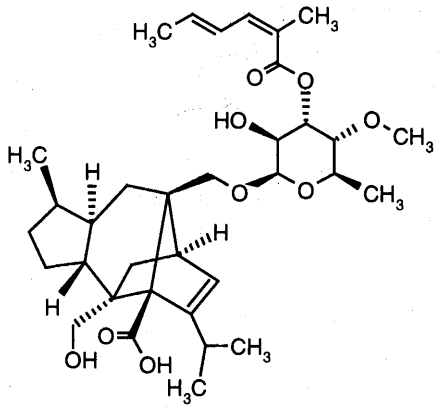
The MS data of compound **7** suggested that it contained an extra oxygen atom. The replacement of the 6′ Me signal by 2 extra proton signals in the sugar region of the NMR spectrum confirmed this and identified its position.

Chemical Derivatisation

Acidic hydrolysis of **1** removed the 3′-*O*-acyl side chain to yield compound **8** which is apparently identical to the known compound sordarin. Reduction of **1** with sodium borohydride reduced the aldehyde functionality to a



8, Sordarin



9

Inhibition of protein synthesis in a cell-free system derived from *Candida albicans*.

Table 5. *In vitro* antifungal activity of compounds **1**~**9**.

Test organism	MIC (μg/ml)							
	1	**2**	**3**	**5**	**6**	**7**	**8**	**9**
Candida albicans C316	0.03	<0.06	0.25	1	0.5	2	16	1
Candida albicans ATCC 10231	0.008	<0.06	0.13	1	0.25	4	8	1
Candida albicans 2402E	0.06	0.25	0.5	2	8	8	31	4
Candida pseudotropicalis 2371E	0.03	<0.06	<0.06	0.25	2	2	16	>31
Aspergillus fumigatus 48238	>125	>125	>125	>125	>125	>125	>125	>31
Cryptococcus neoformans ATCC 32045	1	2	16	16	>125	8	>125	>31

hydroxymethyl (**9**). Table 4 shows the activities of compounds **1**~**9** as inhibitors of protein synthesis in *Candida albicans*.

In Vitro Antifungal Activity of Compounds **1**~**9**

The *in vitro* antifungal activity of compounds **1**~**9** is shown in Table 5.

Discussion

A series of compounds related to **1** has been isolated from *Graphium putredinis*. Compounds **1**~**6** differ only in the nature of the 3′-*O*-acyl substituent. Compounds containing all four geometric isomers of the 2-methyl-2,4-hexadienoyl side chain have been isolated as well as the known compound, zofimarin (**5**), which appears identical to **1** except for the absence of the methyl group at the 2″ position. A simple 3′-*O*-acetyl derivative **6** was also isolated. Compound **7** is the 6′-hydroxy analogue of **1**.

The compounds show interesting differences in biological activity. All the natural compounds tested are potent inhibitors of protein synthesis in *Candida albicans* and indeed the known compound sordarin (**8**), which lacks the 3′-*O*-acyl side chain, is equipotent with **1**. The aldehyde group appears to be essential for protein synthesis inhibition as the borohydride reduction product, **9**, was inactive.

In contrast, as regards whole cell antifungal activity *in vitro*, the nature of the 3′-*O*-acyl side chain had a profound effect on the potency of the compounds. Compound **3** was significantly less potent than **1** against all the fungi examined except for *Candida pseudotropicalis*. This suggests that the configuration of the double-bond at the 2″,3″ position, but less so that at the 4″,5″ position (see data for compound **2**), is important for the *in vitro* antifungal activity. Zofimarin (**5**) lacking the 2″-methyl group, and the simple 3′-*O*-acetyl analogue, **6**, were also

substantially less potent than **1**, as was the 6′-hydroxy compound, **7**. The important role of the 3′-*O*-acyl group in conferring potent antifungal activity is further demonstrated by the modest potency of sordarin (**8**). The fact that compound **9** has some activity against *Candida albicans*, whilst not inhibiting protein synthesis, suggests that this might not be the sole mode-of-action of these compounds.

The importance of the 3′-*O*-acyl side-chain for whole cell antifungal activity together with its lack of influence on the protein synthesis inhibition may suggest that this moiety is important for uptake of the compounds into the fungi.

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