Pergamon

Tetrahedron 58 (2002) 7875–7882

TETRAHEDRON

A cytotoxic sesquiterpene and unprecedented sesquiterpene-bisbibenzyl compounds from the liverwort *Schistochila glaucescens*

Jochen M. Scher,[a] Elaine J. Burgess,[b] Stephen D. Lorimer[b] and Nigel B. Perry[b,*]

[a]*Pharmakognosie und Analytische Phytochemie, Universität des Saarlandes, Im Stadtwald, Gebäude 32, D-66041 Saarbrücken, Germany*
[b]*Plant Extracts Research Unit, Department of Chemistry, New Zealand Institute for Crop & Food Research Limited, University of Otago, P.O. Box 56, Dunedin, New Zealand*

Received 21 May 2002; revised 2 July 2002; accepted 25 July 2002

Abstract—Extracts of the New Zealand liverwort *Schistochila glaucescens* were cytotoxic. Bioactivity-directed isolation led to the new sesquiterpene lactone glaucescenolide **1** as the main cytotoxic component with an IC_{50} of 2.3 μg/ml against P388 leukaemia cells. Also isolated were three known bisbibenzyls, neomarchantins A **2** and B **3** and marchantin C **4**, which were also cytotoxic (P388 IC_{50}s 8–18 μg/ml). Two new compounds GBB A **5** and B **6** are the first examples of a bisbibenzyl connected to a sesquiterpene moiety. A biosynthetic route to **1**, **5** and **6**, via a shared intermediate, furanosesquiterpene **7**, is proposed.

1. Introduction

During our screening of New Zealand liverworts for biologically active natural products,[1,2] an extract of *Schistochila glaucescens* (Hook.) Evans was found to be active against leukaemia cells in vitro. This species (family Schistochilaceae, order Jungermanniales)[3] is endemic[4] and can be found on the ground, old logs or the bases of trees in rain forest throughout New Zealand, at elevations from sea level to 800 m. *S. glaucescens* is a most distinctive plant, named after its glistening and bluish appearance when wet.[5]

The only previous chemical investigation of *S. glaucescens* led to the isolation of three bisbibenzyls neomarchantins A **2** and B **3**, and marchantin C **4**.[6] Cytotoxic activity has been reported for some bisbibenzyls,[7] as we note below for **2**–**4**, but we now report that a new sesquiterpene lactone **1** was the main bioactive compound in *S. glaucescens*. We also report two new compounds **5** and **6**, which are an unprecedented combination of sesquiterpene and bisbibenzyl moieties.

2. Results and discussion

2.1. Cytotoxic sesquiterpene lactone 1

Screening of an extract of *S. glaucescens* showed activity against P388 mouse leukaemia cells. The P388 assay was used to direct the isolation of the active compound from a bulk extract. Two stages of silica gel chromatography then preparative diol HPLC gave the main active compound, glaucescenolide **1**.

The high resolution MS of glaucescenolide supported a molecular formula of $C_{15}H_{22}O_3$. The IR spectrum revealed a hydroxyl absorption at 3378 cm^{-1}, plus a carbonyl absorption at 1740 cm^{-1} consistent with the presence of an α,β-unsaturated γ-lactone.[8] The ^{13}C NMR spectrum exhibited fifteen carbon resonances, assigned by a DEPT experiment to five quaternary carbons, two methines, five methylenes and three methyls (Table 1). These, plus one hydroxyl, accounted for the expected 22 protons. The quaternary carbons at 171.28, 168.28 and 105.58 ppm along with the methine at 114.65 ppm were further evidence for the presence of an α,β-unsaturated γ-lactone hydroxylated in the γ-position.[8] The ^1H NMR resonance at 5.67 ppm (Table 2) was consistent with the α-proton of such a system.[9]

The ^{13}C NMR resonances at 41.63 (CH_2), 18.97 (CH_2), 41.77 (CH_2), 33.15 (C), 48.76 (CH), 38.53 (C), 32.93 (CH_3), 21.40 (CH_3) and 18.75 (CH_3) ppm, along with the three methyl resonances at 0.78, 0.82 and 0.94 ppm in the ^1H NMR spectrum, were consistent with the presence of a trimethyl-cyclohexane moiety, as found in many diterpenes.[10] This substructure was also supported by the observation of $C_9H_{15}^+$ as the main MS fragment ion.

These two moieties were confirmed and linked via the remaining two methylene carbons based on the HMBC results to give the proposed 2D structure in Fig. 1.

Keywords: *Schistochila glaucescens*; liverwort; cytotoxic; sesquiterpene; lactone; bisbibenzyl.

* Corresponding author. Fax: +64-3-479-8543; e-mail: perryn@crop.cri.nz

0040–4020/02/$ - see front matter
PII: S0040-4020(02)00899-2

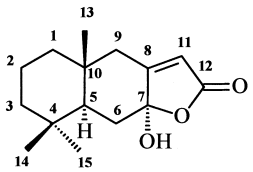
Table 1. ^{13}C NMR data for **1**, **3**, **5** and **6**

C	1	3	5	6
Bisbibenzyl moiety				
1		152.66	152.83	153.22
2		120.43	120.32	119.79
3		130.25	130.25	130.24
4		137.71	137.25	137.46
5		130.25	130.25	130.24
6		120.43	120.32	119.79
7		33.17[a]	33.73	33.15
8		31.07[a]	29.75	30.96
9		122.88	125.60	126.33
10		140.10	144.67	143.58
11		134.93	135.42	137.86
12		144.75	142.55	142.59
13		111.25	114.55	116.50
14		121.79	123.40	123.38
1′		143.36	143.89	145.88
2′		146.32	146.15	144.12
3′		114.93	115.14	115.69
4′		134.83	135.07	134.95
5′		121.44	121.62	121.03
6′		115.02	115.03	115.15
7′		39.79	39.68	42.17
8′		40.79	40.96	39.86
9′		143.86	144.19	143.85
10′		114.57	114.39	113.58
11′		158.03	158.95	159.53
12′		115.36	115.44	115.30
13′		129.86	129.58	129.77
14′		121.97	121.25	121.76
Sesquiterpene moiety				
1″	41.63		41.52	41.91
2″	18.97		18.71	18.46
3″	41.77		42.18	40.74
4″	33.15		33.11	33.01
5″	48.76		48.85	48.89
6″	35.32		21.53	21.50
7″	105.58		156.52	156.02
8″	168.28		104.01	103.84
9″	44.79		41.15	40.00
10″	38.53		33.96	33.53
11″	114.65		80.89	81.98
12″	171.28		100.30	101.11
13″	18.75		19.47	19.34
14″	21.40		21.38	21.56
15″	32.93		32.65	32.49

In $CDCl_3$, 125 MHz, shifts in ppm.
[a] Assignments may be interchanged.

This structure contains three chiral centers. The absolute stereochemistry at C-10 is arbitrarily shown as R as in compounds with the same carbon skeleton from marine sources,[11] but this assumption may be incorrect. The key NOESY and 1D-NOESY interactions used to assign the stereochemistry at C-5 and C-7 are shown in Fig. 2.

The C-5 to C-10 ring junction was assigned as *trans* because of a 1,3-diaxial interaction between Me-13 and Me-14, and the strong interaction between Me-15 and H-6eq. The relative stereochemistry at C-7 was assigned after conformational searching and molecular modeling[12] to predict the most stable conformations of the two possible diastereoisomers. Diastereoisomer **1** gave a predicted most stable conformation (Fig. 2) consistent with the NMR data. In particular, the NOE interaction between Me-13 and H-11 (Fig. 2) is best explained by the predicted proton–proton separation of 3.5 Å in **1**, compared to 5.5 Å in the C-7 epimer.

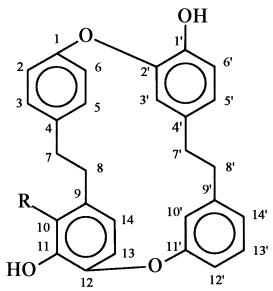


1

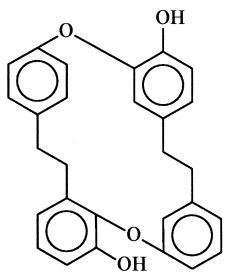
Glaucescenolide **1** is a new compound with a sesquiterpenoid carbon skeleton not previously reported from a liverwort or from any other plant, but a few compounds with this skeleton are known from marine mollusks and sponges.[11] This is also the first report of a sesquiterpene lactone from the genus *Schistochila*.[13] The biological activities and possible biosynthetic origins of **1** are discussed below, along with those of the other compounds purified from *S. glaucescens*.

2.2. Bisbibenzyls 2–4

The three bisbibenzyls previously reported from *S. glaucescens*, neomarchantins A **2** and B **3** and marchantin C **4**, were also isolated from our collection and identified by their NMR spectra[6,14] (unpublished ^{13}C NMR data for **2** and **3** were supplied by Dr M. Tori). It was important to fully assign the NMR spectra of **2** and **3** for the structural work on the two new bisbibenzyl compounds from *S. glaucescens* described below. This was done with the aid of COSY, HMQC, HMBC and NOE experiments (see Supplementary Material), and assignments for **3** (Tables 1 and 2) were made by comparison.



2, R = H
3, R= OH



4

2.3. Sesquiterpene-bisbibenzyls 5 and 6

During the isolation of sesquiterpene **1** and bisbibenzyls **2**–**4** we noticed that other fractions gave NMR spectra with signals for both of these classes of compound. Preparative reversed phase HPLC gave two pure compounds which retained these NMR signals and therefore contained the unprecedented combination of sesquiterpene and bisbibenzyl moieties in single molecules. The two new compounds, named Glaucescens Bis Bibenzyl (GBB) A **5** and B **6**, were shown to be isomers by electrospray ionisation (ESI) MS. Both compounds gave ions appropriate

Table 2. ^{1}H NMR spectral data for **1**, **3**, **5** and **6**

Position	1	3	5	6
Bisbibenzyl moiety				
2		6.94 d (8)	6.92 d (8)	6.91 d (8)
3		7.13 d (8)	7.09 d (8)	7.16 d (8)
5		7.13 d (8)	7.09 d (8)	7.16 d (8)
6		6.94 d (8)	6.92 d (8)	6.91 d (8)
7		3.0–3.2 mNR	3.03–3.10 m NR	3. 18ddd (14,12,5)
		3.0–3.2 m NR	3.03–3.10 m NR	3.10 ddd (13,5,5)
8		3.0–3.2 m NR	2.91 ddd (13,9,3)	2.79 m NR
		3.0–3.2 m NR	3.10–3.12 m NR	3.33 ddd (13,4,4)
13		6.32 d (8)	6.51 d (8)	6.49 d (8)
14		6.36 d (8)	6.49 d (8)	6.36 d (8)
3′		6.22 d (2)	6.20 d (2)	6.27 d (2)
5′		6.70 dd (8,2)	6.70 dd (8,2)	6.70 dd (8,2)
6′		6.88 d (8)	6.86 d (8)	6.86 d (8)
7′		2.4–2.6 m NR	2.53 m NR	2.80 br m NR
		2.4–2.6 m NR	2.45 m NR	2.25 t NR
8′		2.4–2.6 m NR	2.46 m NR	2.72 m NR
		2.4–2.6 m NR	2.56 m NR	2.28 t NR
10′		6.34 dd (1,1)	6.22 dd (2,1)	6.14 dd (2,1)
12′		6.99 ddd (8,2,1)	7.03 ddd (8,2,1)	7.01 ddd (8,2,1)
13′		7.24 dd (8,8)	7.20 dd (8,7)	7.21 dd (8,7)
14′		6.84 br d (8)	6.77 ddd (8,1,1)	6.79 ddd (8,1,1)
Sesquiterpene moiety				
1″ax	1.32 ddd (13,13,3)		1.18 ddd (13,13,4)	0.91 br t NR
1″eq	1.57 m NR[a]		1.62 br d (14)	1.28 br d NR
2″	1.5 m NR		1.51 m NR	1.27 br s NR
	1.6 m NR		1.51 m NR	1.42 br d NR
3″ax	1.23 ddd (13,13,4)		1.12 ddd (13,13,3)	0.61 br t NR
3″eq	1.46 dm (13)		1.41 d NR	1.27 br d NR
5″	1.55 m NR		1.25 dd (12,5)	0.95 m NR
6″ax	1.58 m NR		1.78 br dd (14,14)	1.84 br dd (15,15)
6″eq	2.35 d (12)		2.08 br dd (17,4)	2.10 br ddd (17,5,1)
9″eq	2.26 br s		1.83 d (16)	1.38 d NR
9″ax	2.26 br s		1.90 br d (16)	1.09 br d NR
11″	5.67 br s		5.17 dd (6,2)	5.31 dd (7,2)
12″	–		6.05 d (6)	6.27 d (7)
13″	0.82 s		0.86 s	0.73 s
14″	0.78 s		0.82 s	0.77 s
15″	0.94 s		0.72 s	0.56 s

In $CDCl_3$ at 500 MHz, shifts in ppm (d=doublet, br=broad, s=singlet, m=multiplet), couplings in Hz.
[a] NR=not resolved, multiplicity from HSQC experiment.

for MH^+, MNa^+ and MK^+ of $C_{43}H_{44}O_6$ molecules. This formula again supported the combination of C_{15} sesquiterpene and C_{28} bisbibenzyl moieties.

The NMR data of the bisbenzyl moieties of **5** and **6** largely matched those of neomarchantin B **3** (Tables 1 and 2). The main differences were in the signals of C-9 to C-14 (Table 1), indicating the ring connected to the sesquiterpene moiety. The ^{13}C NMR data of the sesquiterpene moieties of **5** and **6** included signals matching those of the trimethylcyclohexane moiety of glaucescenolide **1** (C-1″ to C-5″, Me-13″, Me-14″ and Me-15″, Table 1). However, there were no carbonyl signals in the IR or ^{13}C NMR spectra of **5** and **6**.

The linkages of the sesquiterpene to bisbibenzyl units were obtained from HMBC results. In **5** a proton signal at 5.17 ppm (H-11″, dd, J=6, 2 Hz) showed a correlation with bisbibenzyl carbon signal C-10 (144.67 ppm), and a

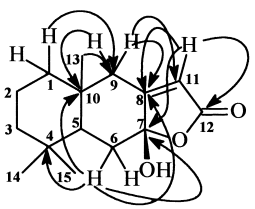


Figure 1. Key HMBC couplings for glaucescenolide **1**.

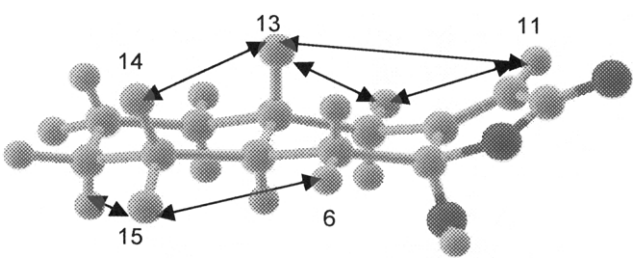


Figure 2. Key NOE interactions and predicted conformation for glaucescenolide **1** (methyl protons omitted for clarity).

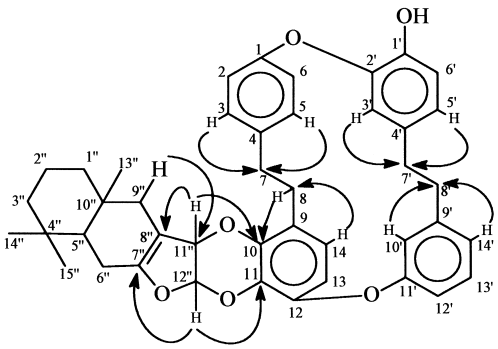


Figure 3. Key HMBC couplings for GBB A **5**.

vicinally-coupled proton signal at 6.05 ppm (H-12″, d, *J*=6 Hz) showed a correlation with C-11 (135.42 ppm, see Fig. 3). These correlations were reversed in **6**: H-11″ (5.31 ppm, dd, *J*=7, 2 Hz) showed a correlation with C-11 (137.86 ppm), and H-12″ (6.25 ppm, d, *J*=7 Hz) showed a correlation with C-10 (143.58 ppm, see Fig. 4).

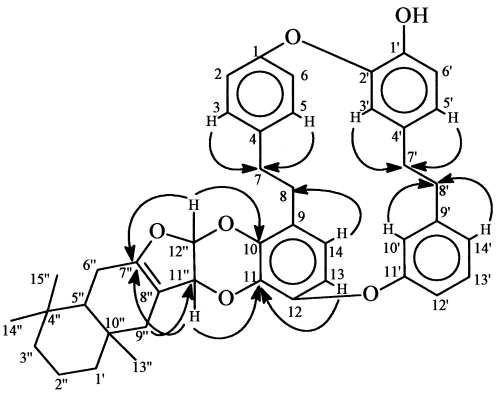


Figure 4. Key HMBC couplings for GBB B **6**.

The ^{13}C NMR shifts of the C-11″ (80.89 ppm in **5**/81.98 ppm in **6**) and C-12″ (100.30 ppm in **5**/101.11 ppm in **6**) signals were consistent with O–CH(O)–CH(O)–C units. The linkages of these to O–C(C)=C(C)–C units and on to the rest of the sesquiterpene moieties were shown by further HMBC correlations (Figs. 3 and 4). The structures of the sesquiterpene units of GBB A **5** and GBB B **6** were also supported by comparison of the ^{1}H and ^{13}C NMR data (Tables 1 and 2) with data reported for the synthetic furanosesquiterpene **7**.[15]

The proposed structures for GBB A **5** and B **6** contain four chiral centers. The absolute stereochemistries at C-10″ are

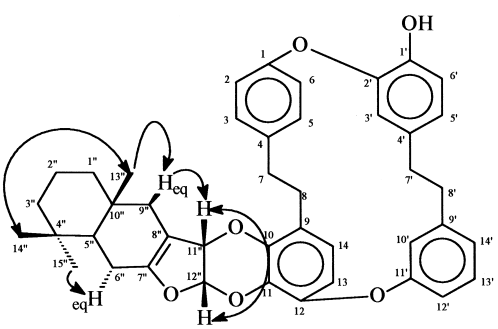


Figure 5. Key NOE interactions for GBB A **5**.

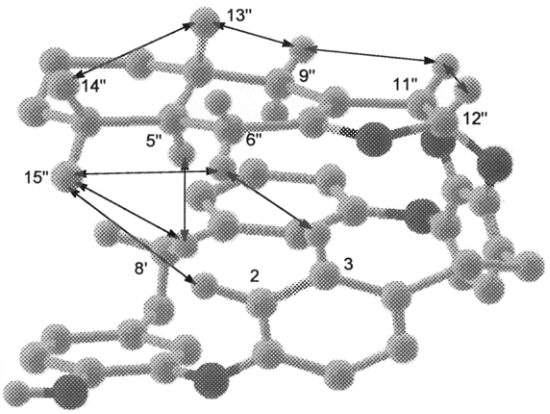
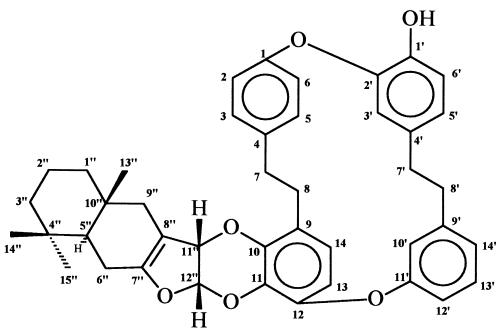


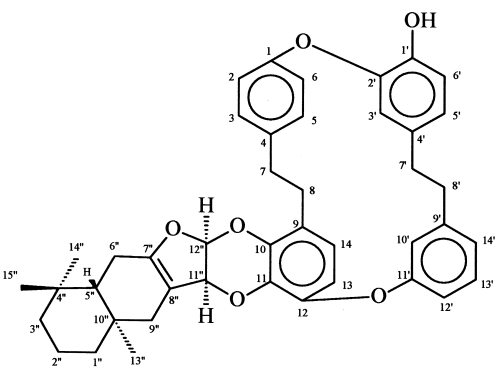
Figure 6. Key NOE interactions and a possible conformation for GBB B **6** (methyl and some other protons omitted for clarity).

again arbitrarily shown as *R*, as in glaucescenolide **1**. The C-5″ to C-10″ ring junctions were assigned as *trans* from the NOESY spectra which showed 1,3-diaxial interactions between Me-13″ and Me-14″, and strong interactions between Me-15″ and H-6″eq (Figs. 5 and 6). The C-11″ to C-12″ ring junctions were assigned as *cis* because of the strong NOESY interactions between H-11″ and H-12″ (Figs. 5 and 6).

This stereochemistry was supported by the similarity of the coupling constants of H-11″ and H-12″ (Table 2) with those reported for synthetic *cis* dihydrofurobenzodioxins.[16] Both H-11″ and Me-13″ showed strong NOE interactions with the same H-9″eq signal (Figs. 5 and 6) so these are all on the same face of the sesquiterpene moieties, giving the proposed structures **5** for GBB A and **6** for GBB B.



5



6

Table 3. Assay results for **1**–**6**

Compound	P388[a]	Antimicrobial[b]	
		Bs	Tm
1	2.3	0	3
2	18	1.5	0.5
3	7.6	2	1
4	8.5	2	0.5
5+**6**[c]	10.3	0	0
Reference[d]	0.02	10	10

Averages of two separate assays.
[a] IC_{50} in μg/ml.
[b] Disc assay dosed at 60 μg/disk, width of inhibition zone given in mm; Bs=*Bacillus subtilis*, Tm=*Trichophyton mentagrophytes*; no inhibition was observed against *Escherichia coli*, *Pseudomonas aeruginosa*, or *Candida albicans*.
[c] A 1:1 mixture.
[d] Reference compounds and doses: Tm, nystatin (100 units/disk); Bs, chloramphenicol (30 μg/disk); P388, mitomycin C.

We searched for NOE interactions between the sesquiterpene and bisbibenzyl moieties of **5** and **6** to get some information about the conformations of these polycyclic but flexible molecules. GBB A **5** did not show any such interactions, but the NOESY spectrum of GBB B **6** showed the long-range interactions shown in Fig. 6. These require a well-populated conformation (or conformations) with the sesquiterpene and bisbibenzyl moieties folded in together (Fig. 6). Such a conformation could also explain some of the differences in NMR shifts between **5** and **6**, through some protons of the sesquiterpene moieties lying in shielding or deshielding regions of the aromatic rings of the bisbibenzyl moieties. For example, the signal of H-9″ax in GBB B **6** is shielded by 0.8 ppm compared to the same signal in GBB A **5** (Table 2). This could be due to a dominant conformation such as that shown in Fig. 6, which has H-9″ax in the shielding zone about 2.7 Å above the center of one of the aromatic rings.

The conformation of GBB B **6** shown in Fig. 6 is a minimized structure derived from molecular modeling, but it is not the result of a full conformational search. Keseru and various co-workers have been successful in applying conformational searching and molecular modeling to bisbibenzyls, including pakyonol with the same macrocylic ring as in neomarchantins A **2** and B **3**.[17] However, the combination of bisbenzyl and sesquiterpene moieties in **5** and **6** presents a greater challenge for exhaustive conformational searching and for force field parameterisation.

3. Biological activities

Glaucescenolide **1** was the most cytotoxic compound isolated from *S. glaucescens* with an IC_{50} of 2.3 μg/ml against P388 leukemia cells (Table 3). This could be due to alkylating activity, through Michael addition of biological nucleophiles,[2] as shown for other α,β-unsaturated sesquiterpene lactones.[18] For example, we found that a sesquiterpene lactone (from two other liverworts) with a conjugated exocyclic methylene had a P388 IC_{50} of 0.4 μg/ml.[19] On the other hand El-Gamal reported P388 IC_{50}s of 42–88 μg/ml for α-methyl-γ-hydroxyl α,β-unsaturated sesquiterpene lactones.[8] These different activities may represent increasing steric hindrance to Michael addition, i.e. the exocyclic methylene is less hindered than C-8 of **1**, which is in turn less hindered than the β-carbon of α-methyl substituted compounds. Glaucescenolide **1** also showed some antimicrobial activity against the dermatophytic fungus *Trichophyton mentagrophytes*.

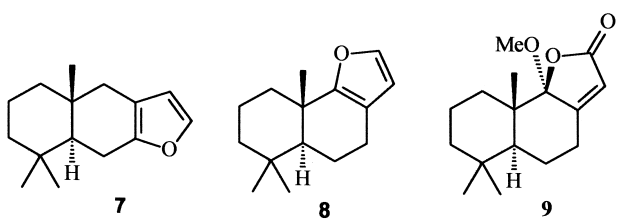
Bisbibenzyls **2**–**4** showed weak to moderate cytotoxicity in the P388 assay, plus some antimicrobial activity against the Gram-positive bacterium *Bacillus subtilis* and against *T. mentagrophytes* (Table 3). This is the first report of biological activity for neomarchantins A **2** and B **3**. Marchantin C **4** has previously been reported to be cytotoxic against KB cells.[7] The related marchantin A also showed cytotoxicity against KB cells (IC_{50} 8.39 μg/ml), and antimicrobial activity against the Gram-positive bacterium *Staphylococcus aureus* (MIC 3.13 μg/ml) and against *T. mentagrophytes* (MIC 3.13 μg/ml).[7,20]

Assays of a mixture of the sesquiterpene-bisbibenzyls **5** and **6** showed that these compounds were less cytotoxic than the sesquiterpene lactone **1** (Table 3). The mixture showed similar cytotoxicity to the bisbibenzyls **2**–**4**, but no antimicrobial activity at the level tested.

4. Biosynthetic hypothesis

No bisbibenzyls linked to sesquiterpenes have been reported before,[7,20] although sesquiterpenes linked to simpler phenolic compounds are known.[1] We propose that furan **7**, which has been synthesized[15] but has not been reported as a natural product, could be an intermediate linking the biosynthetic pathways for glaucescenolide **1** and for GBB A **5** and B **6**.

The carbon skeleton of glaucescenolide **1**, incorporated in the sesquiterpene portions of **5** and **6**, has not previously been reported from liverworts or from any other plant, but it is known from marine organisms.[11,15] For example, pallescensin A **8** has been isolated together with the γ-methoxy lactone **9** from a sponge.[23] These sesquiterpenes seem to require a very unusual polyene cyclisation of a farnesa-3(15),7,10-triene derivative (Fig. 7) which has been achieved in the chemical synthesis of **7**.[15]



7 **8** **9**

Furans such as **7** and **8** can react with singlet oxygen to give furan endoperoxides (Fig. 7) which can react further by a variety of routes.[24,27] Lactone **9** was probably formed by reaction of the endoperoxide of furan **8** with solvent methanol during extraction of the sponge.[23] We suggest that the endoperoxide **10** of furan **7** could rearrange in the presence of water to give glaucescenolide **1** (Fig. 7).

Bisbibenzyls have only been reported from liverworts[7,20]

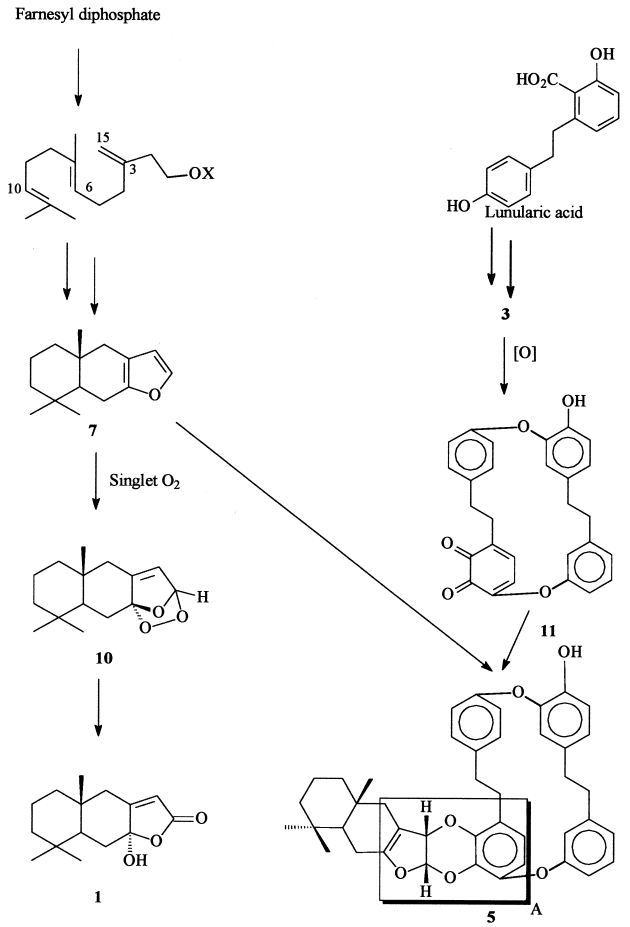


Figure 7. Proposed biosynthetic pathways for glaucescenolide **1** and GBB A **5**.

(apart from one report from a fern[21]) and neomarchantin B **3** has only been isolated from *S. glaucescens*.[7,20] The biosynthetic pathway of some bisbibenzyls, including marchantin C **4**, has been shown to involve oxidative coupling of two molecules of lunularic acid (Fig. 7).[22] Formation of neomarchantin B **3** requires a different coupling and further oxidation to give the 10,11-diphenol.

The proposed structures of GBB A **5** and GBB B **6** contain a dihydrofurobenzodioxin sub-structure (A in Fig. 7). This is unprecedented in natural products, but has been synthesized by reaction of 1,2-benzoquinones with furans.[16] We propose that GBB A **5** and GBB B **6** could be formed by an analogous reaction, i.e. oxidation of neomarchantin B **3** to an *ortho*-benzoquinone **11** (Fig. 7), which could then react with furan **7**. Horspool et al., suggested a two-step mechanism, rather than a concerted process, for this reaction.[16] The linkages could be either C-10 to C-11″ and C-11 to C-12″ to give GBB A **5** (Fig. 7), or C-10 to C-12″ and C-11 to C-11″ to give GBB B **6**.

The stereochemistries of glaucescenolide **1** and of GBB A **5** and B **6** suggest that attack on the furan **7** occurs preferentially away from the axial methyl C-13″. Despite the proposed oxidative routes to these compounds **1**, **5** and **6**, they do not seem to be artifacts of the isolation procedure since their ^{1}H NMR signals could be seen in fresh extracts of *S. glaucescens*. We searched for the reported[15] ^{1}H NMR signals of furan **7** in low polarity fractions from our isolation work, but could not detect this compound.

The proposed biosynthetic pathways (Fig. 7) could also afford biomimetic synthetic routes to the new cytotoxic lactone **1** and to the structurally intriguing new compounds **5** and **6**. Synthesis would be particularly useful if GBB A **5** and B **6**, which may adopt capped macrocycle conformations (Fig. 6), are shown to have potent activities in other biological assays.

5. Experimental

5.1. General methods

All solvents were distilled before use and were removed by rotary evaporation at temperatures up to 35°C. Davisil, 35–70 mm, 150 Å was used for silica gel flash chromatography. Silica gel 60 TLC grade was used for vacuum liquid chromatography. TLC was carried out using Merck DC-Plastikfolien Kieselgel 60 F254, first visualized with a UV lamp, and then by dipping in a vanillin solution (1% vanillin, 1% H_2SO_4 in EtOH) and heating. NMR spectra, at 25°C, were recorded at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR. Chemical shifts are given in parts per million (ppm) on the δ scale referenced to the solvent peaks $CHCl_3$, at 7.25 and $CDCl_3$ at 77.00.

5.2. Molecular modeling

Conformational searching and molecular modeling used the Monte Carlo methods of Mohamadi et al.[12] and the MM3 force field,[25] as implemented in PCMODEL (Version 7.50.00, Serena Software, Bloomington, Indiana, USA).

5.3. Biological assays

Details of the P388 and antimicrobial assays have been published elsewhere.[2,26]

5.4. Collection

S. glaucescens was collected from the West Coast of the South Island of New Zealand (near to Haast), in bush behind Cascade Flats (November 1995) (PERU Code 951101-03, University of Otago Herbarium (OTA) specimen 050442). Initial screening was carried out using extracts produced by shaking air-dried (30°C), ground material (5 g) overnight in EtOH (50 ml).

5.5. Extraction and isolation

*Bioactivity-directed isolation of glaucescenolide **1***. A bulk extract of collection 951102-02 (49 g dry) was prepared by blending with CH_2Cl_2 (2×490 ml) then EtOH (2×490 ml). The solvent was removed from the filtered extracts. The more cytotoxic CH_2Cl_2 extract, a green gum (1.92 g, P388 IC_{50} 10 μg/ml), was subjected to normal-phase vacuum liquid chromatography (1.92 g precoated on 8 g Davisil silica gel, loaded on a 20 g silica gel column), developed in 100 ml steps from 100% cyclohexane through EtOAc to methanol. The fractions eluted with cyclohexane/EtOAc

(6:4–5:5) had a P388 IC_{50} of 6 μg/ml. This fraction (141 mg) was subjected to vacuum liquid chromatography on Si gel, developed in 50 ml steps from cyclohexane to EtOAc. Fractions eluted with 1.5:8.5 and 2:8 EtOAc/cyclohexane, which showed a UV-active spot on TLC (1:1 cyclohexane/EtOAc, R_f 0.6) were combined (54 mg). The main component in this sample was purified by diol HPLC (Merck Lichrospher Diol 10 mm, 250×10 mm^2, mobile phase 5 ml/min 5% IPA/95% hexane, detection at 206 nm). Compound **1** (22 mg) eluted with t_R 7.6 min.

*Isolation of bisbibenzyls **2**–**4***. After the normal-phase vacuum liquid chromatography, as described for **1**, neomarchantins A **2** and B **3** were separated by flash chromatography on silica gel, developed in steps from cyclohexane/CH_2Cl_2 (50:50), CH_2Cl_2 to CH_2Cl_2/EtOAc (70:30). Fractions eluted with 2:8 to 1:9 cyclohexane/CH_2Cl_2, which showed a UV active spot on TLC (1:1 cyclohexane/EtOAc, R_f 0.84) were combined (25 mg neomarchantin A **2**). Fractions eluted with CH_2Cl_2, which showed a UV active spot on TLC (1:1 cyclohexane/EtOAc, R_f 0.65) were combined (21 mg neomarchantin B **3**). Marchantin C was separated by flash chromatography on silica gel in steps from cyclohexane/CH_2Cl_2 (40:60) to CH_2Cl_2. Fractions eluted with CH_2Cl_2, which showed a UV active spot on TLC (1:1 cyclohexane/EtOAc, R_f 0.75) were combined (30 mg marchantin C **4**).

*Isolation of GBB A **5** and B **6***. The CH_2Cl_2 extract was subjected to vacuum liquid chromatography (1.92 g precoated on 8 g Davisil silica gel, loaded on a 20 g silica gel column), developed in 100 ml steps from 100% cyclohexane through EtOAc to methanol. The fractions eluted with cyclohexane/EtOAc (0.75:9.25 to 1.0:9.0), which showed a UV-active, dipped in vanillin/H_2SO_4 pink spot on TLC (4:1 cyclohexane/EtOAc, R_f 0.5) were combined (40 mg). The two main compounds in this sample were separated by RP-18 HPLC (Merck Lichrospher RP18 5 mm, 250×4 mm^2, mobile phase 1.2 ml/min 10% H_2O/90% acetonitrile, detection at 206 nm). Compound **5** (6 mg) eluted with t_R 16 min and compound **6** (5 mg) with t_R 14 min.

5.5.1. Glaucescenolide 1. White gum; $[\alpha]_D^{20}=+60$ (*c* 5%, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 217 (1.182), 340 (shoulder) nm; IR (film) ν_{max} 3377, 2933, 2856, 1740, 1656 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HREIMS *m/z* 250.1576 (M^+, 100%, $C_{15}H_{22}O_3$ requires 250.1569), 235.1357 (M^+-CH_3, 11), 232.1458 (M^+-H_2O, 21), 222.1602 (M^+-CO, 17), 217.1220 ($M^+-H_2O-CH_3$, 27), 123.1182 (C_9H_{15}, 77).

5.5.2. Neomarchantin A 2. IR (film) ν_{max} 3550 cm^{-1}; UV (EtOH) λ_{max} (log ε) 272 (3.57), 278 (3.56) nm; EIMS *m/z* 424 (M^+, 100%), 317 (8), 225 (11), 212 (M^{2+}, 75), 211 (77), 107 (32).

5.5.3. Neomarchantin B 3. IR (film) ν_{max} 3550 cm^{-1}; UV (EtOH) λ_{max} (log ε) 272 (3.87), 278 (3.83) nm; ^1H and ^{13}C NMR, see Tables 1 and 2; EIMS *m/z* 440 (M^+, 100%), 228 (21), 220 (M^{2+}, 23), 213 (42), 211 (15); 153 (18), 107 (15).

5.5.4. Marchantin C 4. Data match literature.[6]

5.5.5. GBB A 5. White gum; $[\alpha]_D^{20}=+20$ (*c* 2.0%, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 273 (1.348) nm; IR (film) ν_{max} 3424, 2917, 2848, 1583, 1502, 1456, 1364, 1272, 1220, 1157, 1105, 754 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HR-ESI-MS *m/z* 679.3044 (M^+, 100%, $C_{43}H_{44}O_6Na$ requires 679.3036).

5.5.6. GBB B 6. White gum; $[\alpha]_D^{20}=+26$ (*c* 1.5%, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 271 (1.756) nm; IR (film) ν_{max} 3442, 2925, 2848, 1591, 1506, 1461, 1379, 1276, 1217, 1165, 1106, 756 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HR-ESI-MS *m/z* 679.3032 (M^+, 100%, $C_{43}H_{44}O_6Na$ requires 679.3036).

Acknowledgements

We thank the New Zealand Department of Conservation and Timberlands for permission to collect; R. Tangney for taxonomic expertise; G. Ellis for biological assays; M. Thomas and W. Redmond for NMR assistance; M. Tori for NMR data on **2** and **3**; B. Clark for MS; R. Weavers for advice; and H. Becker for support. We are grateful to the Deutscher Akademischer Austauschdienst (DAAD) and the New Zealand Foundation for Research, Science and Technology (CO2X0002) for financial support.

References

1. Perry, N. B.; Foster, L. M. *J. Nat. Prod.* **1995**, *58*, 1131–1135.
2. Perry, N. B.; Burgess, E. J.; Baek, S.-H.; Weavers, R. T.; Geis, W.; Mauger, A. B. *Phytochemistry* **1999**, *50*, 423–433.
3. Schuster, R. M.; Engel, J. J. *J. Hattori Bot. Lab.* **1985**, *58*, 239–539.
4. New Zealand plant names database: http://nzflora.landcare.cri.nz/plantnames.
5. Allison, K. W.; Child, J. *The Liverworts of New Zealand*. University of Otago: Dunedin, 1975.
6. Tori, M.; Masuya, T.; Asakawa, Y. *J. Chem. Res. (S)* **1990**, 36–37.
7. Asakawa, Y.; Toyota, M.; Tori, M.; Hashimoto, T. *Spectroscopy* **2000**, *14*, 149–175.
8. El-Gamal, A. A. *Phytochemistry* **2001**, *57*, 1197–1200.
9. Fascio, M.; Mors, W. B.; Gilbert, B.; Mahajan, J. R.; Monteiro, M. B.; Santos Filho, D. D.; Vichnewski, W. *Phytochemistry* **1976**, *15*, 201–203.
10. Wehrli, F. W.; Nishida, T. *Fortschr. Chem. Org. Naturst.* **1979**, *36*, 1–229.
11. Musman, M.; Tanaka, J.; Higa, T. *J. Nat. Prod.* **2001**, *64*, 111–113.
12. Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comp. Chem.* **1990**, *11*, 440–467.
13. Asakawa, Y.; Toyota, M.; Nagashima, F.; Hashimoto, T.; El Hassane, L. *Heterocycles* **2001**, *54*, 1057–1093.
14. Tori, M.; Toyota, M.; Harrison, L. J.; Takikawa, K.; Asakawa, Y. *Tetrahedron Lett.* **1985**, *26*, 4735–4738.
15. Moiseenkov, A. M.; Lozanova, A. V.; Surkova, A. A.; Strelenko, Y. A.; Buevich, A. V. *Russ. Chem. Bull.* **1994**, *43*, 153–160.
16. Horspool, W. M.; Tedder, J. M.; Din, Z. U. *J. Chem. Soc. (C)* **1969**, 1694–1697.

17. Bocskei, Z.; Keseru, G. M. *J. Chem. Soc., Perkin Trans. 2* **1994**, 595–597.
18. Rodriguez, E.; Towers, G. H. N.; Mitchell, J. C. *Phytochemistry* **1976**, *15*, 1573–1580.
19. Lorimer, S. D.; Burgess, E. J.; Perry, N. B. *Phytomedicine* **1997**, *4*, 273–275.
20. Keseru, G. M.; Nogradi, M. *Nat. Prod. Rep.* **1995**, *12*, 69–75.
21. Oiso, Y.; Toyota, M.; Asakawa, Y. *Chem. Pharm. Bull.* **1999**, *47*, 297–298.
22. Friederich, S.; Rueffer, M.; Asakawa, Y.; Zenk, M. H. *Phytochemistry* **1999**, *52*, 1195–1202.
23. Walker, R. P.; Rosser, R. M.; Faulkner, D. J. *J. Org. Chem.* **1984**, *49*, 5160–5163.
24. Barrow, C. J.; Blunt, J. W.; Munro, M. H. G. *J. Nat. Prod.* **1989**, *52*, 346–359, and references therein.
25. Allinger, N. L.; Yuh, Y. H.; Lii, J.-H. *J. Am. Chem. Soc.* **1989**, *111*, 8551–8566.
26. Lorimer, S. D.; Perry, N. B.; Tangney, R. S. *J. Nat. Prod.* **1993**, *56*, 1444–1450.
27. Gopalakrishnan, G.; Singh, N. D. P.; Kasinath, V. *Molecules* **2001**, *6*, 551–556.