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Secondary metabolites from a *Gloeophyllum* species

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Abstract

Six new sesquiterpenoids, four rearranged illudalanes, one rearranged protoilludane and one sterpurane, were isolated from fermentations of *Gloeophyllum* sp. 97022. Their structures were elucidated by spectroscopy. Gloeophyllol B and C show weak antifungal activity, while 1-hydroxy-3-sterpurene shows weak antifungal, antibacterial and cytotoxic activities. © 2000 Published by Elsevier Science Ltd.

Keywords: *Gloeophyllum*; Basidiomycete; New sesquiterpenoids; Rearranged illudalanes; Rearranged protoilludane; Sterpurane

1. Introduction

Gloeophyllum species are conspicuous fungi causing an intensive brown rot of the colonised wood. The genus is cosmopolitan and can be found in temperate and tropical climates. Previously, the antifungal and cytotoxic oosponol was isolated from *G. abietinum* (Umezawa et al., 1972), while dual cultures of *G. abietinum* and *Heterobasidion annosum* yielded oosponol (Sonnenbichler et al., 1994), oospolactone (Nakajima et al., 1976), oospoglycol (Sonnenbichler et al., 1989), 8-hydroxy-3-(hydroxymethyl)-1H-2-benzopyran-1-one, 5-formyl-2-(1-hydroxyisopropyl)benzofuran, 5-formyl-2-(1-hydroxyisopropyl)-2, 3-dihydrobenzofuran and fomannoxinalcohol (Sonnenbichler et al., 1993). Trametin, a brown pigment derived from fruiting bodies by extraction with methanol has a fluorone structure (Gill and Steglich, 1987).

In our search for new compounds from basidiomycetes, we detected — besides oosponol (**1**) and tyrosol (**2**) (Cross et al., 1963) — six new compounds in submerged cultures of *Gloeophyllum* sp. 97022. In the following, we wish to describe the isolation, structure elucidation and biological activities of the new sesquiterpenoids gloeophyllol A (**3**), B (**4b**), C (**5a**), D (**6**), gloeophyllone (**7**) and 1-hydroxy-3-sterpurene (**8**) (structures and atomic numbering are shown in Fig. 1).

2. Results and discussion

Oosponol (**1**), tyrosol (**2**), gloeophyllol A (**3**), B (**4b**), C (**5a**), D (**6**), gloeophyllone (**7**) and 1-hydroxy-3-sterpurene (**8**) were produced and isolated as described in Section 3. While the structures of oosponol (**1**) and tyrosol (**2**) could be established by comparing their spectral data (NMR data are given in Section 3) with those reported in the literature (e.g. Yamamoto and Nitta, 1962), the structures of the new compounds were elucidated from the NMR data obtained in 1D and 2D experiments. The ^{1}H and ^{13}C NMR data are given in Tables 1 and 2, while the pertinent HMBC correlations are indicated in Fig. 2. Four of the new compounds isolated in this investigation are rearranged illudalane sesuiterpenes, one is a rearranged protoilludane while the last is a sterpurane, and it is

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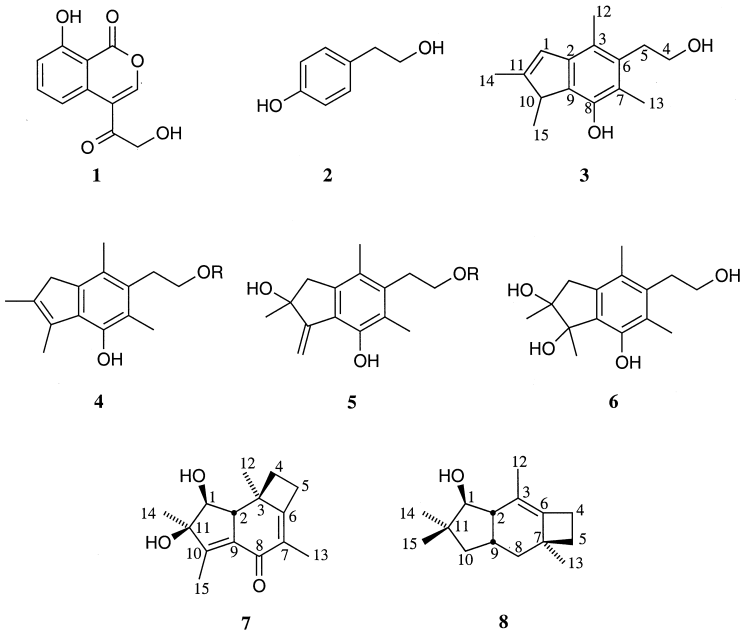

Fig. 1. a: R = H; b: R = CH_3.

interesting to note that rearranged protoilludanes and illudalanes are uncommon.

The mass spectrum of gloeophyllol A (**3**) suggests that its molecular weight is 232, and high resolution experiments show that the exact mass of *m*/*z* 232 corresponds to the elemental composition $C_{15}H_{20}O_2$ and to a molecule with six unsaturations. COSY correlations only reveal the $–CH_2–CH_2–$ unit of C-4/C-5 and the attachment of a methyl group (C-15) to a methine group (C-10). However, revealing HMBC correlations were observed from all the methyl groups (from 12-H_3 to C-2, C-3 and C-6, from 13-H_3 to C-6, C-7 and C-8, from 14-H_3 to C-1, C-10 and C-11, and from 15-H_3 to C-9, C-10 and C-11) as well as from the methylene protons (from 4-H_2 to C-6 and from 5-H_2 to C-3, C-6 and C-7, see also Fig. 2). In addition, 1-H gives HMBC correlations to C-9 and C-10, establishing that gloeophyllol A (**3**) is a substituted indene.

The chiral centre of gloeophyllol A (**3**) has been lost in gloeophyllol B (**4b**) due to the isomerisation of the double bond, and the latter is consequently not optically active. The presence of a methoxy instead of a

Table 1
^{1}H (500 MHz) NMR data (δ multiplicity; *J*) for gleophyllol A (**3**), B (**4b**), C (**5a**), D (**6**), gloeophyllone (**7**) and 1-hydroxy-3-sterpurene (**8**)[a]

H	3	4b	5a	6	7	8
1a	6.43; *s*	3.14; *s*	2.91; *s*	2.86; *d*; 16	3.62; *d*; 6.5	3.24; *d*; 6.1
1b	–	–	–	2.82; *d*; 16	–	–
2	–	–	–	–	3.07; *dd*; 2.6, 6.5	2.12; *m*
4a	3.70; *t*; 7.5	3.42; *t*; 8.1	3.58; *t*; 7.8	3.63; *t*; 8.0	2.08; *m*	2.74; *m*
4b		–	–		2.06; *m*	2.56; *m*
5a	2.97; *t*; 7.5	2.99; *t*; 8.1	2.89; *t*; 7.8	2.86; *t*; 8	3.10; *m*	1.82; *ddd*; 3.5, 9.5, 10.7
5b	–	–	–	–	2.80: *ddd*; 3.4, 7.7, 16.2	1.75; *m*
8	–	–	–	–	–	2.10; *m*/1.76; *m*
9	–	–	–	–	–	2.14; *m*
10a	3.30; *q*; 6.5	–	–	–	–	1.46; *dd*; 8.1, 12.7
10b	–	–	–	–	–	1.31; *dd*; 9.7, 12.7
12	2.25; *s*	2.24; *s*	2.10; *s*	2.08; *s*	1.23; *s*	1.58; *s*
13	2.29; *s*	2.26; *s*	2.18; *s*	2.15; *s*	1.67; *s*	1.04; *s*
14	2.20; *s*	2.01; *s*	1.44; *s*	1.37; *s*	1.38; *s*	1.02; *s*
15	1.30; *d*; 6.5	2.26; *s*	5.78/5.30; 2*s*	1.33; *s*	2.21; *d*; 2.6	0.91; *s*
OMe		3.38; *s*				
OH		4.76; *s*				3.49; *s*

[a] The spectra of **4**, **7**, and **8** were recorded in $CDCl_3$, those of **3** and **5a** in $CDCl_3$:CD_3OD 19:1, and that of **6** in CD_3COCD_3, and the solvent signals (7.25 ppm for $CHCl_3$ and 2.05 ppm for CHD_2COCD_3) were used as reference. The coupling constants *J* are given in Hz.

hydroxy substituent on C-4 increases the molecular weight of gloeophyllol B (**4b**) by 14, and a HMBC correlation from the methoxy protons to C-4 establish its position. Again, HMBC correlations from the methyl groups (from 12-H_3 to C-2, C-3 and C-6, from 13-H_3 to C-6, C-7 and C-8, from 14-H_3 to C-1, C-10 and C-11, and from 15-H_3 to C-9, C-10 and C-11) as well as from the methylene protons (from 4-H_2 to C-6 and from 5-H_2 to C-3, C-6 and C-7) are important for the structure determination, and show that compounds **3** and **4b** share the same skeleton. 1-H_2 give HMBC correlations to C-2 and C-11, which is expected as C-1 is a saturated carbon.

Gloeophyllol B (**4b**) is readily autoxidised to compound **5b**, a process that takes a few days in $CDCl_3$ at room temperature, although interestingly no trace of **5b** was found in the extracts (the NMR data of **5b** is given in Section 3). On the other hand, while compound **4a** was not observed as a metabolite in this investigation, gloeophyllol C (**5a**) could be isolated. Its spectroscopic data are very similar to those of **5b**, except for the absence of signals of the methoxy group and the presence of the signals of an exocyclic methyleme group, and data from mass spectrometry show that **5a** contains one additional oxygen as compared to gloeophyllol A (**3**). The corresponding HMBC correlations from the methyl (except C-15) and methylene protons (as discussed for **3** above) were observed, and the chemical shift of C-11 reveals that it has been oxidised. The exocyclic double bond can be positioned between C-10 and C-15 as 15-H_2 give HMBC correlations to C-9, C-10 and C-11. The fact that gloeophyllol B (**4b**) contains a methoxy group and **5b** is formed chemically from gloeophyllol B (**4b**) makes it uncertain whether **4b** and **5a** are actually true metabolites, especially since methanol was used for the isolation. Protoilludanes similar to gloeophyllone (**7**) are known to be unstable and react with nucleophiles to form aromatic illudalanes according to Fig. 3 (Clericuzio et al., 1997), and it is possible that the true metabolite actually is the protoilludane **9**. However, no trace of **9** was ever observed in this investigation.

High resolution EIMS experiments with gloeophyllol D (**6**) suggest that its chemical composition is $C_{15}H_{22}O_4$, and spectral similarities with compounds **3**, **4b** and **5a** indicate that it is also a rearranged illudalane. The HMBC correlations (corresponding to those discussed above) reveal that gloeophyllol D (**6**) has a second tertiary alcohol function positioned in the five-membered ring. Due to overlapping signals, the relative stereochemistry of gloeophyllol D (**6**) could not be determined.

Gloeophyllone (**7**) is a rearranged protoilludane with a different hydroxylation pattern as compared to gloeophyllol D (**6**). High resolution mass spectrometry indicated that the elemental composition of **7** is $C_{15}H_{20}O_3$, which gives it six unsaturations. Three of these are taken by two carbon–carbon double bonds and one keto function, which means that gloeophyl-

Table 2
^{13}C (125 MHz) NMR data (δ multiplicity) for gloeophyllol A (**3**), B (**4b**), C (**5a**), D (**6**), gloeophyllone (**7**) and 1-hydroxy-3-sterpurene (**8**)[a]

C	3	4b	5a	6	7	8
1	124.2; *d*	42.4; *t*	46.6; *t*	44.5; *t*	75.9; *d*	87.2; *d*
2	147.8; *s*	140.7; *s*	139.5; *s*	136.0; *s*	60.4; *d*	31.8; *d*
3	119.0; *s*	123.9; *s*	124.8; *s*	124.5; *s*	45.4; *s*	122.3; *s*
4	61.9; *t*	71.9; *t*	61.2; *t*	61.9; *t*	32.6; *t*	25.4; *t*
5	33.1; *t*	30.2; *t*	33.2; *t*	34.5; *t*	29.0; *t*	36.4; *t*
6	134.0; *s*	131.3; *s*	136.2; *s*	136.8; *s*	168.2; *s*	142.0; *s*
7	120.7; *s*	121.0; *s*	122.0; *s*	122.2; *s*	127.6; *s*	45.1; *s*
8	142.6; *s*	145.8; *s*	149.4; *s*	151.1; *s*	188.9; *s*	42.7; *t*
9	131.0; *s*	130.5; *s*	123.3; *s*	130.0; *s*	132.2; *s*	46.4; *d*
10	45.7; *d*	131.2; *s*	155.7; *s*	84.3; *s*	150.7; *s*	38.1; *t*
11	149.8; *s*	136.0; *s*	78.6; *s*	81.8; *s*	81.7; *s*	41.4; *s*
12	11.7; *q*	15.3; *q*	15.2; *q*	15.2; *q*	19.6; *q*	17.3; *q*
13	15.2; *q*	11.6; *q*	11.5; *q*	11.4; *q*	9.7; *q*	19.9; *q*
14	14.7; *q*	13.6; *q*	28.0; *q*	21.1; *q*	23.1; *q*	27.1; *q*
15	14.4; *q*	13.2; *q*	105.9; *t*	23.1; *q*	11.4; *q*	20.3; *q*
4-OMe		58.6; *q*				

[a] The spectra of **4**, **7**, and **8** were recorded in $CDCl_3$, those of **3** and **5a** in $CDCl_3$:CD_3OD 19:1, and that of **6** in CD_3COCD_3, and the solvent signals (77.00 ppm for $CDCl_3$ and 29.92 ppm for CD_3COCD_3) were used as reference. The multiplicities of the carbon signals were determined indirectly, by establishing how many protons are attached to each carbon according to a HMQC experiment.

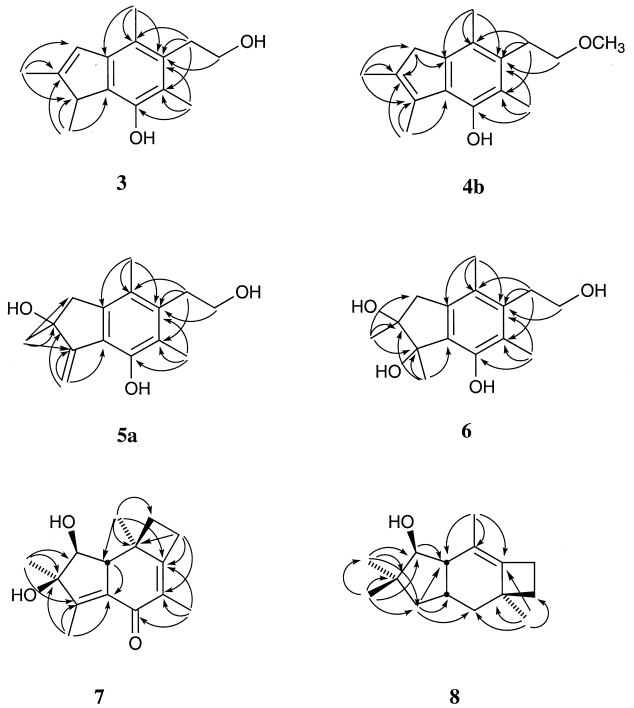

Fig. 2. Pertinent HMBC correlations observed with compounds **3**, **4b**, **5a**, **6**, **7**, and **8**. a: R = H; b: R = CH_3.

lone (**7**) contains three rings. The keto function is obviously unsaturated, as judged by the chemical shift of C-8, and HMBC correlations from 13-H_3 to C-6, C-7 and C-8 show that the α,β-unsaturated ketone is substituted in the α position with a methyl group. Also, 12-H_3, 4-H_2 and 5-H_2 give HMBC correlations to C-6, as well as to C-3, and this together with the strong COSY correlations between 4-H_2 and 5-H_2 prove the existence of a four-membered ring. 12-H_3 and 4-H_2 give additional HMBC correlations, to C-2, which is connected to C-1 by a COSY correlation between 1-H and 2-H, and to C-9 by a HMBC correlation from 2-H to C-9. The remaining two methyl groups give HMBC correlations to C-10, the remaining olefinic carbons that must be connected to C-9, and to C-11. The HMBC correlation from 14-H_3 to C-1 closes the five-membered ring, and the only remaining connection is between C-8 and C-9. Although there are no 2D NMR correlations supporting this bond, its existence is the only alternative that would satisfy the other data. The two hydroxyl groups are suggested to be *cis*, because 2-H only gives a NOESY correlation to 4-Hβ, while 1-Hα correlates to both methyl groups 12-H_3 and 14-H_3.

Finally, analysis of the spectral data of 1-hydroxy-3-sterpurene (**8**) revealed that it is a sterpurane, not obviously related to the other sesquiterpenoids isolated here. Both C-14 and C-15 are connected to the same quaternary carbon, as 14-H_3 as well as 15-H_3 (both appearing as singlets in the ^{1}H NMR spectrum) give HMBC correlations to C-1, C-10 and C-11. C-12 is obviously attached to the carbon–carbon double bond, as 12-H_3 give HMBC correlations to C-2, C-3 and C-6, while 13-H_3 correlate to C-5, C-6, C-7 and C-8. The COSY correlation between 4-H_2 and 5-H_2 as well as their HMBC correlations to C-6 and C-7 show that they are part of a four-membered ring. C-1 is connected to C-2 (by a COSY correlation between 1-H and 2-H, and by a HMBC correlation between 1-H and C-2), while C-10 is connected to C-9 (by a COSY correlation between 10-H_2 and 9-H, and a HMBC correlation between 10-H_2 and C-9). 10-H_2 also give HMBC correlations with C-8, which establishes the C-8-to-C-9 connection. The presence of the remaining bond (between C-2 and C-9) is the only possibility left. NOESY correlations between 15-H_3 and 2-H, 9-H as well as 10-Hβ, and between 10-Hα and 1-Hα as well as 13-H_3 determine the relative stereochemistry of 1-hydroxy-3-sterpurene (**8**).

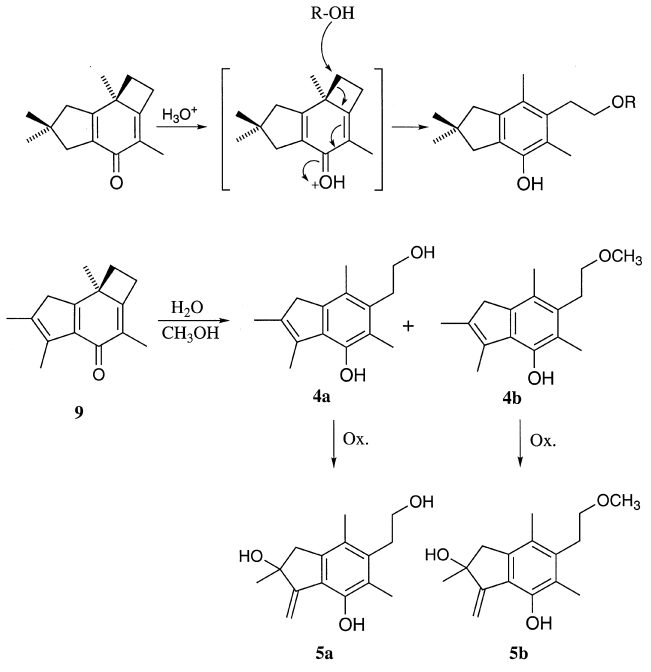

Fig. 3. Compound **9** is a plausible precursor of the isolated gloeophyllols B (**4b**) and C (**5a**), although it was never observed.

In the agar diffusion assay, gloeophyllol B (**4b**), gloeophyllol C (**5a**) and 1-hydroxy-3-sterpurene (**8**) show weak antifungal activities against *Mucor miehei* and *Penicillium notatum* starting from 50 μg/filter disc. In addition, **8** exhibits antibacterial activities against *Bacillus subtilis* and *B. brevis* with 50 μg added to the filter discs. Gloeophyllol A (**3**), gloeophyllol D (**6**) and gloeophyllone (**7**) do not exhibit antibacterial (*B. brevis*, *B. subtilis*, *Enterobacter dissolvens* and *Micrococcus luteus*) or antifungal (*M. miehei*, *P. notatum*, *Paecilomyces variotii* and *Nematospora coryli*) activities at concentrations of up to 100 μg/filter disc. Compound **8** shows weak cytotoxic activities. The IC_{50} for HeLa S3-(epitheloid carcinoma, cervix, human; ATCC CCL 2.2) and HL60-(promyelocytic leukemia, human; ATCC CCL 240) cells were determined to be 50 μg/ml, while COS-7-(Kidney fibroblast, African green monkey; ATCC CRC-1651) cells were somewhat less sensitive (IC_{50} 50–100 μg/ml). The other isolated compounds do not exhibit any cytotoxic activity.

3. Experimental

3.1. Organism

Gloeophyllum sp. 97022 was isolated from fruiting bodies collected from a mangrove in FL, USA. The sporophores show the characteristics of the genus (Jülich, 1984). Its morphological characteristics closely resemble *G. sepiarium* for which, however, an occurrence on mangrove wood would seem unlikely. This species mainly grows on conifer wood. Voucher specimen and strain 97022 are deposited in the culture collection of the LB Biotechnologie, University of Kaiserslautern, Germany. For maintenance on agar slants and fermentation, the fungus was grown in YMG medium. Mycelial cultures were obtained from

spore prints. YMG medium composed of (g/l): yeast extract 4, malt extract 10, glucose 4, pH 5.5 and agar 2.0% for solid media was used for maintenance and submerged culture.

3.2. Fermentation

Fermentations were carried out in 20 l of YMG medium in a Biolafitte C6 fermentation apparatus at 22°C with an aeration rate of 3 l air/min and agitation (120 rpm). A well grown culture of the fungus (250 ml) was used as inoculum. After 180 h of fermentation, the culture fluid was separated from the mycelia by filtration.

3.3. Isolation

The compounds **1**–**8** were extracted from the culture filtrate by adsorption onto Mitsubishi DIAION HP 21 resin. Elution with methanol and acetone yielded crude extracts which were combined and fractionated by chromatography on silica gel (Merck 60, 0.063–0.02 mm). Elution with cyclohexane–ethylacetate 3:7 yielded enriched products containing the compounds oosponol (**1**), tyrosol (**2**), gloeophyllol A (**3**), gloeophyllol C (**5a**), gloeophyllol D (**6**) and gloeophyllone (**7**). An enriched product containing gloeophyllol B (**4b**) and 1-hydroxy-3-sterpurene (**8**) was eluted with cyclohexane–ethylacetate 1:9. The two fractions were subjected to preparative HPLC [Jasco model PU-980 with diode array detector; column: Macherey and Nagel, 250 × 21.2 mm containing Nucleosil C18 (7 μm); flow rate: 5 ml/min]. Elution with water/methanol 80:20 v/v yielded 5.5 mg of **2**, 63:37 v/v yielded 2 mg of **1**, 56:44 v/v yielded 18.2 mg of **5a**, 50:50 v/v yielded 3.9 mg of **6**, 48:52 v/v yielded 4.6 mg of **7** and 40:60 v/v yielded 5.2 mg of **3**. Compound **4b** was isolated with water/methanol 55:45 v/v (yield: 2.9 mg). The cytotoxic compound **8** was eluted last with water/methanol 20:80 v/v, yield: 14.4 mg.

3.4. Analytical methods

For analytical HPLC, a Hewlett Packard 1090 series II instrument (Column: Merck LiChrocart 125-4 filled with LiChrospher 100 RP18) was used. TLC analyses were performed on Macherey–Nagel Alugram Sil G/UV_{254} precoated plates and visualised with anisaldehyde/sulfuric acid 1:1 (5% in ethanol) and heating up to 120°C.

3.5. Biological assays

Assays for antimicrobial (Anke et al., 1989) and cytotoxic (Zapf et al., 1995) activities were carried out as described previously.

3.6. Spectroscopy

^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) were recorded at room temperature with a Bruker ARX500 spectrometer with an inverse multinuclear 5 mm probehead equipped with a shielded gradient coil. The spectra were recorded in acetone-d_6 or $CDCl_3$, and the solvent signals were used as reference. COSY, HMQC and HMBC experiments were recorded with gradient enhancements using sine shaped gradient pulses. For the 2D heteronuclear correlation spectroscopy, the refocussing delays were optimised for $^1J_{CH} = 145$ Hz and $^nJ_{CH} = 10$ Hz. The raw data were transformed and the spectra were evaluated with the standard Bruker UXNMR software (rev. 941001). Mass spectra were recorded with a Jeol SX102 spectrometer, while the UV and the IR spectra were recorded with a Perkin–Elmer λ 16 and a Bruker IFS 48 spectrometer. The optical rotation was measured with a Perkin–Elmer 141 polarimeter at 22°C.

Oosponol (**1**) was identified by comparing its ^{1}H and ^{13}C NMR data (given below) with those reported in the literature (Yamamoto and Nitta 1962). ^{1}H NMR (500 MHz, $CDCl_3$): 10.85, *s*, –O**H**; 8.08, *dd*, $J =$ 8.0 and 0.8, C(OH)═CH–CH═C**H**–C; 7.91, *s*, CO–O–CH═C; 7.75, *dd*, $J = 8$ and 8, C(OH)═CH–C**H**═CH; 7.13, *dd*, $J = 8.4$ and 0.8, C(OH)═C**H**–CH; 4.66; *s*, –CH_2–. ^{13}C NMR (125 MHz, $CDCl_3$): 196.1 C═O; 164.1 O–C═O; 162.1 C–OH; 150.5 O–CH═; 138.6 –CH═**CH**–CH═; 134.2 O–CH═C–**C**═C; 117.6 HO–C═CH–; 116.2 HO–C═CH–CH═**C**H–; 116.0 O–CH═**C**; 106.0 HO–C═**C**–CO; 65.6 CH_2.

Tyrosol (**2**) was identified by comparing its ^{1}H NMR data (given below) with those reported in the literature (Yamamoto and Nitta, 1962). ^{1}H NMR (500 MHz, CD_3OD): 6.99, *d*, $J = 8.4$, HO–C═C**H**–; 6.70, *d*, $J = 8.4$, HO–C═CH–C**H**═; 3.70, *t*, $J = 6.8$, HO–C**H**$_2$–; 2.79, *t*, $J = 6.8$, HO–CH_2–C**H**$_2$–.

Gloeophyllol A (**3**) was obtained as a colourless oil. $[\alpha]_D$ −33° (*c* 0.2 in $CHCl_3$: CH_3OH 19:1). UV (MeOH), λ_{max} (ε): 300 nm (1500) and 265 nm (4100). IR (KBr): 3410, 2965, 1635, 1450, 1380, 1280, 1085, 1040 and 890 cm^{-1}. See Tables 1 and 2 for NMR data. EIMS (70 eV), *m*/*z* (rel. int.): 232.1483 (57%, M$^+$, $C_{15}H_{20}O_2$ requires 232.1463), 201 (100%), 187 (22%).

Gloeophyllol B (**4b**) was obtained as a colourless oil with no optical activity. UV (MeOH), λ_{max} (ε): 302 nm (800) and 264 nm (2800). IR (KBr): 3465, 2960, 2930, 1620, 1440, 1375, 1285, 1260, 1100, 1040 and 845 cm^{-1}. See Tables 1 and 2 for NMR data. EIMS (70 eV), *m*/*z* (rel. int.): 246.1631 (25%, M$^+$, $C_{16}H_{22}O_2$ requires 246.1620).

Gloeophyllol C (**5a**) was obtained as a colourless oil with no optical activity (*c* 0.6 in $CHCl_3$). UV (MeOH), λ_{max} (ε): 300 nm (650), 262 nm (5000) and

222 nm (15,500). IR (KBr): 3410, 2965, 1635, 1450, 1380, 1280, 1085, 1040 and 890 cm^{-1}. See Tables 1 and 2 for NMR data. EIMS (70 eV), *m/z* (rel. int.): 248.1427 (76%, M^+, $C_{15}H_{20}O_3$ requires 248.1412), 233 (15%), 230 (12%), 217 (100%), 205 (14%), 199 (19%) 175 (12%).

Compound **5b** was obtained as a colourless oil with no optical activity, formed from compound **4b** when it was left on a laboratory bench in $CDCl_3$ in a NMR tube for 2 days at room temperature. ^1H NMR (500 MHz, $CDCl_3$): 5.96, *s*, 15-Ha; 5.40, *s*, 15-Hb; 3.42, *t*, $J_{4-5} = 8.0$, 4-H_2; 3.37, *s*, 4-OCH_3; 3.39, *d*, $J_{1a-1b} = 17.1$, 1-Ha; 2.98, *t*, $J_{4-5} = 8.0$, 5-H_2; 2.89, *d*, $J_{1a-1b} = 17.1$, 1-Hb; 2.24, *s*, 13-H_3; 2.19, *s*, 12-H_3; 1.48, *s*, 14-H_3. ^{13}C NMR (125 MHz, $CDCl_3$): 150.7 C-10; 149.3 C-8; 140.6 C-2; 136.8 C-6; 125.0 C-3; 122.7 C-9; 120.6 C-7; 107.8 C-15; 90.4 C-11; 71.4 C-4; 58.7 4-OCH3; 41.1 C-1; 30.6 C-5; 24.3 C-14; 15.4 C-12; 11.4 C-13. EIMS (70 eV), *m/z* (rel. int.): 262.1555 (69%, M^+, $C_{16}H_{22}O_3$ requires 262.1569) 217 (100%), 201 (25%), 187 (16%).

Gloeophyllol D (**6**) was obtained as a colourless oil with no optical activity (*c* 0.4 in $CHCl_3$). UV (MeOH), λ_{max} (ε): 283 nm (1500). IR (KBr): 3430, 2970, 1630, 1440, 1380, 1260, 1135, 1045 and 935 cm^{-1}. See Tables 1 and 2 for NMR data. EIMS (70 eV), *m/z* (rel. int.): 266.1525 (21%, M^+, $C_{15}H_{22}O_4$ requires 266.1518), 248 (100%), 233 (24%), 217 (93%), 205 (20%).

Gloeophyllone (**7**) was obtained as a colourless oil. $[\alpha]_D$ -140° (*c* 0.3 in $CHCl_3$). UV (MeOH), λ_{max} (ε): 286 nm (14,100). IR (KBr): 3380, 2940, 1460, 1440, 1370, 1070 and 1035 cm^{-1}. See Tables 1 and 2 for NMR data. EIMS (70 eV), *m/z* (rel. int.): 248.1421 (18%, M^+, $C_{15}H_{20}O_3$ requires 248.1412), 230 (65%), 215 (42%), 187 (100%), 173 (48%), 159 (43%).

1-Hydroxy-3-sterpurene (**8**) was obtained as a colourless oil. $[\alpha]_D$ -28° (*c* 0.3 in $CHCl_3$). UV (MeOH), λ_{max} (ε): No maxima above 210 nm. IR (KBr): 3380, 2940, 1460, 1440, 1370, 1070 and 1035 cm^{-1}. See Tables 1 and 2 for NMR data. EIMS (70 eV), *m/z* (rel. int.): 220.1812 (82%, M^+, $C_{15}H_{24}O$ requires 220.1827), 205 (28%), 191 (41%), 187 (40%), 173 (78%), 135 (46%), 133 (53%), 119 (100%).

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