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PERENNIPORIOL DERIVATIVES, SIX TRITERPENOIDS FROM THE CULTURED MYCELIA OF *PERENNIPORIA OCHROLEUCA**

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Key Word Index—*Perenniporia ochroleuca*; Basidiomycetes; higher fungi; triterpenoids; perenniporiol; 3-acetylperenniporiol; 15-deacetoxy-7,11-dihydroperenniporiol; (26*S*)-26-*O*-methylperenniporiol; (26*S*)-3-acetyl-26-*O*-methylperenniporiol; (26*S*)-15-deacetoxy-7,11-dihydro-26-*O*-methylperenniporiol.

Abstract—Six new triterpenoids, (26*S*)-26-*O*-methylperenniporiol, (26*S*)-3-acetyl-26-*O*-methylperenniporiol, perenniporiol, 3-acetylperenniporiol, (26*S*)-15-deacetoxy-7,11-dihydro-26-*O*-methylperenniporiol and 15-deacetoxy-7,11-dihydroperenniporiol were isolated from the cultured mycelia of *Perenniporia ochroleuca*. The structures of the first two compounds were determined using spectroscopic and X-ray analyses, and the structures of the other compounds were elucidated by spectroscopic data.

INTRODUCTION

Wood-rotting fungi belonging to the Polyporaceae (Basidiomycetes) are a rich source of lanostane-type tetracyclic triterpenoids [1]. In the course of studies on cultured Polyporaceae, many new triterpenoid derivatives have been found in the methanol and benzene extracts of the cultured mycelia of *Perenniporia ochroleuca* (Japanese name: Uzuratake). A previous chemical investigation [2] of the fruiting bodies of *Perenniporia ochroleuca* led to the identification of ergost-7-en-3β-ol and ergosta-7,22-dien-3β-ol, but the chemical constituents of the cultured mycelia of *P. ochroleuca* have not been studied.

In this paper we wish to report the structural elucidation of three compounds (**1a**, **1c** and **2a**) from the methanol extract and three compounds (**1b**, **1d** and **2b**) from the benzene extract, respectively. The *O*-methyl derivatives (**1a**, **1c** and **2a**) appear to be artefacts formed during the extraction process.

RESULTS AND DISCUSSION

The chloroform extract (28.4 g) of the cultured mycelia was chromatographed on silica gel and separated into fractions A–E. Each fraction was rechromatographed on silica gel and HPLC, and compounds **1a** (350.9 mg), **1c** (191.2 mg) and **2a** (333.9 mg) were isolated from fractions D, B and C, respectively.

Compound **1a**, (26*S*)-26-*O*-methylperenniporiol, $C_{33}H_{50}O_5$, showed absorptions in the UV spectrum at 235, 243 and 251 nm. These values and intensities are characteristic of a heteroannular diene. Compound **1a** had hydroxyl (3560 cm^{-1}) and ester carbonyl (1715 cm^{-1}) absorptions in the IR spectrum. The ^1H NMR spectrum of **1a** (Table 1) clearly showed the presence of one secondary and five tertiary methyl groups, thus suggesting a lanostane skeleton. This was supported by a positive Liebermann–Burchard colour reaction (brown-yellow). The various oxygenated carbon signals gave rise in the ^{13}C NMR spectrum to four lines: 68.1 *d* (C-22), 77.1 *d* (C-15), 78.8 *d* (C-3), 99.5 *d* (C-26). The last signal indicates a carbon atom bearing two oxygens, such as an acetal type. The signal at δ78.8 was identical to the C-3 signal of lanosterol and suggested that the hydroxyl group at C-3 of **1a** was β [3]. The proton signals at δ3.84 and 4.63 were identified as H-22 and H-26, respectively, by $\{^{13}C\}$, $\{H\}$ selective decoupling. Furthermore, the abundant ion at *m/z* 127 (50%) corresponded to the fragment of the side chain resulting from cleavages between C-20 and C-22. All these data indicated that **1a** was a 3β-hydroxy-Δ^7, $\Delta^{9(11)}$-lanostane derivative with a six-membered acetal structure in the side chain. Acetylation of **1a** (pyridine–Ac_2O) gave the acetate (colourless needles, mp 208–209°, $C_{35}H_{52}O_6$), which was identical to **1c**. The relative configurations at the respective asymmetric centres of **1c** were determined by X-ray analysis. Crystal data: orthorhombic, space group $P2_12_12_1$, $a = 20.608$ (5), $b = 24.917$ (7), $c = 6.417$ (1) Å, $Z = 4$. Three-dimensional intensity data were collected on a Rigaku diffractometer with graphite-monochromated Cu Kα radiation ($\lambda = 1.54178$ Å) using a crystal of dimensions $0.3 \times 0.1 \times 0.1$ mm. Integrated intensities were measured in the range $\theta \leqslant 65°$ with an $\omega - 2\theta$ scan. The intensities of 3221 independent reflections were corrected for Lorentz and polarization factors, but not for absorption effects. The structure (Fig. 1) was solved by the direct method, and refined by the block-diagonal least-squares technique to an *R* value of 0.098 for 1968 reflections with $|F_o| \geqslant 3\sigma(F_o)$. The absolute configuration of the molecule is based on the ORD of **1e**, obtained by oxidation of **1a** with pyridinium dichromate, which showed a strong positive Cotton effect ($[\phi]^{20}_{254} + 20370$) like that of lanosta-$\Delta^7$, $\Delta^{9(11)}$-diene-3-one [4]. Thus **1a** and **1c** are (22*S*, 26*S*)-15α-

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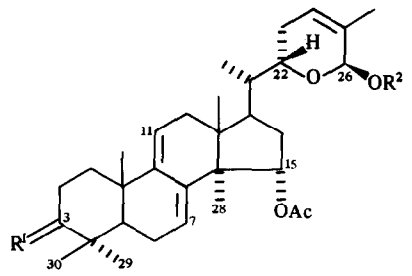


1a R^1 =  R^2 = Me

(26*S*) – 26 – O – methylperenniporiol

1b R^1 =  R^2 = H Perenniporiol

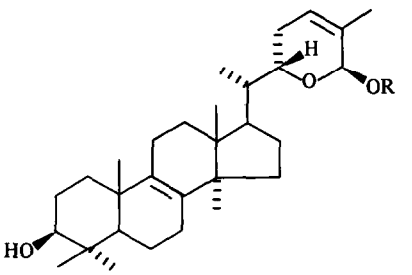
1c R^1 =  R^2 = Me

(26*S*) – 3 – acetyl– 26 – *O* – methylperenniporiol

1d R^1 =  R^2 = H 3 – acetylperenniporiol

1e R^1 = O R^2 = Me

(26*S*) – 26 – *O* – methyl – 3 – oxoperenniporiol



2a R = Me

(26*S*) – 15 – deacetoxy – 7, 11 – dihydro – 26 – *O* – methylperenniporiol

2b R = H

15 – deacetoxy – 7, 11 – dihydroperenniporiol

acetoxy-22,26-epoxy-3β-hydroxy-26-methoxy-5α-lanosta-7,9(11),24-triene and the corresponding acetate, respectively.

Compound **2a**, (26*S*)-15-deacetoxy-7,11-dihydro-26-*O*-methylperenniporiol, colourless needles, mp 170–171°, showed no UV absorption. The molecular formula was established as $C_{31}H_{50}O_3$ by high resolution mass spectroscopy. In the mass spectrum of **2a**, the fragment at *m/z* 127 (cleavage between C-20 and C-22) suggested that **2a** had the same acetal structure in the side chain as that of **1a** and **1c**. In compound **2a**, the lack of UV absorption and the loss of two olefinic protons and two trisubstituted olefinic carbon signals in the ^{1}H NMR and ^{13}C NMR spectra indicated that the diene moiety of compound **1a** had been replaced by a monoene structure. Furthermore, there was no evidence for the presence of a 15α-acetoxy group. From these spectral data compound **2a** was established as (26*S*)-15-deacetoxy-7,11-dihydro-26-*O*-methylperenniporiol.

In the second experiment the benzene extract of cultured mycelia was separated by silica gel column chromatography (see Experimental). After repeating the silica gel column chromatography and HPLC, compound **1b**, perenniporiol, was obtained as a colourless solid, mp 186–187°, which could not be crystallized. The molecular formula was established as $C_{32}H_{48}O_5$ by high resolution mass spectroscopy. The UV spectrum of **1b** showed the presence of a heteroannular diene [232, 242, 251 nm (log ε 4.11, 4.17, 4.0)]. Compound **1b** exhibited hydroxyl (3420 cm^{-1}) and ester carbonyl (1710 cm^{-1}) absorptions in its IR spectrum. The ^{1}H NMR spectrum of **1b** showed the presence of one secondary (21-Me) and five tertiary methyl groups (Table 1). In addition, a new hydroxyl proton signal (δ2.61) coupled with the H-26 proton (δ5.14) was observed. The methoxy methyl signal of **1a** was absent. In the ^{13}C NMR spectrum, the signal of the hemiacetal carbon of **1b** shifted upfield (δ92.8) relative to the signal in the spectrum of **1a**. These data suggested that **1b** had a hydroxyl group instead of a methoxy group attached to C-26. In order to verify the proposed structure, a mixture of **1b** and *p*-toluenesulphonic acid in methanol was left at room temperature for 10 min. Purification by HPLC and recrystallization gave methylated **1b**, which was identical in all respects to **1a**. The stereochemistry of the C-26 hydroxyl group was deduced unambiguously by the carbon shifts of the hemiacetal moiety [5]. The configuration of C-22 of **1b** was assigned as *S*(β) by comparison with **1a**. In the ^{13}C NMR spectrum, the chemical shift value (δ68.2) of C-22 of **1b** suggested that there was a 1,3-diaxial relationship between H-22 and the C-26 hydroxyl group [6]. Thus the C-26 hydroxyl group is axial and β. From these results, the structure of compound **1b**, named perenniporiol, was established as (22*S*,26*S*)-15α-acetoxy-22,26-epoxy-3β,26-dihydroxy-5α-lanosta-7,9(11),24-triene.

After repeated silica gel column chromatography and HPLC, the second compound, 3-acetylperenniporiol (**1d**), was obtained as colourless needles. The molecular formula was established as $C_{34}H_{50}O_6$ by high resolution mass spectroscopy. The UV spectrum showed absorptions at 236, 243, 252 nm (log ε 4.31, 4.36, 4.20). The IR spectrum and ^{1}H NMR data showed the presence of a hydroxyl group (3500 cm^{-1}; δ2.53, disappearing with D_2O) and two acetate methyl groups (δ2.06 and 2.08). In the ^{13}C NMR spectrum, the signal of the hemiacetal carbon shifted upfield (δ92.8) relative to the signal for **1c** and the methoxy methyl carbon which was observed in the spectrum of **1c** was absent. These data suggested that **1d** was the hemiacetal analogue of **1c**. In order to verify the proposed structure, a mixture of **1d** and *p*-toluenesulphonic acid in methanol was left at room temperature for 10 min. Recrystallization of the product gave methylated **1d**, identical to **1c** in all respects. Consideration of the ^{13}C NMR chemical shifts indicated that **1d** had the

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Table 1. ^{1}H NMR spectral data of compounds **1a–1e**, **2a** and **2b** (400 MHz, $CDCl_3$, TMS as internal standard)

Assignments	1a	1b	1c	1d	1e	2a	2b
H-18	0.67 *s*	0.66 *s*	0.69 *s*	0.66 *s*	0.70 *s*	0.71 *s*	0.70 *s*
H-19	0.88 *s*	0.88 *s*	0.80 *s*	0.90 *s*	1.12 *s*	0.91 *s*	0.93 *s*
H-21	0.93 *d*	0.91 *d*	0.93 *d*	0.91 *d*	0.94 *d*	0.96 *d*	0.94 *d*
	J = 6.8 Hz	*J* = 6.8 Hz	*J* = 6.8 Hz	*J* = 7.1 Hz	*J* = 6.8 Hz	*J* = 6.8 Hz	*J* = 6.8 Hz
H-14	0.97 *s*	0.97 *s*	0.94 *s*	0.95 *s*	1.03 *s*	0.81 *s*	0.81 *s*
H-29 (4α)	1.03 *s*	1.05 *s*	1.03 *s*	1.05 *s*	1.10 *s*	1.00 *s*	1.00 *s*
H-30 (4β)	1.02 *s*	1.01 *s*	0.99 *s*	0.99 *s*	1.19 *s*	0.99 *s*	0.97 *s*
H-20	1.43 *m*	1.43 *m*	1.43 *m*	1.45 *m*	1.45 *m*	1.44 *m*	1.40 *m*
C-15 $C\underline{H}_3COO$	2.07 *s*	2.08 *s*	2.07 *s*	2.08 *s*	2.08 *s*	—	—
C-3 $C\underline{H}_3COO$	—	—	2.06 *s*	2.06 *s*	—	—	—
C-26 O<u>H</u>	—	2.61 *bd*	—	2.53 *d*	—	—	2.55 *d*
		J = 3.1 Hz		*J* = 5.4 Hz			*J* = 4.9 Hz
H-3α	3.26 *dd*	3.24 *dd*	4.50 *dd*	4.51 *dd*	—	3.24 *dd*	3.25 *bd*
	J = 10, 4.1 Hz	*J* = 11.1, 4.5 Hz	*J* = 11.2, 4.4 Hz	*J* = 11.4, 4.5 Hz		*J* = 11.6, 4.3 Hz	*J* = 13 Hz
C-26 OMe	3.46 *s*	—	3.46 *s*	—	3.46 *s*	3.46 *s*	—
H-22	3.84 *dd*	3.91 *dd*	3.84 *dd*	3.92 *dd*	3.84 *dd*	3.95 *dd*	4.03 *dd*
	J = 11.3, 2.0 Hz	*J* = 11.4, 2.2 Hz	*J* = 11.3, 2.0 Hz	*J* = 9.5, 2.0 Hz	*J* = 9.3, 2.4 Hz	*J* = 11.9, 2.3 Hz	*J* = 11.5, 2.0 Hz
H-26α	4.63 *s*	5.14 *d*	4.63 *s*	5.14 *d*	4.63 *s*	4.63 *s*	5.16 *d*
		J = 3.1 Hz		*J* = 5.4 Hz			*J* = 4.9 Hz
H-15β	5.10 *dd*	5.08 *dd*	5.10 *dd*	5.10 *dd*	5.12 *dd*	—	—
	J = 9.5, 6.0 Hz	*J* = 9.3, 5.6 Hz	*J* = 9.2, 5.9 Hz	*J* = 9.3, 5.6 Hz	*J* = 9.5, 5.6 Hz		
H-7	5.34 *d*	5.32 *d*	5.33 *d*	5.33 *d*	5.41 *d*	—	—
or	J = 6.3 Hz	*J* = 6.3 Hz	*J* = 6.3 Hz	*J* = 6.3 Hz	*J* = 5.9 Hz		
H-11	5.50 *d*	5.49 *d*	5.47 *d*	5.48 *d*	5.53 *d*	—	—
	J = 5.9 Hz	*J* = 6.1 Hz	*J* = 5.9 Hz	*J* = 5.9 Hz	*J* = 6.3 Hz		
H-24	5.66 *d*	5.66 *d*	5.64 *d*	5.66 *d*	5.65 *d*	5.66 *d*	5.68 *d*
	J = 5.4 Hz	*J* = 5.4 Hz	*J* = 5.9 Hz	*J* = 5.6 Hz	*J* = 5.4 Hz	*J* = 5.4 Hz	*J* = 5.6 Hz

—, Indicates no signal.

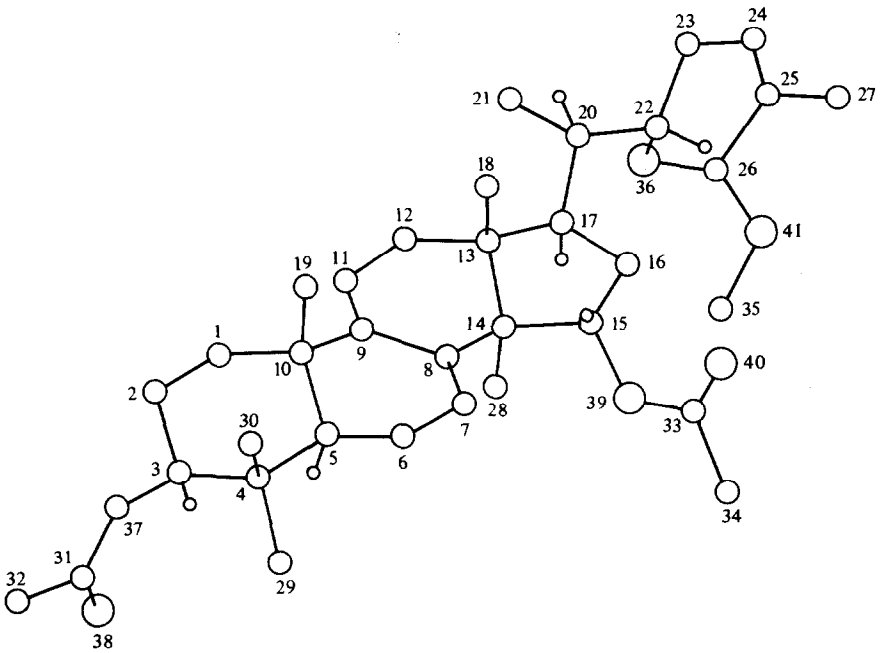

Fig. 1. A computer-generated perspective drawing of **1c**.

same relative stereochemistry as **1b**. From these results the structure of compound **1d** was established as 3-acetylperenniporiol.

The third of the triterpenoids from the benzene extract, 15-deacetoxy-7,11-dihydroperenniporiol (**2b**), $C_{30}H_{48}O_3$, was obtained as colourless needles, mp 187–188°. The UV spectrum showed no absorption while the IR spectrum had hydroxyl absorption (3360 cm^{-1}). The ^1H NMR and ^{13}C NMR spectra of compound **2b** (see Table 1 and Experimental) suggested a structure closely related to compound **2a**. In fact, the difference between **2b** and **2a** was the presence in the ^1H NMR spectrum of compound **2b** of an additional hydroxyl group (δ2.55, disappearing with D_2O) coupled to the signal due to hemiacetal methine H-26 (δ5.16) and the absence of a methoxyl group. Again the ^{13}C NMR chemical shifts of the hemiacetal moiety indicated the same relative stereochemistry as in **2a**. Finally, methylated compound **2b** was identical in all respects to **2a**. Thus **2b** is 15-deacetoxy-7,11-dihydroperenniporiol.

The fact that **1a**, **1c** and **2a**, containing a methoxyl group at C-26, were obtained by methanol extraction and were not present in the benzene extract suggests that they are artefacts.

It is well known that lanostane derivatives are widely distributed in Basidiomycetes, but lanostane derivatives with a hemiacetal or lactone structure in the side chain like the withanolides [7] are unknown. Batta and Rangaswami [8] have reported the presence of oxidosenexone, which has a five-membered 22,25-epoxy structure in the side chain, in *Fomes senex*. The occurrence of lanostane derivatives such as **1a**, **1b**, **1c**, **1d**, **2a** and **2b** with a six-membered 22,26-epoxy structure from Basidiomycetes has been demonstrated for the first time.

EXPERIMENTAL

Mps were determined in a Büchi apparatus and Kofler hot plate, and are uncorr. NMR spectra were taken in $CDCl_3$ at 25° using TMS as internal standard; ^{13}C NMR at 25.2 MHz and ^1H NMR at 400 MHz. MS were run using a direct insertion probe.

Isolation and culture conditions of Perenniporia ochroleuca. After sterilizing the fruiting bodies (collected at Kyoto in July 1979) of *Perenniporia ochroleuca*, a small piece was inoculated onto a malt agar medium. Developing colonies of the new hyphae from the fruiting body were transferred to malt agar in Petri dishes. The formation of the clamp connection of the pure cultured hyphae was observed under a microscope. The pure mycelium was subcultured for 2–3 weeks and grown in modified Raulin's medium (according to the methods of Bassett *et al.* [9]) containing 46.6 g sucrose, 2.66 g tartaric acid, 0.4 g KH_2PO_4, 2.7 g NH_4NO_3, 0.4 g K_2CO_3, 0.26 g $MgCO_3$, 0.16 g $CaSO_4$, 0.06 g $ZnSO_4$, 0.06 g $FeSO_4$ and 0.1 mg thiamine hydrochloride in 1 l. H_2O. The medium was adjusted to pH 4.5 with 1 M NaOH, dispensed in 1 l. Roux flasks (200 ml each flask) and autoclaved at 121° for 20 min. Usually, each flask was seeded with 5 or 7 of the 10 mm plugs cut from the malt agar culture. The culture was maintained in the dark at 25°.

Extraction procedure and separation of the MeOH *extract of the mycelia.* After 56 days' culture (190 Roux flasks), the mycelia (4.9 kg fr. wt) were harvested with nylon cloth, homogenized with MeOH (18 l.) in a Waring blender and allowed to stand for 1 week at room temp. The homogenate was filtered, and the residue was extracted 2 × with fresh solvent. The filtrates were combined, and the organic solvent was removed under red. pres. The residue was extracted with $CHCl_3$ (500 ml × 3), dried and evapd to dryness. The $CHCl_3$ extract (28.4 g) was subjected to chromatography over silica gel (900 g Wako gel C-200). Elution with 3% EtOAc in C_6H_6 2.0 l. (fraction A), 1.3 l. (fraction B), 2.8 l. (fraction C), 3–20% EtOAc in C_6H_6 8.9 l. (fraction D) and 20% EtOAc in C_6H_6 2.4 l. + 30% EtOAc in C_6H_6 0.9 l. (fraction E) yielded the crude mixture of compound **1c** (fraction B 2.57 g), compound **2a** (fraction C 4.07 g) and compound **1a** (fraction D 9.98 g), respectively.

Isolation and structure elucidation of compound **1a**. Fraction D

(9.98 g) was rechromatographed on a silica gel column (370 g Wako gel C-200) and eluted with a C_6H_6–EtOAc solvent system. The amorphous powder, compound **1a** (878 mg), which showed one spot on TLC (R_f 0.4; C_6H_6–EtOAc, 4:1) was obtained but it was a mixture of two components showing two peaks on HPLC (R_t 11.0, 13.1 min; see the conditions of HPLC). Further purification was achieved on HPLC, and compound **1a** was isolated from the fraction containing the peak at 11.0 min. Compound **1a** (350.9 mg), colourless prisms, mp 188–189°, $C_{33}H_{50}O_5$ (required 526.3658, $[M]^+$ *m/z* 526.3647), $[\alpha]_D^{28}$ + 138° ($CHCl_3$, *c* 0.58); IR ν_{max}^{KBr} cm^{-1}: 3560 (OH), 2940 (CH), 1715 (COO), 1260, 1035; UV λ_{max}^{EtOH} nm (log ε): 235 (4.28), 243 (4.34), 251 (4.17). ^1H NMR (400 MHz, $CDCl_3$): see Results and Discussion and Table 1; ^{13}C NMR (25.2 MHz, $CDCl_3$): δ12.6 (*q*), 15.7 (*q* × 2), 18.5 (*q*), 18.9 (*q*), 21.4 (*q*), 22.8 (*q*), 23.0 (*t*), 27.7 (*t*), 28.1 (*q*), 28.2 (*t*), 35.7 (*t*), 37.1 (*t*), 37.4 (*s*), 38.0 (*s*), 38.7 (*t*), 39.8 (*d*), 43.8 (*s*), 45.1 (*d*), 48.8 (*d*), 51.4 (*s*) and 55.4 (*q*), 68.1 (*d*), 77.1 (*d*), 78.8 (*d*), 99.5 (*d*), 115.9 (*d*), 121.3 (*d*), 123.7 (*d*), 132.1 (*s*), 140.1 (*s*), 145.9 (*s*), 170.8 (*s*). In the selective decoupling experiments of compound **1a**, each proton signal (from δ0.67 to 5.66) was irradiated and the decoupled carbon signal was observed. In the experiment of **1a**, when the proton signals at δ3.84 and 4.63 were irradiated, the singlet carbon signals at δ68.1 and 99.5 were clearly observed. EIMS (direct inlet) 20 eV, *m/z* (rel. int.): 526 $[M]^+$ (20), 494 $[M - MeOH]^+$ (100), 479 (26), 438 (26), 434 $[M - MeOH - HOAc]^+$ (54), 420 (28), 419 (81), 401 (13), 377 (14), 369 (35), 309 (13), 127 $[C_7H_{11}O_2]^+$ (50), 95 (50).

Isolation and structure elucidation of compound **1c**. After repeating silica gel CC and HPLC of fraction B, compound **1c** (191.2 mg) was obtained as colourless needles from MeOH, mp 208–209°; $C_{35}H_{52}O_6$ (required 568.3764, $[M]^+$ *m/z* 568.3768); $[\alpha]_D^{28}$ + 174° ($CHCl_3$, *c* 0.52); IR ν_{max}^{KBr} cm^{-1}: 2920 (CH), 1730 (COO), 1250, 1240; UV λ_{max}^{MeOH} nm (log ε): 236 (4.27), 243 (4.34), 252 (4.16). ^1H NMR (400 MHz, $CDCl_3$): see Results and Discussion and Table 1. ^{13}C NMR (25.2 MHz, $CDCl_3$): δ12.6 (*q*), 15.7 (*q*), 16.9 (*q*), 18.4 (*q*), 18.9 (*q*), 21.3 (*q* × 2). 22.9 (*q*, *t*), 24.2 (*t*), 28.1 (*t*, *q*), 35.4 (*t*), 37.1 (*t*), 37.3 (*s*), 37.6 (*s*), 38.0 (*t*), 39.9 (*d*), 43.8 (*s*), 45.2 (*d*), 49.0 (*d*), 51.4 (*s*), 55.4 (*q*), 68.1 (*d*), 77.0 (*d*), 80.7 (*d*), 99.5 (*d*), 116.2 (*d*), 121.1 (*d*), 123.7 (*d*), 132.1 (*s*), 140.2 (*s*), 145.6 (*s*), 170.9 (*s* × 2). EIMS (direct inlet) 20 eV, *m/z* (rel. int.): 568 $[M]^+$ (7), 536 $[M - MeOH]^+$ (60), 476 $[M - MeOH - HOAc]^+$ (36), 461 $[M - MeOH - HOAc - Me]^+$ (47), 411 $[M - side\ chain]^+$ (26), 351 $[M - side\ chain - HOAc]^+$ (9), 291 $[M - side\ chain - 2HOAc]^+$ (11), 127 $[C_7H_{11}O_2]^+$ (46).

Isolation and structure elucidation of compound **2a**. After repeating silica gel CC and HPLC with a part of fraction C, compound **2a** (333.9 mg) was obtained as colourless needles. Mp 170–171°, $C_{31}H_{50}O_3$ (required 470.3760, $[M]^+$ *m/z* 470.3771); $[\alpha]_D^{22}$ + 68° ($CHCl_3$, *c* 0.2); IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 2930, 2860, 2820 (CH), 1450, 1370, 1090, 1050, 1025; UV absorbption was not observed. ^1H NMR (400 MHz, $CDCl_3$): see Results and Discussion and Table 1. ^{13}C NMR (25.2 MHz, $CDCl_3$): δ12.9 (*q*), 15.5 (*q* × 2), 18.3 (*q*), 19.0 (*t*), 19.2 (*q*), 21.1 (*t*), 24.3 (*q*), 26.5 (*t*), 27.9 (*t*), 28.0 (*q*), 28.1 (*t*), 28.2 (*t*), 30.9 (*t*), 31.0 (*t*), 35.6 (*t*), 37.1 (*s*), 38.9 (*s*), 40.4 (*d*), 44.3 (*s*), 46.7 (*d*), 49.9 (*s*), 50.4 (*d*), 55.3 (*q*), 68.1 (*d*), 78.9 (*d*), 99.5 (*d*), 123.8 (*d*), 132.2 (*s*), 134.2 (*s*), 134.6 (*s*). EIMS (direct inlet) 25 eV, *m/z* (rel. int.): 470 $[M]^+$ (64), 455 $[M - Me]^+$ (19), 439 $[M - MeO]^+$ (13), 423 $[M - Me - MeO]^+$ (100), 357 (33), 341 (16), 299 (13), 127 $[C_7H_{11}O_2]^+$ (42).

X-Ray data of compound **1a** *acetate* (*compound* **1c**). Compound **1a** (104 mg) was acetylated by reacting with Ac_2O–pyridine and allowing the mixture to stand at room temp. overnight. The acetate was recrystallized from MeOH to afford colourless needles (66.2 mg), mp 208–209°. All spectroscopic data were completely consistent with those of compound **1c**. X-Ray data are described in the Results and Discussion, and atomic coordinates have been deposited at the Cambridge Crystallographic Data Centre.

Synthesis of compound **1e** (*3-oxo-compound* **1a**) [10]. Compound **1a** (50 mg) was reacted with pyridinium dichromate (35 mg) in 5 ml CH_2Cl_2 for 17 hr at room temp. The reaction mixture was filtered, and the filtrate was then further filtered through a small amount of silica gel to remove the last trace of Cr species. After removing the CH_2Cl_2, the residue was recrystallized from MeOH to give colourless needles (31.9 mg, yield 64%), mp 170–171°, $C_{33}H_{48}O_5$ (required 524.3502, $[M]^+$ *m/z* 524.3511); $[\alpha]_D^{28}$ + 121° ($CHCl_3$, *c* 0.35); IR ν_{max}^{KBr} cm^{-1}: 2930 (CH), 1735 (COO), 1710 (CO), 1250, 1025; UV λ_{max}^{EtOH} nm (log ε): 235 (4.01), 241 (4.06), 251 (3.91). ORD, $[M]^{22}$ (EtOH, 0.00051) (nm): + 1080 (589), + 18 640 (250), − 16 680 (230). ^1H NMR (400 MHz, $CDCl_3$): see Table 1. ^{13}C NMR (25.2 MHz, $CDCl_3$): δ12.7 (*q*), 15.8 (*q*), 18.4 (*q*), 18.9 (*q*), 21.4 (*q*), 22.2 (*q*), 22.5 (*q*), 23.7 (*t*), 25.5 (*q*), 28.2 (*t*), 34.9 (*t*), 36.7 (*t*), 37.2 (*t*), 37.4 (*s*), 38.1 (*t*), 40.0 (*d*), 44.0 (*s*), 45.3 (*d*), 47.6 (*s*), 50.6 (*s*), 51.6 (*s*), 55.6 (*q*), 68.4 (*d*), 77.2 (*d*), 99.8 (*d*), 117.4 (*d*), 121.4 (*d*), 124.0 (*d*), 132.6 (*s*), 140.8 (*s*), 144.9 (*s*), 171.4 (*s*), 217.0 (*s*). EIMS (direct inlet) 25 eV, *m/z* (rel. int.): 524 $[M]^+$ (7), 492 $[M - MeOH]^+$ (42), 432 $[M - MeOH - HOAc]^+$ (31), 417 (23), 367 (26), 307 (24), 293 (10), 269 (14), 255 (10), 170 (11), 127 (77), 99 (100), 95 (90).

Extraction procedure and separation of the C_6H_6 *extract*. After 56 days' culture (100 Roux flasks), the mycelia (fr. wt 2.04 kg) were lyophilized, and the dried mycelia (193.3 g) were extracted with C_6H_6 (2.2 l.) at room temp. for 3 days. The C_6H_6 soln was filtered, the residue was re-extracted with C_6H_6 (3 l.) according to the same method as described above, and the filtration residue was refluxed with C_6H_6 (2 l.) for 5 hr. After filtration, the C_6H_6 solns were combined and evapd under red. pres. and the C_6H_6 extract (7.8 g) was obtained, subjected to chromatography over silica gel (450 Wako gel C200) and eluted as follows: fraction 1, 2% Me_2CO in C_6H_6 0.6 l. and 5% Me_2CO in C_6H_6 1.2 l.; fraction 2, 5% Me_2CO in C_6H_6 1 l.; fraction 3, 5% Me_2CO in C_6H_6 0.7 l. and 10% Me_2CO in C_6H_6 2.1 l.; fraction 4, 10% Me_2CO in C_6H_6 0.1 l. and 15% Me_2CO in C_6H_6 1.3 l.; fraction 5, 15% Me_2CO in C_6H_6 0.5 l. Fractions 2 and 4 contained the mixture of compound **1d**, **2b** (2.4 g) and compound **1b** (1.1 g) respectively.

Isolation and structure elucidation of compound **1d**. Fraction 2 (2.4 g) was rechromatographed on silica gel (300 g) and eluted with the Me_2CO–C_6H_6 solvent system. Compound **1d** (220 mg) showed one spot on TLC but two peaks on HPLC. Separation was achieved by repeated HPLC (see conditions of HPLC). The pure compound **1d** gave colourless needles (120 mg), mp 208–210° (MeCN), $C_{34}H_{50}O_6$ (required 554.3607, $[M]^+$ *m/z* 554.3580), $[\alpha]_D^{28}$ + 117° ($CHCl_3$, *c* 0.56); IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 2920 (CH), 1720, 1710 (COO), 1250, 1025; UV λ_{max}^{EtOH} nm (log ε): 236 (4.31), 243 (4.36), 252 (4.20). ^1H NMR (400 MHz, $CDCl_3$): see Results and Discussion and Table 1. ^{13}C NMR (25.2 MHz, $CDCl_3$): δ12.9 (*q*), 15.9 (*q*), 17.0 (*q*), 18.5 (*q*), 19.1 (*q*), 21.4 (*q*), 21.5 (*q*), 22.9 (*t*, *q*), 24.3 (*t*), 28.2 (*t*, *q*), 35.5 (*t*), 36.7 (*t*), 37.4 (*s*), 37.7 (*s*), 38.2 (*t*), 39.7 (*d*), 44.0 (*s*), 45.1 (*d*), 49.2 (*d*), 51.5 (*s*), 68.2 (*d*), 77.5 (*d*), 81.0 (*d*), 92.8 (*d*), 116.6 (*d*), 121.3 (*d*), 123.9 (*d*), 133.2 (*s*), 140.7 (*s*), 146.0 (*s*), 171.3 (*s*), 171.4 (*s*). In a gated decoupling experiment, the value of the CH coupling constant of C-26 of **1d** was 182 Hz. EIMS (direct inlet) 20 eV, *m/z* (rel. int.): 554 $[M]^+$ (18), 536 $[M - H_2O]^+$ (32), 476 $[M - H_2O - HOAc]^+$ (13), 401 $[M - H_2O - 2HOAc - Me]^+$ (11), 352 $[M - side\ chain - HOAc]^+$ (36), 293 $[M - side\ chain - HOAc - OAc]^+$ (16), 239 $[C_{18}H_{23}]^+$ (16), 95 $[C_6H_7O_1]^+$ (100). The mixture of **1d** (41 mg) and *p*-toluenesulphonic acid (36 mg) in MeOH (20 ml) was left at room temp. for 10 min. After reaction, H_2O (40 ml) was added and the soln evapd to a small vol. (*ca* 10 ml), extracted with $CHCl_3$, dried with Na_2SO_4 and evapd to dryness under red.

Table 2. HPLC conditions for separation of compounds **1a–1d**, **2a** and **2b**

Compound*	Solvent	Flow rate (ml/min)	R_t (min) Diene	Monoene
1a	90% MeOH soln	4	11.0	13.1
1c	95% MeOH soln	4	9.8	11.7
2a	93% MeOH soln	4	12.8	15.2
1b	80% MeCN soln	4	10.0	—
1d	95% MeCN soln	4	5.5	6.5
2b	85% MeCN soln	3.5	11.4	14.0

*Before purification by HPLC, each compound (**1a**, **1c**, **1d**) contained the monoene derivatives, and **2a** and **2b** contained diene-type compounds.

pres. The residue was crystallized from MeOH to give 30.9 mg colourless needles. The methylated **1d**, mp 208–209°, was identical in all respects (mp, mmp, $[\alpha]_D$, IR, UV, ^{1}H NMR and MS) to compound **1c**.

Isolation and structure elucidation of compound **1b**. Impure compound **1b** from fraction 4 was subjected to repeated HPLC (see conditions of HPLC). Pure compound **1b** on HPLC was obtained as an amorphous powder (37 mg), mp 186–187°; $C_{32}H_{48}O_5$ requires 512.3502, $[M]^+$ m/z 512.3503; $[\alpha]_D^{28}$ +100° ($CHCl_3$, c 0.092); UV λ_{max}^{EtOH} nm (log ε): 232 (4.11), 242 (4.17), 251 (4.00); IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 2960 (CH), 1710 (COO), 1260; ^{1}H NMR (400 MHz, $CDCl_3$): see Results and Discussion and Table 1. ^{13}C NMR (25.2 MHz, $CDCl_3$): δ12.8, 15.9, 15.9, 18.7, 19.0, 21.5, 22.9, 23.1, 27.9, 28.2, 28.3, 35.8, 36.7, 37.6, 38.2, 38.8, 39.7, 44.0, 45.1, 49.0, 51.5, 68.2, 77.5, 79.1, 92.8, 116.2, 121.6, 123.9, 133.2, 140.6, 146.3, 171.8. Compound **1b** is very insoluble in $CDCl_3$ and the multiplicities in the single frequency off-resonance decoupling spectrum were not measured. EIMS (direct inlet) 20 eV, m/z (rel. int.): 512 $[M]^+$ (12), 494 $[M-H_2O]^+$ (26), 434 $[M-H_2O-HOAc]^+$ (7), 369 [M – side chain]$^+$ (4), 309 [M – side chain – HOAc]$^+$ (11), 291 [M – side chain – HOAc $-H_2O]^+$ (7), 253 $[C_{19}H_{25}]^+$ (4), 95 $[C_6H_7O_1]^+$ (100). After reaction of **1b** and MeOH using *p*-toluenesulphonic acid in the same way as for compound **1d**, methylated **1b** was identical in all respects to compound **1a**.

Isolation and structure elucidation of compound **2b**. After removing compound **1d** from fraction 2 by CC, the fraction containing compound **2b** was obtained (683 mg). The compound gave one spot on TLC but two peaks on HPLC, and was further purified by HPLC. After purification, compound **2b** was obtained as colourless needles (184 mg), mp 187–188° (MeCN), $C_{30}H_{48}O_3$ (required 456.3603, $[M]^+$ m/z 456.3596); $[\alpha]_D^{22}$ +43° ($CHCl_3$, c 0.076); IR ν_{max}^{KBr} cm^{-1}: 3360 (OH), 2920, 2860, 2820 (CH), 1370, 1075, 1010; UV absorption was not observed. ^{1}H NMR (400 MHz, $CDCl_3$): see Table 1. ^{13}C NMR (25.2 MHz, $CDCl_3$): δ 13.2 (*q*), 15.5 (*q*), 15.7 (*q*), 18.4 (*q*), 19.1 (*q*, *t*), 21.2 (*t*), 24.9 (*q*), 26.6 (*t*), 27.9 (*t*), 28.1 (*q*, *t*), 28.3 (*t*), 31.0 (*t*), 31.2 (*t*), 35.8 (*t*), 37.2 (*s*), 39.0 (*s*), 40.1 (*d*), 44.5 (*s*), 46.8 (*d*), 50.1 (*s*), 50.6 (*d*), 68.4 (*d*), 79.2 (*d*), 92.9 (*d*), 124.1 (*d*), 133.2 (*s*), 134.7 (*s*), 134.9 (*s*). EIMS (direct inlet) 20 eV, m/z (rel. int.): 456 $[M]^+$ (38), 441 $[M-Me]^+$ (29), 438 $[M-H_2O]^+$ (12), 423 $[M-Me-H_2O]^+$ (100), 405 $[M-Me-2H_2O]^+$ (18), 309 (14), 299 (17), 281 (9), 95 $[C_6H_7O_1]^+$ (38). After methylation of **2b** in the same way as described above, methylated **2b** was identical in all respects to compound **2a**.

Conditions for HPLC and data of perenniporiol derivatives. HPLC analysis was run on a Shimadzu liquid chromatograph LC-3A instrument with a Shimadzu absorbance detector model SPD-2A and a differential refractometer. A column (7.6 × 300 mm) packed with Unisil Q C18 was operated under the conditions shown in Table 2.

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