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TWO PERENNIPORIOL DERIVATIVES, LANOSTANE-TYPE TRITERPENOIDS, FROM THE CULTURED MYCELIA OF *PERENNIPORIA OCHROLEUCA**

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Key Word Index—*Perenniporia ochroleuca*; Basidiomycetes; higher fungi; triterpenoids; 3-*O*-acetyl-7,11-dihydroperenniporiol; 12β-acetoxyperenniporiol.

Abstract—Two new perenniporiol derivatives, lanostane-type triterpenoids, were isolated from the benzene extract of cultured mycelia of *Perenniporia ochroleuca*. The structures of the two new compounds were elucidated by spectral data to be 3-*O*-acetyl-7,11-dihydroperenniporiol and 12β-acetoxyperenniporiol.

INTRODUCTION

In a previous paper, we reported the isolation and structure determination of new triterpenoids (**1a**, **1b**, **2a**) from the benzene extract of cultured mycelia of *P. ochroleuca* [1]. These new triterpenoids had novel structures which have not been found in Basidiomycetes, i.e. lanostane derivatives having a six-membered hemiacetal side chain.

The structure of perenniporiol (**1a**) was determined as (22*S*,26*S*)-15α-acetoxy-22,26-epoxy-3β,26-dihydroxy-5α-lanosta-7,9(11),24-triene, the others as 3-*O*-acetylperenniporiol (**1b**) and 15-deacetoxy-7,11-dihydroperenniporiol (**2a**).

In this paper we report the isolation and structure elucidation of additional new triterpenoids, 3-*O*-acetyl-7,11-dihydroperenniporiol (**2b**) and 12β-acetoxyperenniporiol (**1c**), and the assignments of the ^{13}C NMR spectral signals of the perenniporiol derivatives. The ^{13}C NMR assignments were performed in comparison with the published spectral data of lanostenol and γ-lanostadienol [2]. The ^{1}H NMR assignments of the perenniporiol derivatives were also made by reference to the assignments of **1c**, which were determined by selective decoupling experiments.

RESULTS AND DISCUSSION

The benzene extract (7.8 g) of the dried cultured mycelia was subjected to silica gel column chromatography and separated into five fractions as described in the Experimental. Rechromatography on a silica gel column and further purification by reversed-phase HPLC afforded compounds **2b** and **1c** from fractions 2 and 5, respectively. Compound **2b**, 3-*O*-acetyl-7,11-dihydroperenniporiol, colourless needles, mp 197–200°, was deduced to have the molecular formula $C_{34}H_{52}O_6$ from high-resolution mass spectrometry. In the mass spectrum of **2b**, an *m/z* 95 fragment ion peak was observed as the base peak, suggesting the presence of a hemiacetal side chain characteristic of perenniporiol derivatives. In the IR spectrum, **2b** showed absorptions at 3500 (OH) and 1717 (COO) cm^{-1}. In the UV spectrum, **2b** showed no absorption. The ^{1}H NMR and ^{13}C NMR spectral patterns of **2b** were very similar to those of **1b** except for the olefinic region. In the ^{13}C NMR spectrum of **2b**, four olefinic carbon signals were observed at δ 123.9 (*d*, C-24), 133.2 (*s*, C-25), 135.8 (*s*, C-8) and 133.4 (*s*, C-9). On the other hand, six olefinic carbon signals were observed at δ 116.6 (*d*, C-11), 121.3 (*d*, C-7), 123.9 (*d*, C-24), 133.2 (*s*, C-25), 140.7 (*s*, C-8) and 146.0 (*s*, C-9) in the spectrum of **1b**. The loss of two olefinic carbon signals in the spectrum of **2b** relative to that of **1b** indicated that the diene moiety of **1b** had been replaced by a monoene structure like compound **2a** (15-deacetoxy-7,11-dihydroperenniporiol). This suggestion was supported by the ^{1}H NMR spectrum, which showed only one olefinic proton signal at δ 5.65 (24-H). From these spectral investigations, compound **2b** was established as 3-*O*-acetyl-7,11-dihydroperenniporiol.

Compound **1c**, mp 217–218° showed absorptions at 237, 244 and 253 nm (log ε 4.48, 4.55 and 4.37) in the UV spectrum which indicated the presence of a heteroannular diene moiety. The IR spectrum of **1c** showed absorptions at 3410 (OH) and 1728 (COO) cm^{-1}. The molecular formula of **1c** was established as $C_{34}H_{52}O_7$ by high-resolution mass spectrometry. In the mass spectrum of **1c**, the fragment ion peaks were observed at *m/z* 510 $[M - HOAc]^+$, 309 $[M - \text{side chain} - 2 \times HOAc]^+$ and 95 $[C_6H_7O_1]^+$, suggesting **1c** was an acetoxyperenniporiol. This suggestion was consistent with the ^{13}C NMR spectrum of **1c**, which contained one additional acetoxy group (21.7, *q* and 171.1, *s*) relative to that of **1a**.

Furthermore, the position and configuration of the additional acetoxy group of **1c** were elucidated from its ^{1}H NMR spectral data. In the ^{1}H NMR spectrum of **1c** a new signal at δ 5.50, which was absent in **1a**, appeared as a singlet and the H-11 signal, observed at 5.49 in **1a**, was shifted upfield and appeared at 5.0 as a singlet. The assignment of H-11 was confirmed by a selective decoup-

*Part 3 in the series "Studies on the Metabolites of Higher Fungi". For Part 2 see ref. [1].

ling experiment. The upfield shift of the H-11 signal in **1c** was considered to be due to the anisotropic effect of a carbonyl group (12β-acetoxy group).

Also the change of multiplicity of H-11 (from a doublet in **1a** to a singlet in **1c**) indicated that the additional acetoxy group was attached at C-12, and H-11 made a 90° dihedral angle with 12-H. This evidence led to the conclusion that the additional acetoxy group was attached at C-12 with a β-configuration.

Therefore, the structure of **1c** was elucidated as 12β-acetoxyperenniporiol, (22*S*,26*S*)-12β,15α-diacetoxy-22,26-epoxy-3β,26-dihydroxy-5α-lanosta-7,9(11),24-triene.

The assignments of the ^{13}C NMR spectral signals of perenniporiol and its derivatives were made by comparison with the ^{13}C NMR spectral data of lanostenol and γ-lanostadienol [2], on the basis of off-resonance decoupling (SFORD), empirical shift rules such as α-, β-, γ- and δ-substituent effects, and by means of the acetylation shift [3].

The signal assignments of the carbon atoms situated in the A/B/C rings of **1a** were made according to the corresponding values observed for γ-lanostadienol. In the D-ring, the carbon atom signals were assigned after consideration of the substituent effect of an acetoxy group at C-15 and the corresponding data of steroids reported previously [4]. The assignments of the side chain carbon signals were facilitated by the characteristic 22,26-epoxy and 26-hydroxy structures.

The hydroxy group at C-26 gave a downfield shift to this carbon and provided the basis for the assignment of C-26 to the signal at 92.8. Another remaining oxygenated carbon signal (68.2) was due to C-22. The olefinic carbon signals due to C-24 and C-25 were confirmed by SFORD spectral data, which assigned the band at 123.9 (*d*) to C-24 and that at 133.2 (*s*) to C-25; this assignment was consistent with the ^{13}C NMR spectral data of **2b** which has a 7,11-dihydro structure.

The conformation of the C-26 hydroxy group of **1a** was predicted to be axial from the C-22 carbon chemical shift value of 68.2, with reference to the ^{13}C NMR spectral data of withanolides [5]. The conformation of H-22 was revealed to be axial by its coupling constants 11.4 and 2.2 Hz to the axial and equatorial protons of C-23. The chemical shift value of C-22 was considered to be reflected by the 1,3-diaxial relationship between H-22 and 26-OH (γ-effect).

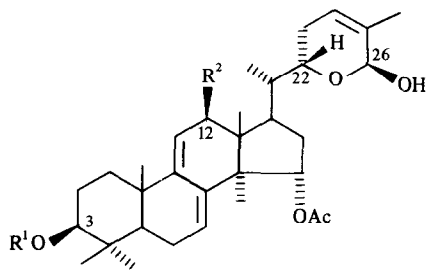
In perenniporiol derivatives the C-22 carbon bands were in the region of 67.9–68.4, suggesting that the hemiacetal hydroxy group preferred an axial orientation. This suggestion was supported by the anomeric effect [6]. The ^1H NMR signal assignments of **1c** were made by selective decoupling experiments and is shown in Table 1. With regard to other perenniporiol derivatives, the proton signal assignments were based upon those of **1c** and they are also summarized in Table 1.

Five new novel triterpenoids were isolated from cul-

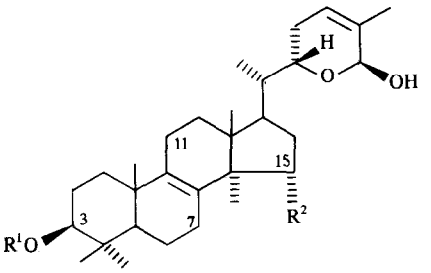
Table 1. ^1H NMR spectral data of **1a**, **1b**, **1c**, **2a** and **2b** (400 MHz, $CDCl_3$)

Proton	1b	1a	1c	2b	2a
18-H_3	0.66 *s*	0.66 *s*	0.75 *s**	0.78 *s*	0.71 *s*
19-H_3	0.99 *s*	1.01 *s*	1.01 *s*	1.00 *s*	0.99 *s*
21-H_3	0.91 *d*	0.91 *d*	0.91 *d**	0.91 *d*	0.96 *d*
	(6.8)	(6.8)	(6.8)	(6.8)	(6.8)
27-H_3	1.68 *s*	1.70 *s*	1.67 *s**	1.68 *s*	1.67 *s*
28-H_3	1.05 *s*	1.05 *s*	1.18 *s**	1.04 *s*	0.91 *s*
29-H_3	0.89 *s*	0.88 *s*	0.87 *s*	0.87 *s*	1.00 *s*
30-H_3	0.95 *s*	0.97 *s*	1.00 *s*	0.87 *s*	0.81 *s*
3-MeCOO	2.06 *s*	—	—	2.05 *s*	—
12-MeCOO	—	—	2.06 *s**	—	—
15-MeCOO	2.07 *s*	2.08 *s*	2.08 *s**	2.03 *s*	—
3-H	4.52 *dd*	3.24 *dd*	3.30 *dd**	4.50 *dd*	3.25 *dd*
	(11.4, 4.5)	(11.1, 4.5)	(11.2, 4.4)	(11.7, 4.5)	(11.3, 4.4)
7-H	5.33 *d*	5.32 *d*	5.60 *d**	—	—
	(6.3)	(6.3)	(6.3)		
11-H	5.48 *d*	5.49 *d*	5.0 *s**	—	—
	(5.9)	(6.1)			
12-H	—	—	5.50 *s**	—	—
15-H	5.10 *dd*	5.08 *dd*	5.13*	5.05 *dd*	—
	(9.3, 5.6)	(9.3, 5.6)		(9.3, 5.6)	
22-H	3.92 *dd*	3.91 *dd*	4.03 *dd**	3.91 *dd*	4.03 *dd*
	(9.8, 2.2)	(11.4, 2.2)	(11.3, 2.4)	(11.7, 2.4)	(11.5, 2.0)
24-H	5.66 *d*	5.66 *d*	5.64 *d**	5.65 *d*	5.68 *d*
	(5.6)	(5.4)	(5.3)	(5.6)	(5.6)
26-OH	2.61 *d*	2.52 *d*		2.53 *d*	2.55 *d*
	(3.1)	(5.4)		(5.6)	(4.9)
26-H	5.14 *d*	5.14 *d*	5.13*	5.14 *d*	5.16 *d*
	(3.1)	(5.4)		(5.6)	(4.9)

Figures in parentheses are coupling constants in Hz.
*Assignments were confirmed by selective spin-decoupling.



1a $R^1 = R^2 = H$
1b $R^1 = Ac$, $R^2 = H$
1c $R^1 = H$, $R^2 = OAc$



2a $R^1 = R^2 = H$
2b $R^1 = Ac$, $R^2 = OAc$

Table 2. ^{13}C NMR spectral data of compounds **1a**, **1b**, **1c**, **2a** and **2b** (25.2 MHz, $CDCl_3$)

Carbon	1c	1b	1a	I*	II*	2a	2b
1	36.0 (*t*)†	36.7 (*t*)†	36.7 (*t*)†	35.8 (*t*)	35.8 (*t*)	35.8 (*t*)	36.3 (*t*)†
2	27.7 (*t*)	24.3 (*t*)	27.8 (*t*)	28.2 (*t*)	27.9 (*t*)	27.9 (*t*)	24.2 (*t*)
3	78.8 (*d*)	81.0 (*d*)	79.1 (*d*)	79.0 (*d*)	79.0 (*d*)	79.2 (*d*)	81.0 (*d*)
4	38.8 (*s*)	37.7 (*s*)	38.2 (*s*)	38.5 (*s*)	39.0 (*s*)	39.0 (*s*)	37.9 (*s*)
5	48.8 (*d*)	49.2 (*d*)	49.0 (*d*)	49.2 (*d*)	50.5 (*d*)	50.6 (*d*)	50.5 (*d*)
6	23.2 (*t*)	22.9 (*t*)	23.1 (*t*)	23.0 (*t*)	19.2 (*t*)	19.1 (*t*)	19.0 (*t*)
7	123.7 (*d*)	121.3 (*d*)	121.6 (*d*)	120.1 (*d*)	28.3 (*t*)	28.1 (*t*)	31.2 (*t*)
8	148.3 (*s*)	140.7 (*s*)	140.6 (*s*)	142.7 (*s*)	134.4 (*s*)	134.7 (*s*)	135.8 (*s*)
9	139.1 (*s*)	146.0 (*s*)	146.2 (*s*)	145.9 (*s*)	134.4 (*s*)	134.9 (*s*)	133.4 (*s*)
10	37.5 (*s*)	37.4 (*s*)	37.6 (*s*)	37.4 (*s*)	37.2 (*s*)	37.2 (*s*)	37.1 (*s*)
11	117.7 (*d*)	116.6 (*d*)	116.2 (*d*)	116.3 (*d*)	21.1 (*t*)	21.7 (*t*)	20.9 (*t*)
12	76.5 (*d*)	38.2 (*t*)	38.9 (*t*)	37.9 (*t*)	26.7 (*t*)	26.6 (*t*)	26.4 (*t*)
13	47.8 (*s*)	44.0 (*s*)	44.0 (*s*)	43.8 (*s*)	44.6 (*s*)	44.5 (*s*)	44.7 (*s*)
14	52.7 (*s*)	51.5 (*s*)	51.5 (*s*)	50.4 (*s*)	49.9 (*s*)	50.1 (*s*)	51.3 (*s*)
15	77.3 (*d*)	77.5 (*d*)	77.5 (*d*)	28.1 (*t*)	31.2 (*t*)	31.1 (*t*)	76.2 (*d*)
16	35.6 (*t*)†	35.5 (*t*)†	35.8 (*t*)†	31.6 (*t*)	31.0 (*t*)	31.0 (*t*)	35.3 (*t*)†
17	46.4 (*d*)	45.1 (*d*)	45.1 (*d*)	51.1 (*d*)	50.7 (*d*)	46.8 (*d*)	45.4 (*d*)
18	11.2 (*q*)	15.9 (*q*)	15.9 (*q*)	15.7 (*q*)	15.9 (*q*)	15.7 (*q*)	16.0 (*q*)
19	22.6 (*q*)	22.9 (*q*)	22.9 (*q*)	22.8 (*q*)	18.3 (*q*)	18.4 (*q*)	18.2 (*q*)
20	38.4 (*d*)	39.7 (*d*)	39.7 (*d*)	36.5 (*d*)	36.5 (*d*)	40.1 (*d*)	40.0 (*d*)
21	13.7 (*q*)	12.9 (*q*)	12.8 (*q*)	18.6 (*q*)	18.8 (*q*)	13.2 (*q*)	12.9 (*q*)
22	67.9 (*d*)	68.2 (*d*)	68.2 (*d*)	36.3 (*t*)	36.5 (*t*)	68.4 (*d*)	68.2 (*d*)
23	28.6 (*t*)	28.2 (*t*)	28.3 (*t*)	24.1 (*t*)	24.2 (*t*)	28.3 (*t*)	28.2 (*t*)
24	123.7 (*d*)	123.9 (*d*)	123.9 (*d*)	39.5 (*t*)	39.6 (*t*)	124.1 (*d*)	123.9 (*d*)
25	133.3 (*s*)	133.2 (*s*)	133.2 (*s*)	28.1 (*d*)	28.1 (*d*)	133.2 (*s*)	133.2 (*s*)
26	92.8 (*d*)	92.8 (*d*)	92.8 (*d*)	22.8 (*q*)	22.6 (*q*)	92.9 (*d*)	92.8 (*d*)
27	19.1 (*q*)‡	19.1 (*q*)‡	19.0 (*q*)‡	22.6 (*q*)	22.8 (*q*)	19.1 (*q*)	19.0 (*q*)‡
28	18.3 (*q*)‡	18.5 (*q*)‡	18.7 (*q*)‡	25.6 (*q*)	24.3 (*q*)	24.7 (*q*)	19.2 (*q*)‡
29	28.2 (*q*)	28.2 (*q*)	28.2 (*q*)	27.9 (*q*)	28.1 (*q*)	28.1 (*q*)	28.0 (*q*)
30	15.9 (*q*)	17.0 (*q*)	15.9 (*q*)	15.8 (*q*)	15.4 (*q*)	15.5 (*q*)	16.6 (*q*)
3 OCOMe		171.3 (*s*)					171.3 (*s*)
12 OCOMe	171.1 (*s*)						
15 OCOMe	171.4 (*s*)	171.4 (*s*)	171.8 (*s*)				172.8 (*s*)
3 $OCOCH_3$		21.4 (*q*)					21.3 (*q*)
12 $OCOCH_3$	21.7 (*q*)						
15 $OCOCH_3$	21.5 (*q*)	21.5 (*q*)	21.5 (*q*)				21.4 (*q*)

*The data of compounds I and II (γ-lanostadienol and lanostenol) are cited from ref. [2].
†‡Assignments in the same vertical column may be interchanged.

tured mycelia of *P. ochroleuca* and further investigation should indicate the relationship of these perenniporiol derivatives to the biosynthetic pathway.

More recently, De Bernardi *et al.* [7] reported the structure elucidation of 3β-acetoxyl-2α-(3′-hydroxy-3′-methyl)glutarylcrustulinol, which has a six-membered hemiacetal structure in the side chain like the perenniporiol derivatives. This compound was isolated from the fruit

body of two *Hebeloma* species. This new triterpenoid was proved to have high activity in the H-60 and P-388 leukaemia tests. The test of biological activity of the perenniporiol derivatives is in progress.

EXPERIMENTAL

Mps were determined in a Büchi apparatus and Kofler hot plate, and are uncorr. UV spectra were recorded in EtOH and IR spectra in KBr discs. 400 MHz ^{1}H NMR and 25.2 MHz ^{13}C NMR spectra were taken in $CDCl_3$ using TMS as internal standard. Selective decoupling experiments were performed with careful irradiation at proton signals from 0.6 to 6.0 ppm. HPLC of compounds **2b** and **1c** were performed using a Unisil Q C-18 column (7.6 × 300), coupled to a UV detector and a differential refractometer.

The isolation and culture methods of *P. ochroleuca* have been previously reported [1]. The cultured mycelia (fr. wt. 2.04 kg) were lyophilized and extracted with C_6H_6 at room temp. for 3 days, × 2 (total 5.2 l.) and filtered; the residue was refluxed with C_6H_6 (2.1 l.) for 5 hr, C_6H_6 solns were evapd under red. pres. and the C_6H_6 extract (7.8 g) was obtained. The C_6H_6 extract was subjected to CC over silica gel eluted with C_6H_6–Me_2CO, and was separated 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.

Fraction 2 (2.4 g) contained compounds **1b**, **2a** and **2b**; fraction 5 (0.5 g) contained compound **1c**. The isolation and structure elucidation of compounds **1b** and **2a** have been reported in a previous paper [1].

Compound **2b**. Fraction 2 was rechromatographed on CC over silica gel (300 g) eluted with C_6H_6–Me_2CO and gave fractions which contained compound **2b**. Further purification by HPLC (mobile phase MeCN H_2O (9:1), flow rate 4 ml/min, R_t 11.2 min) afforded compound **2b** as colourless needles (112 mg). Compound **2b**, mp 197–200°, $[\alpha]_D^{18}$ +102° ($CHCl_3$; *c* 0.47) had molecular formula $C_{34}H_{52}O_6$ (required 556.3748, $[M]^+$ *m/z* 556.3756); EIMS *m/z* (rel. int.): 556 $[M]^+$ (12), 538 $[M-H_2O]^+$ (40), 496 $[M-HOAc]^+$ (8), 478 $[M-HOAc-H_2O]^+$ (28), 422 (13), 204 (13), 258 (32), 95 $[C_6H_7O_1]^+$ (100), IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 1717 (COO), 1250; ^{1}H NMR (400 MHz) and ^{13}C NMR (25.2 MHz) spectra of **2b**, see Tables 1 and 2.

Compound **1c**. Fraction 5 (0.5 g) was rechromatographed on CC over silica gel (200 g) eluted with C_6H_6–Me_2CO and gave the fraction which contained compound **1c**, which was purified by HPLC (mobile phase MeCN–H_2O (3:2), flow rate 4 ml/min, R_t 15.5 min). Compound **1c** was obtained as colourless prisms (78 mg), mp 217–218° (Et_2O). High-resolution MS $C_{34}H_{52}O_7$ (required 570.3507, $[M]^+$ *m/z* 570.3531); UV λ_{max}^{EtOH} nm (log ε): 237 (4.48), 244 (4.55), 253 (4.37), $[\alpha]_D^{22}$ −2.6° ($CHCl_3$; *c* 0.15); IR ν_{max}^{KBr} cm^{-1}: 3410 (OH), 2930 (CH), 1728 (COO); EIMS *m/z* (rel. int.): 570 $[M]^+$ (18), 552 $[M-H_2O]^+$ (13), 510 $[M-HOAc]^+$ (73), 443 [M – side chain]$^+$ (46), 309 [M – side chain $-2\times$ HOAc]$^+$ (13), 271 $[C_{19}H_{27}O_1]^+$ (14), 95 $[C_6H_7O_1]^+$ (100); ^{1}H NMR and ^{13}C NMR spectra of **1c**, see Tables 1 and 2.

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