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ISOLATION OF LANOSTANE-TYPE TRITERPENE ACIDS HAVING AN ACETOXYL GROUP FROM SCLEROTIA OF *PORIA COCOS**

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Key Word Index—*Poria cocos*; Polyporaceae; 3-*o*-acetyl-16α-hydroxytrametenolic acid; 3-*o*-acetyl-16α-hydroxydehydrotrametenolic acid; 3-epidehydropachymic acid; dehydropachymic acid; pachymic acid; N-chloromethylphthalimide; HPLC

Abstract—The separation of lanostane-type triterpene acids carrying an acetoxyl group from sclerotia of *Poria cocos* (hoelen) was effectively achieved by means of preparative HPLC of the phthalimidomethyl esters followed by selective hydrolysis of the phthalimidomethyl ester groupings. By this method, three new triterpene acids were isolated together with pachymic and dehydropachymic acids, which are characteristic compounds of hoelen. The structures of three new compounds were determined to be 3-*o*-acetyl-16α-hydroxytrametenolic acid, 3-*o*-acetyl-16α-hydroxydehydrotrametenolic acid, and 3-epidehydropachymic acid, respectively, based on spectroscopic evidence.

INTRODUCTION

Dried sclerotia of *Poria cocos* Wolf are used as the Chinese crude drug 'hoelen' and are combined in many traditional Chinese prescriptions for use as diuretics, sedatives, and tonics [1]. The medicinal evaluation of hoelen in Chinese prescriptions requires the qualitative and quantitative analyses of diagnostic compounds. In a previous paper [1], we reported the first isolation and characterization of dehydropachymic acid (**4a**) and pachymic acid (**5a**) by prep. HPLC. These characteristic compounds of Hoelen could be useful as diagnostic standards. Due to their poor solubilities in the usual HPLC solvents, however, the previous separation procedure is very inefficient and thus a more effective alternative is needed. Any derivatization procedure must afford derivatives which have suitable solubilities and from which it is possible to regenerate the starting material under mild reaction conditions; in particular any acetoxyl group must be left untouched. Recently, we used HPLC of phthalimidomethyl esters for the separation of four compounds [3β-hydroxylanosta-7,9(11),24-trien-21-oic acid; trametenolic acid; dehydroeburicoic acid; eburicoic acid] from the surface layer of *P. cocos* [2]. Usually N-chloromethylphthalimide (NCMP) is used as a labelling reagent for HPLC analysis. In this paper, we wish to report the isolation of three new triterpenes having an acetoxyl group along with dehydropachymic and pachymic acids from sclerotia of *P. cocos* by the phthalimidomethyl ester method.

RESULTS AND DISCUSSION

Silica gel column chromatography of the MeOH extract of sclerotia of *P. cocos* (hoelen) gave a mixture of monoacetyl triterpene acids. This was esterified with NCMP, and the derivatized mixture separated by means of reversed-phase prep. HPLC to give six compounds, **1b**–**6b**. Among these, **6b** was identified as polyporenic acid C phthalimidomethyl ester based on the fact that alkaline hydrolysis yielded polyporenic acid C (**6a**), mp 273–275° [3].

Compounds **4b** and **5b** gave EIMS molecular ion peaks at *m/z* 685 and 687, respectively. Their molecular formulae were determined as $C_{42}H_{55}O_7N$ and $C_{42}H_{57}O_7N$, respectively, by HR-mass spectrometry measurements. The ^1H and ^{13}C NMR spectra of both compounds showed signals due to an acetoxyl group and a phthalimidomethyl residue (Table 1) and detailed analysis of the NMR spectra with the aid of ^1H–^1H, ^1H–^{13}C, and long-range ^1H–^{13}C COSY's suggested that they were phthalimidomethyl esters of dehydropachymic acid and pachymic acid, respectively.

Selective hydrolysis of the phthalimidomethyl ester grouping in **4b** and **5b** was effectively performed by reaction with 0.1 M NaOH-EtOH under controlled conditions to afford dehydropachymic acid (**4a**) and pachymic acid (**5a**), respectively. Thus the improvement in the

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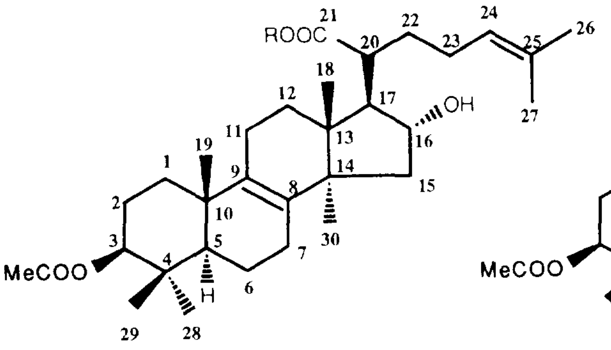
*Part 5 in the series studies on the constituents of *Poria cocos*. For Part 4 see ref. 5.



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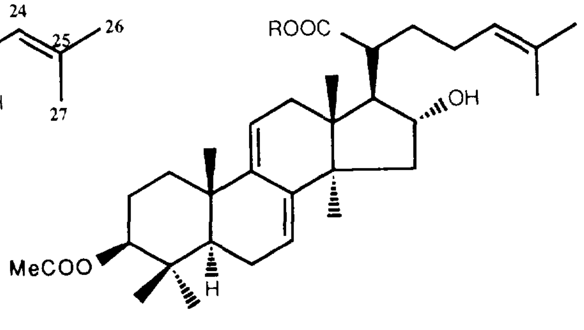

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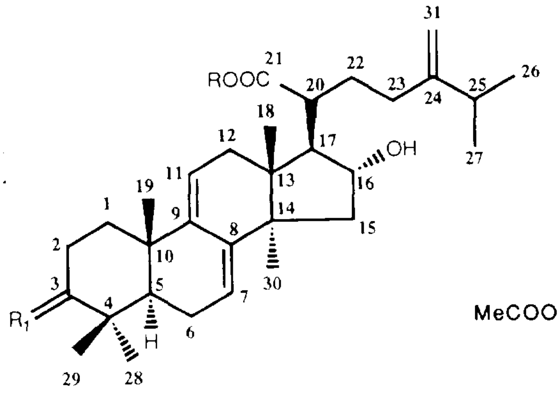

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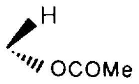

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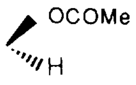

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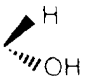
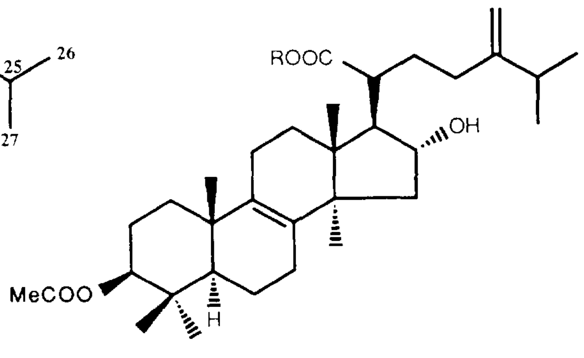
7 R_1 =





5

a : R = H

b : R = phthalimidomethyl

method of isolation of **4a** and **5a** from hoelen was successfully achieved.

Compound **1b**, $[\alpha]_D^{26}$ + 8 ($CHCl_3$), **2b**, $[\alpha]_D^{26}$ + 30 ($CHCl_3$) showed EIMS molecular ion peaks at *m/z* 673 and 671, respectively, and the molecular formulae were determined as $C_{41}H_{55}O_7N$ and $C_{41}H_{53}O_7N$, respectively, by HRMS measurements. The ^1H and ^{13}C NMR spectra of both compounds showed signals due to seven *tert*-methyls, an acetoxyl group, and two oxygen-bearing methines. On partial hydrolysis under controlled conditions, they gave the corresponding acids **1a** and **2a**. In the UV spectra, **2a** revealed an absorption at 242 nm (log ε 4.1), while **1a** showed no absorption.

Eventually, the structures of **1a** and **2a** were determined as 3-*o*-acetyl-16α-hydroxytrametenolic acid and 3-*o*-acetyl-16α-hydroxydehydrotrametenolic acid, respectively, based on detailed analyses of the 2D NMR spectra.

Compound **3b** was assigned the molecular formula $C_{42}H_{55}O_7N$ by HRMS measurement. Furthermore, the MS, UV and IR spectra were almost superimposable to those of **4b**. In the ^1H NMR spectrum of **3b**, the signal assignable to H-3 appeared as a broad singlet (W/2 = 8 Hz) at δ4.68, suggesting a β-equatorial orientation. Moreover, in the ^{13}C NMR spectrum, the chemical shifts of the A-ring carbons showed upfield shifts of C-29 compared with those of **4b** [4]. Thus, **3b** was adjudged to be a C-3 epimer of **4b**. This was proved by alkaline hydrolysis of **3b**, which gave 3-epidehydrotumulosic acid [5].

Table 1. ^{1}H NMR spectral data of compounds **1b**–**5b** in $CDCl_3$ and **1a**–**5a** in pyridine-d_5

H	**1b**	**1a**	**2b**	**2a**	**3b**
1α	1.25 *td* (13, 4)	1.15 *td* (13, 4)	1.47 *td* (13, 4)	1.42 *td* (13, 4)	1.60 *td* (14, 4)
1β	1.70 *dt* (13, 3)	1.53 *dt* (13, 3)	1.92 *dt* (13, 3)	1.87 *dt* (13, 3)	1.67 *dt* (13, 3)
2α	*c*. 1.7	1.63 *m*	1.75 *ddd* (14, 8, 4)	1.77 *ddd* (13, 8, 4)	1.74 *ddd* (15, 6, 3)
2β	1.59 *ddd* (15, 13, 3)	1.71 *ddd* (14, 12, 3)	1.68 *ddd* (14, 12, 3)	1.69 *ddd* (14, 12, 3)	1.91 *ddd* (15, 4, 2)
3	4.49 *dd* (12, 4)	4.68 *dd* (12, 5)	4.50 *dd* (12, 4)	4.70 *dd* (12, 4)	4.68 *brs* (W/2 = 8)
5	1.16 *dd* (13, 2)	1.15 *dd* (10, 2)	1.17 *dd* (11, 4)	1.26 *dd* (10, 6)	1.49 *dd* (10, 6)
6α	*c*. 1.6	1.65 *m*	*c*. 2.0	*c*. 2.1	*c*. 2.0
6β	1.49 *tdd* (13, 7, 2)	1.50 *m*	*c*. 2.0	*c*. 2.1	*c*. 2.0
7	*c*. 1.9	*c*. 2.1	5.41 *d* (6)	5.57 *br s*	5.43 *brs* (W/2 = 6)
	c. 1.9	*c*. 2.1		–	
11	*c*. 1.8	*c*. 1.9	5.16 *d* (6)	5.31 *d* (6)	5.21 *d* (6)
	c. 1.8	*c*. 1.9			—
12α	*c*. 1.7	2.20 *dt* (13, 9)	2.17 *d* (17)	2.69 *d* (18)	2.23 *d* (17)
12β	1.20 *dd* (13, 8)	*c*. 2.0	1.64 *dd* (17, 6)	2.41 *dd* (18, 6)	1.67 *dd* (17, 7)
15α	1.45 *d* (14)	1.70 *d* (13)	1.45 *d* (13)	1.93 *d* (13)	1.47 *d* (13)
15β	2.15 *dd* (14, 6)	2.38 *dd* (13, 8)	2.17 *dd* (13, 8)	2.44 *dd* (13, 8)	2.18 *dd* (13, 8)
16	4.06 *dd* (8, 6)	4.52 *dd* (8, 6)	4.05 *dd* (8, 6)	4.52 *dd* (8, 6)	4.08 *dd* (8, 6)
17	2.07 *dd* (11, 6)	2.79 *dd* (11, 6)	2.14 *dd* (11, 6)	2.86 *dd* (11, 6)	2.18 *dd* (11, 6)
18	0.67 *s*	1.12 *s*	0.54 *s*	1.03 *s*	0.56 *s*
19	0.95 *s*	0.96 *s*	0.95 *s*	1.00 *s*	0.95 *s*
20	2.45 *td* (11, 4)	2.94 *td* (11, 3)	2.46 *td* (11, 4)	2.95 *td* (11, 3)	2.49 *td* (11, 4)
22	1.78 *ddd* (13, 6, 4)	*c*. 2.5	1.81 *ddd* (13, 6, 4)	*c*. 2.5	1.81 *ddd* (13, 6, 4)
	c. 1.7	*c*. 2.4	*c*. 1.7	*c*. 2.4	*c*. 1.9
23	*c*. 1.9	*c*. 2.5	*c*. 1.9	*c*. 2.4	*c*. 2.0
	c. 1.9	*c*. 2.4	*c*. 1.9	*c*. 2.4	*c*. 2.0
24	5.07 *tt* (7, 1)	5.34 *tt* (7, 1)	5.07 *tt* (7, 1)	5.34 *tt* (7, 1)	—
25	—	—	—	—	2.18 *m*
26	1.65 *s*	1.62 *s*	1.65 *s*	1.62 *s*	*0.97 *d* (7)
27	1.54 *s*	1.60 *s*	1.54 *s*	1.60 *s*	*0.98 *d* (7)
28	0.87 *s*	0.92 *s*	0.88 *s*	0.90 *s*	0.88 *s*
29	0.87 *s*	0.93 *s*	0.94 *s*	0.99 *s*	0.98 *s*
30	1.07 *s*	1.47 *s*	1.05 *s*	1.48 *s*	1.11 *s*
31	—	—	—	—	4.67 *d* (1)
	—	—	—	—	4.73 *s*
Ac	2.06 *s*	2.06 *s*	2.06 *s*	2.05 *s*	2.06 *s*

Table 1 (Continued)

H	**3a**	**4b**	**4a**	**5b**	**5a**
1α	1.77 *td* (13, 4)	1.47 *td* (13, 4)	1.42 *td* (13, 4)	1.25 *td* (13, 4)	1.12 *td* (13, 4)
1β	*c.* 1.7	*c.* 1.9	1.88 *dt* (13, 3)	1.70 *dt* (13, 3)	1.57 *dt* (13, 3)
2α	1.69 *ddd* (15, 6, 3)	1.75 *ddd* (14, 8, 4)	1.78 *ddd* (13, 8, 4)	*c.* 1.7	*c.* 1.60 *m*
2β	1.88 *ddd* (15, 4, 2)	1.68 *ddd* (14, 12, 3)	1.69 *ddd* (14, 12, 3)	1.59 *ddd* (15, 13, 3)	1.71 *ddd* (14, 12, 3)
3	†4.85 *br s*	4.50 *dd* (12, 4)	4.70 *dd* (12, 4)	4.49 *dd* (12, 4)	4.67 *dd* (12, 4)
5	1.73 *dd* (10, 6)	1.17 *dd* (11, 4)	1.27 *dd* (9, 7)	1.16 *dd* (13, 2)	1.16 *dd* (10, 2)
6α	*c.* 2.0	*c.* 2.0	*c.* 2.1	*c.* 1.6	1.65 *m*
6β	*c.* 2.0	*c.* 2.0	*c.* 2.1	1.49 *tdd* (13, 7, 2)	1.50 *m*
7	5.61 *brs* (W/2 = 6)	5.41 *d* (6)	5.98 *br s*	*c.* 1.9	*c.* 2.1
	—	—	—	*c.* 1.9	*c.* 2.1
11	5.43 *d* (6)	5.16 *d* (6)	5.32 *d* (6)	*c.* 1.8	*c.* 1.9
	—	—	—	*c.* 1.8	*c.* 1.9
12α	2.69 *d* (17)	2.17 *d* (17)	2.70 *d* (18)	*c.* 1.7	2.20 *dt* (13, 9)
12β	2.45 *dd* (17, 7)	1.64 *dd* (17, 6)	2.42 *dd* (18, 6)	1.20 *dd* (13, 8)	*c.* 2.0
115α	1.92 *d* (13)	1.45 *d* (13)	1.94 *d* (13)	1.45 *d* (14)	1.70 *d* (13)
15β	2.44 *dd* (13, 8)	2.17 *dd* (13, 8)	*c.* 2.4	2.15 *dd* (14, 6)	2.38 *dd* (13, 8)
16	4.52 *dd* (8, 6)	4.06 *dd* (8, 6)	4.55 *dd* (8, 6)	4.06 *dd* (8, 6)	4.52 *dd* (8, 6)
17	2.86 *dd* (11, 6)	2.14 *dd* (11, 6)	2.88 *dd* (11, 6)	2.07 *dd* (11, 6)	2.80 *dd* (11, 6)
18	1.07 *s*	0.54 *s*	1.04 *s*	0.67 *s*	1.12 *s*
19	1.02 *s*	0.95 *s*	1.01 *s*	0.95 *s*	0.97 *s*
20	2.95 *td* (11, 3)	2.48 *td* (11, 4)	2.95 *td* (11, 3)	2.45 *td* (11, 4)	2.93 *td* (11, 3)
22	2.66 *m*	1.80 *ddd* (13, 6, 4)	2.66 *m*	1.80 *ddd* (13, 6, 4)	2.66 *m*
	c. 2.4	*c.* 1.9	*c.* 2.5	*c.* 1.9	*c.* 2.5
23	*c.* 2.4	*c.* 2.0	*c.* 2.4	*c.* 2.0	*c.* 2.4
	2.54 *br t* (12)	*c.* 2.0	2.54 *br t* (12)	*c.* 2.0	2.54 *brt* (12)
24	—	—	—	—	—
25	2.29 *m*	2.18 *m*	2.29 *m*	2.18 *m*	2.29 *m*
26	*0.99 *d* (7)	*0.96 *d* (7)	*0.99 *d* (7)	*0.96 *d* (7)	*0.99 *d* (7)
27	*1.00 *d* (7)	*0.97 *d* (7)	*1.00 *d* (7)	*0.97 *d* (7)	*1.00 *d* (7)
28	0.89 *s*	0.88 *s*	0.90 *s*	0.87 *s*	0.92 *s*
29	0.91 *s*	0.94 *s*	0.99 *s*	0.87 *s*	0.93 *s*
30	1.43 *s*	1.05 *s*	1.50 *s*	1.07 *s*	1.48 *s*
31	4.98 *d* (1)	4.67 *d* (1)	4.99 *d* (1)	4.67 *d* (1)	4.98 *d* (1)
	†4.85 *br s*	4.72 *s*	4.85 *s*	4.73 *s*	4.85 *s*
Ac	1.93 *s*	2.06 *s*	2.05 *s*	2.06 *s*	2.05 *s*

*Assignments in each column may be interchanged.
†Overlapped.

Table 2. ^{13}C NMR spectral data of compounds **1b**–**5b** in $CDCl_3$ and **1a**–**5a** in pyridine-d_5

C	**1b**	**1a**	**2b**	**2a**	**3b**	**3a**	**4b**	**4a**	**5b**	**5a**
1	35.1 *t*	35.4 *t*	35.3 *t*	35.6 *t*	30.5 *t*	31.0 *t*	35.5 *t*	35.6 *t*	35.1 *t*	35.4 *t*
2	24.1 *t*	24.5 *t*	24.2 *t*	24.6 *t*	23.1 *t*	23.4 *t*	24.2 *t*	24.5 *t*	24.1 *t*	24.5 *t*
3	80.8 *d*	80.6 *d*	80.7 *d*	80.6 *d*	78.1 *d*	77.9 *d*	80.8 *d*	80.6 *d*	80.8 *d*	80.6 *d*
4	37.8 *s*	38.0 *s*	37.6 *s*	37.8 *s*	36.5 *s*	36.8 *s*	37.6 *s*	37.8 *s*	37.8 *s*	38.0 *s*
5	50.4 *d*	50.7 *d*	49.2 *d*	49.6 *d*	44.1 *d*	44.8 *d*	49.2 *d*	49.6 *d*	50.4 *d*	50.7 *d*
6	18.0 *t*	18.4 *t*	22.8 *t*	23.1 *t*	22.8 *t*	23.2 *t*	22.8 *t*	23.1 *t*	18.0 *t*	18.4 *t*
7	26.4 *t*	26.7 *t*	120.5 *d*	120.7 *d*	120.6 *d*	120.8 *d*	120.5 *d*	120.7 *d*	26.3 *t*	26.8 *t*
8	134.0 *s*	134.4 *s*	141.7 *s*	142.8 *s*	141.7 *s*	142.8 *s*	141.7 *s*	142.7 *s*	134.4 *s*	134.4 *s*
9	134.3 *s*	135.0 *s*	145.1 *s*	145.8 *s*	145.4 *s*	146.0 *s*	145.2 *s*	145.7 *s*	134.6 *s*	135.0 *s*
10	36.9 *s*	37.1 *s*	37.3 *s*	37.6 *s*	37.3 *s*	37.7 *s*	37.3 *s*	37.6 *s*	36.9 *s*	37.2 *s*
11	20.3 *t*	20.9 *t*	116.2 *d*	117.0 *d*	115.7 *d*	116.6 *d*	116.2 *d*	117.0 *d*	20.3 *t*	20.9 *t*
12	29.0 *t*	29.7 *t*	35.5 *t*	36.3 *t*	35.4 *t*	36.2 *t*	35.5 *t*	36.2 *t*	29.0 *t*	29.7 *t*
13	45.9 *s*	46.3 *s*	44.7 *s*	45.1 *s*	44.7 *s*	45.1 *s*	44.7 *s*	45.0 *s*	45.9 *s*	45.3 *s*
14	48.1 *s*	48.8 *s*	48.6 *s*	49.4 *s*	48.7 *s*	49.5 *s*	48.6 *s*	49.4 *s*	48.1 *s*	48.8 *s*
15	42.7 *t*	43.6 *t*	43.5 *t*	44.3 *t*	43.6 *t*	44.4 *t*	43.6 *t*	44.4 *t*	42.7 *t*	43.6 *t*
16	77.1 *d*	76.6 *d*	77.0 *d*	76.4 *d*	77.0 *d*	76.4 *d*	77.0 *d*	76.4 *d*	77.1 *d*	76.6 *d*
17	57.0 *d*	57.4 *d*	57.2 *d*	57.7 *d*	57.0 *d*	57.6 *d*	57.1 *d*	57.6 *d*	56.9 *d*	57.3 *d*
18	17.4 *q*	17.8 *q*	17.2 *q*	17.6 *q*	17.3 *q*	17.6 *q*	16.9 *q*	17.6 *q*	16.5 *q*	17.8 *q*
19	19.1 *q*	19.2 *q*	22.7 *q*	22.8 *q*	22.4 *q*	22.7 *q*	22.7 *q*	22.8 *q*	19.1 *q*	19.2 *q*
20	46.7 *d*	48.6 *d*	46.6 *d*	48.4 *d*	46.7 *d*	48.5 *d*	46.7 *d*	48.5 *d*	46.8 *d*	48.6 *d*
21	174.8 *s*	178.8 *s*	174.5 *s*	178.6 *s*	174.5 *s*	178.6 *s*	174.4 *s*	178.5 *s*	174.7 *s*	178.6 *s*
22	32.1 *t*	33.2 *t*	32.0 *t*	33.1 *t*	30.6 *t*	31.5 *t*	30.6 *t*	31.4 *t*	30.6 *t*	31.6 *t*
23	26.1 *t*	27.1 *t*	26.0 *t*	27.1 *t*	32.1 *t*	33.2 *t*	32.1 *t*	33.2 *t*	32.1 *t*	33.2 *t*
24	123.5 *d*	125.2 *d*	123.4 *d*	125.2 *d*	155.0 *s*	156.1 *s*	155.0 *s*	156.0 *s*	154.9 *s*	156.1 *s*
25	132.3 *s*	131.5 *s*	132.3 *s*	131.5 *s*	33.7 *d*	34.1 *d*	33.8 *d*	34.1 *d*	33.6 *d*	34.1 *d*
26	25.7 *q*	25.8 *q*	25.7 *q*	25.8 *q*	*21.8 *q*	*22.0 *q*	*21.9 *q*	*22.0 *q*	*21.8 *q*	*22.0 *q*
27	17.6 *q*	17.7 *q*	17.6 *q*	17.7 *q*	*21.7 *q*	*21.9 *q*	*21.7 *q*	*21.9 *q*	*21.7 *q*	*21.9 *q*
28	27.9 *q*	28.0 *q*	28.1 *q*	28.2 *q*	27.8 *q*	27.9 *q*	28.1 *q*	28.2 *q*	27.9 *q*	28.0 *q*
29	16.5 *q*	16.8 *q*	16.9 *q*	17.1 *q*	22.4 *q*	22.5 *q*	17.3 *q*	17.1 *q*	17.4 *q*	16.8 *q*
30	25.1 *q*	25.4 *q*	26.1 *q*	26.6 *q*	26.2 *q*	26.7 *q*	26.1 *q*	26.5 *q*	25.1 *q*	25.4 *q*
31	—	—	—	—	107.0 *t*	107.1 *t*	107.0 *t*	107.0 *t*	106.9 *t*	107.0 *t*
$\underline{C}H_3CO$	21.3 *q*	21.1 *q*	21.3 *q*	21.1 *q*	21.3 *q*	20.9 *q*	21.3 *q*	21.1 *q*	21.3 *q*	21.1 *q*
$CH_3\underline{C}O$	170.0 *s*	170.6 *s*	170.9 *s*	170.6 *s*	170.8 *s*	170.3 *s*	170.8 *s*	170.5 *s*	171.0 *s*	170.5 *s*
CH_2	60.7 *t*	—	60.7 *t*	—	60.7 *t*	—	60.6 *t*	—	60.6 *t*	—

*Assignments in each column may be interchanged.

EXPERIMENTAL

General. Mps: uncorr.; UV: EtOH; IR: K, Pyridine-d_5; EIMS and HRMS: 70 eV; ^1H and ^{13}C NMR: $CDCl_3$ at 500 and 125 MHz, respectively, with TMS as int. standard. Analytical TLC and prep. TLC: Kieselgel 60 F_{254} plate (0.2, 0.5, 1.0, 2.0 mm) detection with 10% H_2SO_4; prep. HPLC: Develosil ODS-7 (20×250 mm) column with a UV monitoring flow system (210 nm) and a flow rate of 10.0 ml min^{-1}.

Fungal material. Dried sclerotia of *Poria cocos* (Hoelen), the surface layers of which had been removed, were purchased in Osaka market, Japan, in 1993.

Extraction and fractionation. Fungal material (30 kg) was extracted twice with MeOH (30 l) under reflux. The combined MeOH soln was concd under reduced pressure to afford an orange-coloured residue (160 g), which was chromatographed on a silica gel (3 kg) column eluting with $CHCl_3$ (5 l) and a MeOH–$CHCl_3$ gradient (1:99–1:4). A fraction eluted with MeOH–$CHCl_3$ (1:49), on concentration, gave a precipitate (4.5 g). This was collected by filtration and suspended in MeCN (100 ml). NCMP (10 g) and Et_3N (2 ml) were added to this suspension and the mixture was refluxed for 1 hr. After concentration under reduced pressure, the residue was chromatographed over silica gel (1 kg) with n-hexane–EtOAc (1:1) to afford a yellowish paste (6 g). The paste was separated by reversed-phase prep. HPLC with MeCN–H_2O (17:3) to give five frs.

Fraction 1 (300 mg) was identified as polyporenic acid C phthalimidomethyl ester (**6b**). Fraction 2 was purified by repeated prep. TLC with MeOH–$CHCl_3$ (1:19) to yield **2b** (30 mg) and **3b** (50 mg). Fraction 3 gave **1b** (200 mg). Fractions 4 and 5 gave **4b** (510 mg) and **5b** (2.0 g) respectively.

Compound **1b** (*3-o-acetyl-16α-hydroxytrametenolic acid phthalimidomethyl ester*). Amorphous powder, $[\alpha]_D^{26}$ + 8 ($CHCl_3$; *c* 1.0). IR ν_{max}^{KBr} cm^{-1}: 1785, 1733; UV λ_{max}^{EtOH} nm (log ε): 218 (4.6); EIMS *m/z* (rel. int.): 673 $[M]^+$ (2), 495 (30), 421 (36), 316 (36), 160 (100); HRMS *m/z*: 673.3995 $[M]^+$, $C_{41}H_{55}O_7N$ requires 673.3978.

Compound **2b** (*3β-acetyl-16α-hydroxydehydrotrametenolic acid phthalimidomethyl ester*). Amorphous powder, $[\alpha]_D^{26}$ + 30 ($CHCl_3$; *c* 1.0). IR ν_{max}^{KBr} cm^{-1}: 1785, 1733; UV λ_{max}^{EtOH} nm (log ε): 218 (4.7); EIMS *m/z* (rel. int.): 671 $[M]^+$ (4), 511 (37), 494 (37), 160 (100); HRMS *m/z*: 671.3849 $[M]^+$, $C_{41}H_{53}O_7N$ requires 671.3822.

Compound **3b** (*3-epidehydropachymic acid phthalimidomethyl ester*). Amorphous powder, $[\alpha]_D^{26}$ − 1 ($CHCl_3$; *c* 1.0). IR ν_{max}^{KBr} cm^{-1}: 1785, 1734; UV λ_{max}^{EtOH} nm (log ε): 218 (4.8); EIMS *m/z* (rel. int.): 685 $[M]^+$ (4), 525 (33), 508 (38), 433 (30), 160 (100); HRMS *m/z*: 685.4013 $[M]^+$, $C_{42}H_{55}O_7N$ requires 685.3979.

Compound **4b** (*dehydropachymic acid phthalimidomethyl ester*). Amorphous powder, $[\alpha]_D^{26}$ + 20 ($CHCl_3$; *c* 1.0). IR ν_{max}^{KBr} cm^{-1}: 1785, 1734; UV λ_{max}^{EtOH} nm (log ε): 218 (4.8); EIMS *m/z* (rel. int.): 685 $[M]^+$ (4), 525 (57), 507 (34), 160 (100); HRMS *m/z*: 685.4004 $[M]^+$, $C_{42}H_{55}O_7N$ requires 685.3979.

Compound **5b** (*pachymic acid phthalimidomethyl ester*). Amorphous powder, $[\alpha]_D^{26}$ + 9 ($CHCl_3$; *c* 1.0). IR ν_{max}^{KBr} cm^{-1}: 1785, 1734; UV λ_{mx}^{EtOH} nm (log ε): 218 (4.7); EIMS *m/z* (rel. int.): 687 $[M]^+$ (10), 654 (17), 160 (100); HRMS *m/z* 687.4157 $[M]^+$, $C_{42}H_{57}O_7N$ requires 687.4135.

Partial hydrolysis of **1b**–**3b**. A drop of 0.1 M NaOH–EtOH soln was added every 1 min into a stirred soln containing **1b** (90 mg) in $CHCl_3$ (5 ml) at room temp until **1b** could not be detected by TLC (n-hexane–EtOAc (2:1), rf. 0.7, detected under UV lamp). Then the reaction mixt. was acidified with dil. HCl and extracted with $CHCl_3$. The $CHCl_3$ soln was concd under reduced pressure and the residue was recrystallized from MeOH to give **1a** (62 mg). Compound **2b** (20 mg) and **3b** (30 mg) were partially hydrolysed in the same way to give **2a** (15 mg) and **3a** (20 mg), respectively.

Compound **1a** (*3-o-acetyl-16α-hydroxytrametenolic acid*). Recrystallized from MeOH. Needles, mp > 300°, $[\alpha]_D^{26}$ + 7 (pyridine; *c* 1.0). IR ν_{max}^{KBr} cm^{-1}: 1737, 1707; EIMS *m/z* (rel. int.): 514 $[M]^+$ (4), 481 (40), 421 (32), 316 (100); HRMS *m/z*: 514.3629 $[M]^+$, $C_{32}H_{50}O_5$ requires 514.3658.

Compound **2a** (*3-o-acetyl-16α-hydroxydehydrotrametenolic acid*). Recrystallized from MeOH. Needles, mp 254–255°, $[\alpha]_D^{26}$ + 20 (pyridine; *c* 0.1). IR ν_{max}^{KBr} cm^{-1}: 1732, 1712; UV λ_{max}^{EtOH} nm (log ε): 242 (4.1); EIMS *m/z* (rel. int.): 512 $[M]^+$ (45), 495 (47), 419 (64), 353 (98), 295 (98), 277 (91), 43 (100); HRMS *m/z*: 512.3474 $[M]^+$, $C_{32}H_{48}O_5$ requires 512.3502.

Compound **3a** (*3-epidehydropachymic acid*). Recrystallized from MeOH. Needles, mp 274–275°, $[\alpha]_D^{26}$ − 8 (pyridine; *c* 0.1). IR ν_{max}^{KBr} cm^{-1}: 1741, 1705; UV λ_{max}^{EtOH} nm (log ε): 242 (4.1); EIMS *m/z* (rel. int.): 526 $[M]^+$ (62), 433 (85), 353 (79), 295 (81), 277 (100); HRMS *m/z*: 526.3636 $[M]^+$, $C_{33}H_{50}O_5$ requires 526.3658.

Complete hydrolysis of **3b**. Compound **3b** (10 mg) in $CHCl_3$ (10 ml) was hydrolyzed with 1 M NaOH–EtOH under reflux for 1 hr. Then, the reaction mixture was acidified with dil. HCl and extracted with $CHCl_3$. The $CHCl_3$ soln was concentrated under reduced pressure and the residue was purified by prep. TLC (MeOH–$CHCl_3$–H_2O; 10:90:1) to give 3-epidehydrotumulosic acid (**7a**, 5 mg) [5].

Partial hydrolysis of **4b** *and* **5b**. Partial hydrolysis of **4b** (30 mg) and **5b** (30 mg) was performed with 0.1 M NaOH–EtOH at room temp in a similar manner as described for **1b** to give dehydropachymic acid (**4a**, 21 mg), mp 267–268°, and pachymic acid (**5a**, 20 mg), mp 296–298°, respectively.

Hydrolysis of **6b**. Hydrolysis of **6b** were performed with 0.1 M NaOH–EtOH at room temp in a similar manner as described above to give polyporenic acid C, mp 273–275°, $[\alpha]_D^{26}$ + 2 (pyridine; *c* 0.1) [3].

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