Studies on Constituents of Fruit Body of *Polyporus umbellatus* and Their Cytotoxic Activity

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From the crude drug Chorei, the fruit body of *Polyporus umbellatus*, seven new components named polyporusterone A, B, C, D, E, F and G, were isolated and their structures were determined on the basis of the spectral data. These compounds showed cytotoxic action on leukemia 1210 cell proliferation.

Keywords Chorei; *Polyporus*; ecdyson; polyporusterone; cytotoxicity; L-1210

The crude drug "Chorei", prepared from the dried fruit body of *Polyporus umbellatus* of Chinese and Japanese origin, is an indispensable material in oriental medicine, and is used for kidney and other such diseases. The components of Chorei have been examined by several authors and ergosterol, α-hydroxy-tetracosanoic acid,[1] ergosta-4,6,8(14),22-tetraen-3-one[2] and polysaccharides[3–8] have so far been reported. However, systematic investigation, including that of its minor components, has not been done. In this study, we isolated the minor components of Chorei and examined their cytotoxic effects on leukemia 1210 (L-1210) cells. Chorei (100 kg) was extracted with 30% ethanol to obtain the extract (2.4 kg) which was partitioned as shown in Chart 1. From fractions C, D and E, seven new compounds were isolated.

Compound **1** from fraction D was positive in both the Liebermann–Burchard reaction and the Sarkowski reaction and was suggested to be a steroidal compound. The molecular formula of **1** was determined as $C_{28}H_{46}O_6$, since the molecular ion peak in the field desorption mass spectrum (FD-MS) was found at *m/z* 478 and the carbon-13 nuclear magnetic resonance (^{13}C-NMR) signals revealed the presence of six methyls, seven methylenes, nine methines and six quaternary carbons (Table I). The ^{13}C-NMR spectrum showed five hydroxylated carbons at δ 67.8 (two overlapping signals), 74.3, 83.7 and 76.5 and a carbonyl carbon at δ 202.4. In the proton nuclear magnetic resonance (^{1}H-NMR) spectrum, the signal at δ 6.28 (1H, d, J=2.5 Hz) reveals an olefinic proton. The infrared (IR) absorption at 1650 cm^{-1} suggests the presence of the conjugated ketone. Based on the above evidence, compound **1** was suggested to be a phytoecdysone analog. Then, both acetyl derivatives of **1** and ecdysterone (**8**) were synthesized[9] and their spectral data were compared.

As shown in Chart 2, **8** was converted to a 20-methyl ketone compound (**8b**), which was known to be caused by the oxidative cleavage between C_{20} and C_{22} of ecdysterone 2-*O*-acetate.[10] Compound **1** was dealt with in the same method as the case of **8** to give **1b** and **1b′**. All of the spectral data of **1b** completely coincided with those of compound **8b**. The another fragment (**1b′**) was converted

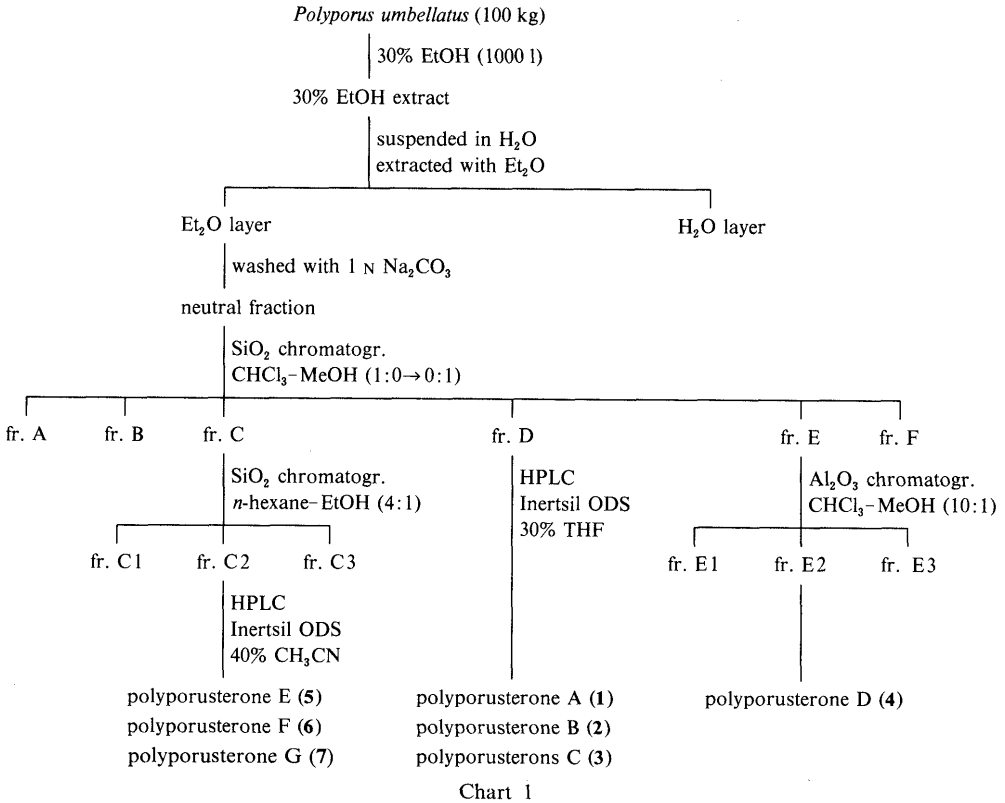

Chart 1

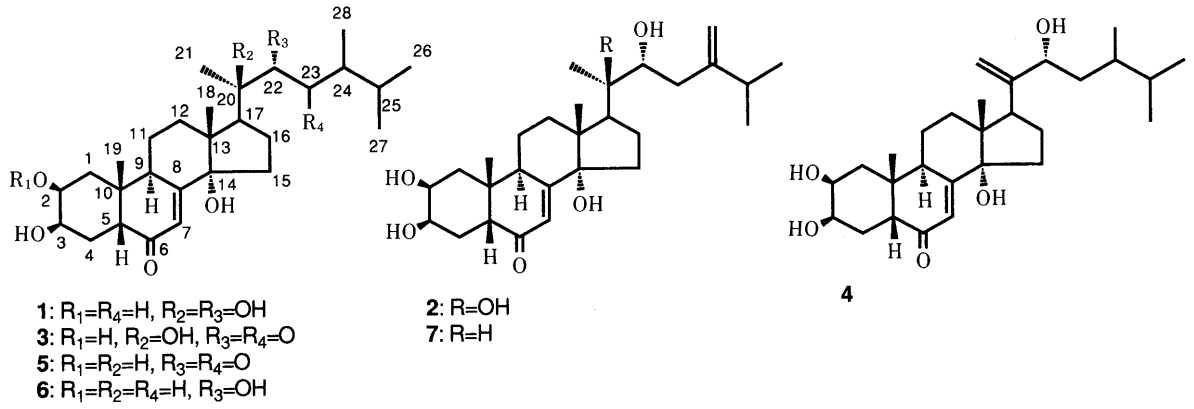

Fig. 1

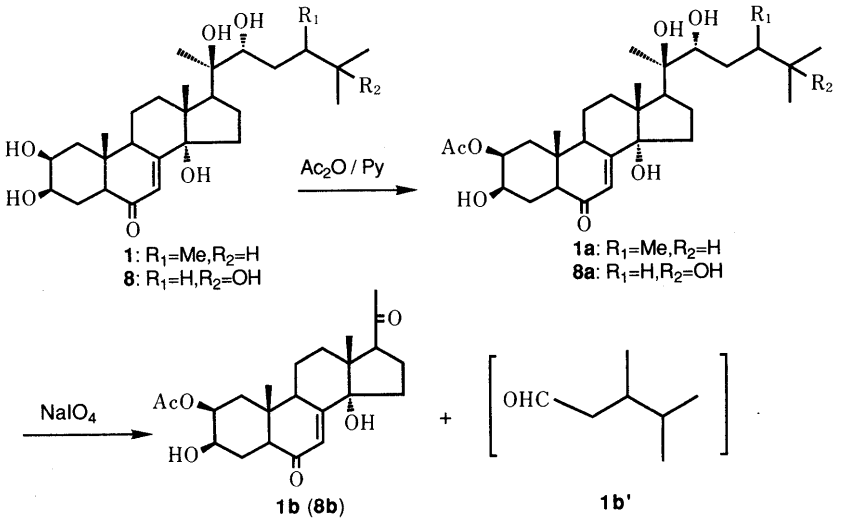

Chart 2

to a 2,4-dinitrohydrazone (**1c**). The ^{1}H-NMR spectrum of **1c** was shown in Fig. 2. This finding supports **1b'** as a 3,4-dimethyl-pentanal derivative. This evidence showed a methyl group attached to C_{24} in the side chain moiety of **1**. Considering the γ-effect to the C_{22} chemical shift caused by the introduction of the methyl group at C_{24}, the ^{13}C-NMR chemical shifts in the moiety of C_{20} to C_{22} of **1** were very similar to those of **8**. This fact suggested that the absolute configuration of both 20- and 22-positions of **1** was elucidated to be *R*. This finding supports the side chain structure of **1** shown in Fig. 1. The structure of **1** was determined and compound **1** was named polyporusterone A.

Compound **2** named polyporusterone B from fraction D was positive in both the Liebermann–Burchard reaction and the Sarkowski reaction, and ultraviolet (UV) and IR spectra were similar to those of **1**, suggesting it to be a polyporusterone A analog. In the ^{1}H-NMR spectrum of **2**, the signal at δ 0.76 found in **1** as a methyl group was displaced by the signals at δ 5.10 and 4.96 assignable to terminal methylene protons. In the ^{13}C-NMR spectrum, the signals at δ 36.0 (methine) and 21.2 (methyl) found in **1** disappeared in **2**, and the signals at δ 154.4 and 108.7 due to the terminal methylene group were found in **2**. In the FD-MS spectrum, the fragment peak (*m/z* 113) due to the side chain of **2** was two mass units less than that of **1** (*m/z* 115). The ^{1}H-NMR spectral data of the side chain in **2** (Table II) was represented in Fig. 2. Accordingly, the structure of polyporusterone B was determined as **2**.

Compound **3**, named polyporusterone C, was isolated from fraction D. In the FD-MS spectrum, the side chain fragment peak (*m/z* 113) with a relatively low intensity was two mass units less than that of **1** (*m/z* 115). IR absorption was found at 907 and 880 cm^{-1}. From these facts, it was assumed that compound **3** contained an epoxy group in the side chain fragment. In addition, the presence of the epoxy group was also supported by ^{1}H-NMR signals found at δ 3.04 and 2.74, which were not found in **1**, and a signal at δ 1.19 assignable to C_{24}-H being shielded to a much higher field than that of **1** (δ 1.88). In the ^{13}C-NMR spectrum of **3**, the signal at δ 37.1 assignable to C_{23} of **1** was displaced by the methine signal at δ 58.1. The coupling with protons of the side chain in **3** was shown in Fig. 2 together with chemical shifts of their protons. These results revealed that the epoxy group was formed between C_{22} and C_{23}. The other spectral data were similar to those of **1**. On the basis of these facts, the structure of polyporusterone C was determined as **3**.

Compound **4**, named polyporusterone D, from fraction E had a molecular ion peak of *m/z* 460 in the MS spectrum eighteen mass units less than that of **1**. In the ^{1}H-NMR

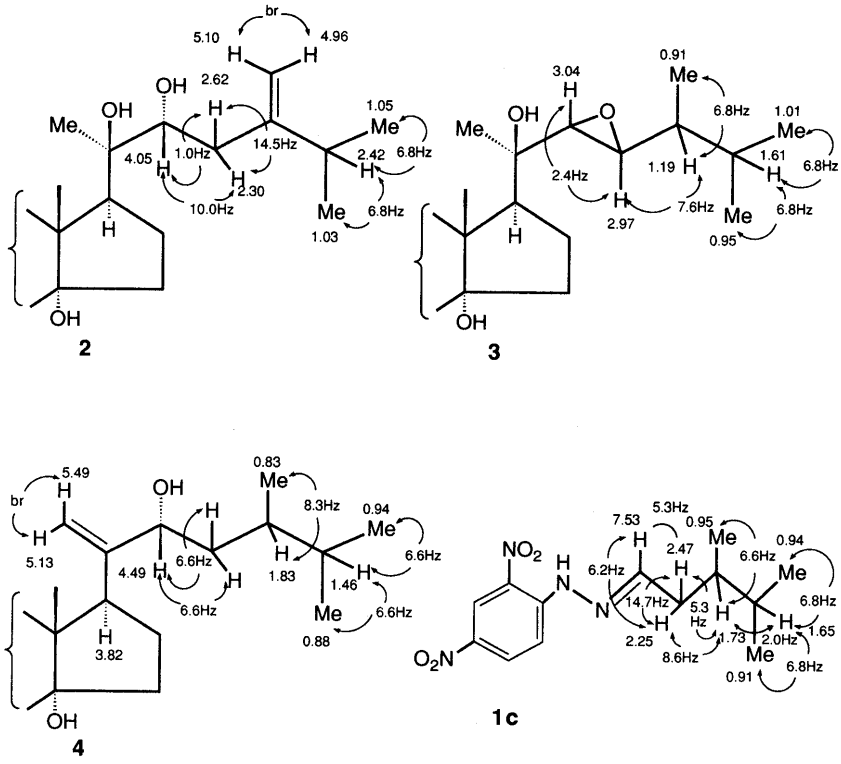

Fig. 2

TABLE I. ^{13}C-NMR Spectral Data of Compounds **1**—**8**, **1a** and **1b** (**8b**)

Compd.	1	1a	2	3	4	5	6	7	8	1b (8b)
C-1	37.9	33.9	38.0	38.0	38.1	38.0	38.1	38.1	37.8	33.7
C-2	67.8	72.7	68.1	68.1	68.2	68.0	68.2	68.2	67.8	72.6
C-3	67.8	65.3	68.1	68.1	68.2	68.0	68.2	68.2	67.7	65.1
C-4	32.3	32.8	32.5	32.5	32.6	32.5	32.6	32.6	32.3	32.6
C-5	51.2	51.3	51.4	51.4	51.6	51.4	51.5	51.5	51.1	51.1
C-6	202.4	202.9	203.5	203.4	203.6	203.4	203.7	203.7	202.4	202.5
C-7	121.1	121.8	121.7	121.7	121.6	121.6	121.7	121.7	121.1	122.2
C-8	165.3	166.4	166.1	165.9	165.9	165.4	165.9	165.7	165.2	164.5
C-9	34.4	34.5	34.5	34.5	34.6	34.6	34.7	34.7	34.3	34.2
C-10	38.5	38.9	38.7	38.7	38.8	38.7	38.8	38.8	38.5	38.9
C-11	21.1	21.3	21.1	21.1	21.2	21.0	21.3	21.3	21.0	21.1
C-12	21.4	21.6	21.5	21.8	28.0	26.0	26.9	26.9	21.4	21.8
C-13	47.9	48.1	48.1	47.9	48.4	47.5	48.3	48.4	47.9	48.1
C-14	83.7	84.2	84.2	84.0	84.1	83.6	83.9	83.9	83.8	83.9
C-15	31.9	32.1	32.0	31.8	32.3	32.0	32.1	32.1	31.9	31.9
C-16	31.6	31.8	31.7	31.7	31.8	32.0	31.6	31.6	31.6	30.5
C-17	49.7	50.0	50.0	54.0	46.8	50.9	47.7	47.7	49.9	59.5
C-18	17.8	18.0	17.9	17.7	16.1	15.9	16.0	16.0	17.8	17.1
C-19	24.4	24.4	24.5	24.4	24.5	24.4	24.6	24.6	24.3	24.2
C-20	76.5	76.9	76.7	72.0	154.1	36.0	43.5	43.0	76.5	209.1
C-21	21.3	21.3	21.5	24.3	111.6	20.5	13.6	13.6	21.6	31.3
C-22	74.3	74.7	75.4	66.2	74.3	64.0	70.9	71.7	77.2	—
C-23	37.1	37.4	38.3	58.1	41.4	59.9	35.3	36.5	27.3	—
C-24	36.0	36.2	154.4	42.1	35.7	42.4	35.8	154.9	42.4	—
C-25	29.6	29.7	33.7	31.5	30.0	31.3	29.8	33.9	69.3	—
C-26	16.1	16.2	22.1	19.7	17.6	15.9	16.3	22.3	29.9	—
C-27	15.8	15.9	21.8	20.5	17.7	16.5	16.0	22.1	29.9	—
C-28	21.2	21.5	108.7	13.9	20.6	13.8	21.6	108.7	—	—
C=O	—	170.5	—	—	—	—	—	—	—	170.4
CH_3	—	21.5	—	—	—	—	—	—	—	21.2

Run at 100 MHz in pyridine-d_5.

spectrum, terminal methylene protons at δ 5.49 and 5.13 were observed, and the methyl at δ 1.59 assignable to C_{21}-methyl in **1** was not found in **4**. In the ^{13}C-NMR spectrum, the signals of the terminal methylene group were found at δ 111.6 (CH_2) and 154.1 (C). In addition, the ^{1}H-NMR signals of C_{17}-H and C_{22}-H of **4** were shifted to a lower field in comparison with those of **1** (Table II). The other spectral data were similar to those of **1**. Accordingly, the structure of polyporusterone D was determined as **4**.

Compounds **5**, **6** and **7** named polyporusterone E, F, G from fraction C had similar spectral data to compounds **3**, **1** and **2** respectively, except for the following findings; (1) Each molecular ion peak of compounds **5**, **6** and **7** was sixteen mass units less than those of compounds **3**, **1** and **2**, respectively; (2) In the ^{1}H-NMR spectra of compounds **5**, **6** and **7**, one tertiary methyl disappeared and a secondary methyl newly appeared in comparison with those of compounds **3**, **1** and **2**, respectively; (3) One methine carbon signal in **5**, **6** and **7** was newly found in a range of δ 43.5 from 36.0 in place of the quaternary carbon signal in a range of δ 76.7 from δ 72.0 of **1**, **2** and **3**; (4) The carbon signals assignable to C_{23} of **5**, **6** and **7** were shifted to down or upfield (*ca.* 1—2 ppm) in comparison with those of **3**, **1** and **2**, respectively (Table I). Judging from these findings, it was concluded that **5**, **6** and **7** were the deoxycompounds at C_{20} of **3**, **1** and **2**, respectively. Accordingly, the structures of polyporusterones E, F and G were determined as **5**, **6** and **7**, respectively.

Next, the cytotoxic effects of these seven compounds were examined using leukemia 1210 cells (L-1210). As shown in Table III, compounds **1** to **7** inhibited the proliferation of L-1210 cells, dose-dependently. Previously, polysaccharides have been reported as the antitumor components of Chorei. The cytotoxic effect suggests that these polyporusterones may participate in the biological action of Chorei, though these compounds are contained in small quantity.

Table II. ^{1}H-NMR Spectral Data of Compounds **1**—**8**

Compd.	**1**	**2**	**3**	**4**
H-2	4.18 (1H, br d, J=11.2 Hz)	4.20 (1H, dt, J=11.4, 4.0 Hz)	4.20 (1H, dt, J=10.2, 3.2 Hz)	4.14 (1H, br d, J=10.9 Hz)
H-3	4.24 (1H, br s)	4.25 (1H, m)	4.28 (1H, m)	4.23 (1H, br s)
H-7	6.28 (1H, d, J=2.5 Hz)	6.28 (1H, d, J=2.2 Hz)	6.24 (1H, d, J=2.2 Hz)	6.27 (1H, d, J=2.3 Hz)
H-9	3.61 (1H, m)	3.61 (1H, m)	3.62 (1H, m)	3.60 (1H, m)
H-17	2.94 (1H, dd, J=9.4, 9.0 Hz)	2.96 (1H, dd, J=9.4, 8.8 Hz)	—	3.82 (1H, m)
H-21	—	—	—	5.49 (1H, br s)
H-21′	—	—	—	5.13 (1H, br s)
H-22	3.93 (1H, br d, J=9.5 Hz)	4.05 (1H, dd, J=10.0, 1.0 Hz)	3.04 (1H, d, J=2.4 Hz)	4.49 (1H, t, J=6.6 Hz)
H-23	—	2.62 (1H, br d, J=14.5 Hz)	2.97 (1H, dd, J=7.6, 2.4 Hz)	—
H-23′	—	2.30 (1H, dd, J=14.5, 10.0 Hz)	—	—
H-24	1.88 (1H, m)	—	1.19 (1H, dq, J=7.6, 6.8 Hz)	1.83 (1H, m)
H-25	1.45 (1H, septed, J=6.2 Hz)	2.42 (1H, septed, J=6.8 Hz)	1.61 (1H, septed, J=6.8 Hz)	1.46 (1H, septed, J=6.5 Hz)
H-28	—	5.10 (1H, br s)	—	—
H-28′	—	4.96 (1H, br s)	—	—
Me-18	1.25 (3H, s)	1.24 (3H, s)	1.16 (3H, s)	0.84 (3H, s)
Me-19	1.09 (3H, s)	1.09 (3H, s)	1.09 (3H, s)	1.05 (3H, s)
Me-21	1.59 (3H, s)	1.60 (3H, s)	1.54 (3H, s)	—
Me-26	0.88 (3H, d, J=6.1 Hz)	1.05 (3H, d, J=6.8 Hz)	1.01 (3H, d, J=6.8 Hz)	0.94 (3H, d, J=6.6 Hz)
Me-27	0.87 (3H, d, J=6.4 Hz)	1.03 (3H, d, J=6.8 Hz)	0.95 (3H, d, J=6.8 Hz)	0.88 (3H, d, J=6.6 Hz)
Me-28	0.76 (3H, d, J=6.8 Hz)	—	0.91 (3H, d, J=6.8 Hz)	0.83 (3H, d, J=8.3 Hz)

	5	**6**	**7**	**8**
H-2	4.18 (1H, br d, J=10.2 Hz)	4.11 (1H, br d, J=10.5 Hz)	4.11 (1H, br d, J=10.5 Hz)	4.20 (1H, br d, J=11.0 Hz)
H-3	4.26 (1H, br s)	4.21 (1H, br s)	4.21 (1H, br s)	4.23 (1H, br s)
H-7	6.23 (1H, d, J=2.2 Hz)	6.24 (1H, d, J=2.5 Hz)	6.24 (1H, d, J=2.5 Hz)	6.26 (1H, d, J=2.5 Hz)
H-9	3.59 (1H, m)	3.57 (1H, m)	3.57 (1H, m)	3.59 (1H, m)
H-17	—	—	—	—
H-21	—	—	—	—
H-21′	—	—	—	—
H-22	2.67 (1H, d, J=6.9 Hz)	4.11 (1H, m)	4.11 (1H, m)	3.89 (1H, d, J=9.4 Hz)
H-23	2.54 (2H, dd, J=6.9, 2.4 Hz)	—	—	—
H-23′	—	—	—	—
H-24	1.26 (1H, m)	—	—	—
H-25	1.45 (1H, septed, J=6.2 Hz)	1.58 (1H, septed, J=6.5 Hz)	2.48 (1H, septed, J=6.8 Hz)	—
H-28	—	—	5.11 (1H, br s)	—
H-28′	—	—	4.99 (1H, br s)	—
Me-18	0.72 (3H, s)	0.73 (3H, s)	0.72 (3H, s)	1.23 (3H, s)
Me-19	1.09 (3H, s)	1.09 (3H, s)	1.09 (3H, s)	1.08 (3H, s)
Me-21	0.94 (3H, d, J=7.9 Hz)	1.28 (3H, d, J=6.9 Hz)	1.28 (3H, d, J=6.9 Hz)	1.60 (3H, s)
Me-26	1.07 (3H, d, J=6.9 Hz)	0.90 (3H, d, J=6.9 Hz)	1.06 (3H, d, J=6.9 Hz)	1.38 (3H, s)
Me-27	1.01 (3H, d, J=6.6 Hz)	0.88 (3H, d, J=6.9 Hz)	1.01 (3H, d, J=6.9 Hz)	1.38 (3H, s)
Me-28	0.92 (3H, d, J=6.9 Hz)	0.73 (3H, d, J=6.8 Hz)	—	—

Run at 270 MHz in pyridine-d_5.

Table III. Effect of Compounds **1**—**7** on the Growth of L-1210 Cells

Substance	IC_{50} μg/ml (95% confidence limits)		
	3	5	7 d
1	26 (8—86)	60 (38—95)	32 (14—75)
2	17 (4—68)	64 (40—103)	42 (24—74)
3	37 (22—63)	26 (16—42)	42 (26—69)
4	46 (24—87)	12 (4—39)	10 (3—31)
5	13 (7—25)	10 (5—31)	13 (9—19)
6	26 (14—47)	17 (7—40)	36 (11—117)
7	26 (13—52)	20 (10—40)	39 (19—83)

n=4.

Experimental

All melting points were taken on a Yanagimoto micromelting point apparatus and are uncorrected. UV absorption spectra were determined on a Shimadzu UV-240 spectrophotometer. IR spectra were recorded on JASCO FT-7000 spectrometer. NMR spectra were measured with JEOL GX-270 using tetramethylsilane (TMS) as an internal standard. Electron impact mass (EI-MS) spectra and FD-MS spectra were recorded on Shimadzu LKB-9000B and JEOL JMS DX-303 mass spectrometers, respectively. Optical rotations were determined on a JASCO DIP-4 digital polarimeter. Column chromatography was carried out on silica gel (Kieselgel 60, Merck) or alumina (Aluminium oxide 90, Merck). High performance liquid chromatography (HPLC) was carried out on Gasukuro Kogyo Model 572P and 502T using Inertsil ODS column (i.d. 20 mm × 250 mm, Gasukuro Kogyo). Thin layer chromatography (TLC) was developed on Merck Silica gel 60 F_{254} plates (0.25 mm thickness) and spots were detected by means of UV absorbance measurement at 254 nm and/or by spraying with 0.5% *p*-anisaldehyde in 10% H_2SO_4 followed by heating.

Extraction and Isolation The dried fruit body (100 kg) of *Polyporus umbellatus*, supplied by Takasago Medical Co., was extracted with boiling 30% ethanol (EtOH) for 1 h. The extract solution was evaporated by heating under reduced pressure to afford the residue as a dark brownish gum (2.4 kg). The residue was suspended in water (1 l) and extracted four

times with ether (Et_2O, 3 l). The combined Et_2O layer was washed twice with 1 N Na_2CO_3 (1 l) and then dried over Na_2SO_4. The organic layer was evaporated *in vacuo* to afford the neutral portion (14.5 g). It was dissolved in methanol (MeOH) and adsorbed on silica gel (50 g). The adsorbed material was transfered to a silica gel (500 g) column packed in $CHCl_3$ and developed with an eluting solvent ($CHCl_3$, 5% MeOH, 7% MeOH, 10% MeOH, 20% MeOH and MeOH) and six fractions, that is, fraction A (2.0 g), B (6.1 g), C (2.8 g), D (1.4 g), E (0.58 g) and F (0.36 g) were obtained. Fraction C was further chromatographed on silica gel (300 g) developing with *n*-hexane–EtOH (4 : 1), and was purified by HPLC to afford polyporusterone E (**5**, 22 mg), polyporusterone F (**6**, 10 mg) and polyporusterone G (**7**, 10 mg). The condition of HPLC was as follows; column; Inertsil ODS (20 × 250 mm), mobile phase; 40% CH_3CN, flow rate; 10 ml/min, detection; UV 242 nm, each compound and retention time (min); **5** (20.3 min): **6** (12.0 min): **7** (10.5 min). The separation of fraction D was performed by HPLC to afford polyporusterone A (**1**, 300 mg), polyporusterone B (**2**, 300 mg) and polyporusterone C (**3**, 26 mg). The condition of HPLC was as follows; column; Inertsil ODS (20 × 250 mm), mobile phase; 30% tetrahydrofuran (THF), flow rate; 10 ml/min, detection; UV 242 nm, each compound and retention time (min); **1** (14.5 min): **2** (12.3 min): **3** (25.0 min). Fraction E was applied to alumina (100 g) column chromatography developing with a mixture of $CHCl_3$–MeOH (10 : 1) to afford polyporusterone D (**4**, 8 mg).

Polyporusterone A (1) Colorless needles (MeOH). mp 261.5 °C. $[\alpha]_D^{20}$ +52.9° (c=0.61, EtOH). UV λ_{max}^{MeOH} nm (log ε): 241 (4.03). IR ν_{max}^{KBr} cm^{-1}: 3300 (OH), 1650 (C=O). ^{1}H- and ^{13}C-NMR data were shown in Tables I and II. FDMS m/z: 501 $(M+Na)^+$, 479 $(M+1)^+$, 478 (M^+), 363 $(M-C_7H_{15}O)^+$, 115 ($C_7H_{15}O$, base peak).

Polyporusterone B (2) Colorless needles (MeOH). mp 250 °C. $[\alpha]_D^{20}$ +56.1° (c=0.46, EtOH). UV λ_{max}^{MeOH} nm (log ε): 241 (4.05). IR ν_{max}^{KBr} cm^{-1}: 3300 (OH), 1650 (C=O), 803 ($C=CH_2$). ^{1}H- and ^{13}C-NMR data were shown in Tables I and II. FDMS m/z: 499 $(M+Na)^+$, 477 $(M+1)^+$, 476 (M^+), 363 $(M-C_7H_{13}O)^+$, 113 ($C_7H_{13}O$, base peak).

Polyporusterone C (3) Colorless needles (MeOH). mp 250 °C. UV λ_{max}^{MeOH} nm (log ε): 241 (4.01). IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1645 (C=O), 907, 880 (epoxy). ^{1}H- and ^{13}C-NMR data were shown in Tables I and II. FDMS m/z: 499 $(M+Na)^+$, 477 $(M+1)^+$, 476 (M^+), 319 $(M-C_9H_{17}O_2)^+$, 157 ($C_9H_{17}O_2$, strong peak), 113 ($M-C_7H_{13}O$, weak peak).

Polyporusterone D (4) Colorless powder. UV λ_{max}^{MeOH} nm (log ε): 241 (4.01). IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1650 (C=O), 803 ($C=CH_2$). ^{1}H- and ^{13}C-NMR data were shown in Tables I and II. EIMS m/z: 460 (M^+), 432, 249 (base peak).

Polyporusterone E (5) Colorless needles (MeOH). mp 232 °C. UV λ_{max}^{MeOH} nm (log ε): 242 (4.02). IR ν_{max}^{KBr} cm^{-1}: 3296 (OH), 1644 (C=O), 907, 880 (epoxy). ^{1}H- and ^{13}C-NMR data were shown in Tables I and II. EIMS m/z: 460 (M^+), 432, 345, 301, 290, 249 (base peak).

Polyporusterone F (6) Colorless needles (MeOH). mp 251 °C. UV λ_{max}^{MeOH} nm (log ε): 242 (4.02). IR ν_{max}^{KBr} cm^{-1}: 3338 (OH), 1649 (C=O). ^{1}H- and ^{13}C-NMR data were shown in Tables I and II. EIMS m/z: 462 (M^+), 434, 250, 249 (base peak).

Polyporusterone G (7) Colorless powder. UV λ_{max}^{MeOH} nm (log ε): 242 (4.02). IR ν_{max}^{KBr} cm^{-1}: 3340 (OH), 1650 (C=O), 803 ($C=CH_2$). ^{1}H- and ^{13}C-NMR data were shown in Tables I and II. EIMS m/z: 460 (M^+), 432, 249 (base peak), 247.

2-*O*-Acetylpolyporusterone A (1a) A mixture of **1** (45 mg), pyridine (10 ml) and Ac_2O (2 ml) was stirred and left for 1 h at room temperature. The reaction mixture was poured into ice-water (30 ml) and extracted twice with Et_2O (50 ml). The extracted solution was evaporated off and the residue was purified with the column chromatography on silica gel (10 g) with 4% MeOH in $CHCl_3$ to afford 2-*O*-acetylpolyporusterone A (**1a**, 25 mg) as a white powder. IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1745 (OAc), 1650 (C=O). ^{1}H- and ^{13}C-NMR data were shown in Tables I and II. EIMS m/z: 520 (M^+).

2-*O*-Acetyl-poststerone (1b)[11] $NaIO_4$ (30 mg) was added to the solution of **1a** (25 mg) in EtOH/H_2O (2.5 ml/5 ml). This solution was stirred for 30 min at room temperature. The reaction mixture was distilled, and the distilled solution and the residue were obtained. The residue was purified by the column chromatography on silica gel (5 g) with $CHCl_3$–MeOH (97 : 3) to afford **1b** (20 mg) as a white powder. IR ν_{max}^{KBr} cm^{-1}: 3474 (OH), 1740 (OAc), 1705 (C=O at C-20), 1659 (C=O at C-6). ^{1}H-NMR (pyridine-d_5) δ: 5.21 (1H, br d, J=11.6 Hz, H-2), 4.35 (1H, br s, H-3), 3.58 (1H, dd, J=8.8, 8.1 Hz, H-17), 2.16 (3H, s, H-21), 1.97 (3H, s, OAc), 1.05 (3H, s, H-19), 0.70 (3H, s, H-18). ^{13}C-NMR (pyridine-d_5) δ: 209.1 (C-20), 202.5 (C-6), 170.4 (OAc), 164.5 (C-8), 122.2 (C-7), 83.9 (C-14), 72.6 (C-2), 65.1 (C-3), 31.3 (C-21), 24.2 (C-19), 17.1 (C-18).

3-Methylisohexanal-2,4-dinitrophenylhydrazone (1c) The distilled solution, which was obtained by the oxidative reaction mixture of **1a** and $NaIO_4$, was added to 1.5% 2,4-dinitrophenyl-hidrazine in 10% H_2SO_4 (2 ml). The reaction mixture was extracted twice with Et_2O. The organic layer was evaporated and the residue was subjected on preparative TLC with a mixture of cyclohexane–Et_2O (5 : 1) to afford **1c** (8 mg) as a yellow powder. ^{1}H-NMR ($CDCl_3$): 11.04 (1H, br s), 9.12 (1H, d, J=2.6 Hz), 8.30 (1H, ddd, J=9.7, 2.6, 0.7 Hz), 7.94 (1H, d, J=9.7 Hz), 7.53 (1H, dd, J=6.2, 5.3 Hz), 2.47 (1H, dt, J=14.7, 5.3 Hz), 2.25 (1H, ddd, J=14.7, 8.6, 6.2 Hz), 1.73 (1H, m), 1.65 (1H, septed, d, J=6.8, 2.0 Hz), 0.95 (3H, d, J=6.6 Hz), 0.94 (3H, d, J=6.8 Hz), 0.91 (3H, d, J=6.8 Hz). ^{13}C-NMR ($CDCl_3$) δ: 152.6 (C=N), 145.2, 137.9, 130.0, 128.9, 123.5, 116.6 (aromatic-C), 37.1 (2C), 32.2, 20.0, 18.2, 15.8.

Synthesis of 1b from Ecdysterone (8) Using **8** instead of **1**, all the same reactions as those of the syntheses of **1a** and **1b** from **1** were performed. The yielded compound (**8b**) was identified with **1b** by the spectral data of TLC, IR, ^{1}H- and ^{13}C-NMR.

Assay for Cytotoxicity to Leukemia L-1210 L-1210 cells were adjusted to 8×10^4 cells/ml with Celgrosser H (Meguro Res.) as a medium. Two milliliters of this cell suspension and 10 μl of dimethyl sulfoxide (DMSO) containing an appropriate amount of a test compound were added to a screw cap test tube. After incubation at 37 °C for 3, 5 and 7 d, the cells which were not stained with 0.5% trypan blue in the phosphate buffered saline solution were counted as surviving cells. Results were represented as the concentration of a compound for 50% inhibition to the cell proliferation (IC_{50}), which was calculated in accordance with the method of Litchfield and Wilcoxon.[12]

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