5-Lipoxygenase Inhibitors Isolated from the Mushroom *Boletopsis leucomelas* (PERS.) FAYOD

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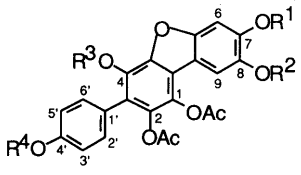
Terphenyl compounds, tentatively named Bl-I (1), Bl-II (2), Bl-III (3), Bl-IV (4) and Bl-V (5), showing 5-lipoxygenase inhibitory activity have been isolated from the mushroom *Boletopsis leucomelas* (PERS.) FAYOD. On the basis of physico-chemical and spectral evidence, they were concluded to be a series of cycloleucomelone-leucoacetates.

Keywords 5-lipoxygenase inhibitor; mushroom; basidiomycete; *Boletopsis leucomelas*; cycloleucomelone-leucoacetate

5-Lipoxygenase is a key enzyme which catalyzes the first step in the biosynthesis of leukotrienes. Leukotrienes are involved in the pathology of a variety of inflammatory and allergic diseases, such as asthma, psoriasis, rheumatic arthritis and so on. Specific inhibitors of 5-lipoxygenase, therefore, are expected to be potential therapeutic drug for these diseases as well as tools for research on the biochemical and physiological roles of leukotrienes.[2)]

During our screening program for biologically active constituents from mushrooms (Basidiomycetes), the MeOH extract of *Boletopsis leucomelas* (PERS.) FAYOD (Japanese name: kurokawa)[3)] was found to show an inhibitory effect on 5-lipoxygenase, and this led us to examine the constituents of this mushroom. As a result, several terphenyl compounds, tentatively named Bl-I (**1**), Bl-II (**2**), Bl-III (**3**), Bl-IV (**4**) and Bl-V (**5**), have been isolated as active principles. They were shown to be a series of cycloleucomelone-leucoacetates, two of which, namely **1** and **4**, were previously isolated from the same mushroom by Steglich and co-workers.[4)] In this paper, we wish to report the isolation and structure elucidation of Bl-I through Bl-V (**1**—**5**), and their inhibitory effects on 5-lipoxygenase.



	R1	R2	R3	R4
Bl-I	Ac	Ac	Ac	Ac
II	Ac	Ac	Ac	H
III	H	H	Ac	Ac
IV	H	H	Ac	H
V	H	H	H	H

Fig. 1. Structures of Bl-I (**1**), Bl-II (**2**), Bl-III (**3**), BI-IV (**4**) and BI-V (**5**)

Results and Discussion

Isolation and Structure Elucidation The MeOH extract of *B. leucomelas* was separated into ethyl acetate-, *n*-butanol- and water-soluble fractions. The ethyl acetate extract, exhibiting significant 5-lipoxygenase inhibitory activity (IC_{50} value: 0.25 μg/ml), was subjected to successive column chromatography on silica gel to afford Bl-I through Bl-V (**1**—**5**, Fig. 1), along with fatty acids, ergosterol, and ergosterol peroxide.

On the basis of the ^{1}H-NMR, IR, UV and electron impact (EI) mass spectra (MS), **1**—**5** were deduced to be a series of terphenyl analogues containing six, five, four, three and two acetyl groups (Tables I and II). Further, acetylation of **2**—**5** with acetic anhydride in pyridine gave the respective acetyl derivatives, which were identical with **1**. Thus, the structure elucidation of **1**—**5** was carried out as follows.

The molecular formula of Bl-I (**1**) was determined to be $C_{30}H_{24}O_{13}$ by high-resolution (HR) EI-MS [*m/z*: 592.1200 (M^+); Calcd: 592.1217]. The IR spectrum of **1** showed absorptions at 3025, 1605, 1520 cm^{-1} (benzene ring) and 1780 cm^{-1} ($OCOCH_3$). In the ^{1}H-NMR spectrum ($CDCl_3$, 500 MHz), **1** exhibited six singlet signals at δ 2.00, 2.16, 2.32, 2.33, 2.34, 2.46 assignable to acetoxymethyl groups. Further, signals in the aromatic region [δ 7.12 (2H, d, J=8.0), 7.32 (2H, d, J=8.0), 7.46 (1H, s), 7.55 (1H, s)] indicated the presence of 1,4-disubstituted and 1,2,4,5-

TABLE I. Physico-Chemical Properties of Bl-I through Bl-V (**1**—**5**)

	Bl-I	Bl-II	Bl-III	Bl-VI	Bl-V
Appearance	Colorless needles	Colorless needles	Pale purple needles	Pale brown needles	Brown amorphous
mp (°C)	202—204 (lit. 203—204)	130—131	194—196	242—245 (dec.)	—
Molecular formula	$C_{30}H_{24}O_{13}$	$C_{28}H_{22}O_{12}$	$C_{26}H_{20}O_{11}$	$C_{24}H_{18}O_{10}$	$C_{22}H_{16}O_9$
HR EI-MS (M^+)					
Found:	592.1200	550.1134	508.0995	466.0925	424.0773
Calcd :	592.1217	550.1111	508.1006	466.0900	424.0793
EI-MS	592 (M^+), 550, 508, 466, 424, 382, 340	550 (M^+), 508, 466, 424, 382, 340	508 (M^+), 466, 424, 382, 340	466 (M^+), 424, 382, 340	424 (M^+), 382, 340
IR ν_{max}^{KBr} cm^{-1}	3030, 1780, 1520	3600—2800, 1780, 1610, 1520	3700—2800, 1770, 1750, 1625, 1520, 1500	3600—2800, 1770 (br), 1625, 1610, 1500	3700—2400 1760, 1615, 1520
UV λ_{max}^{MeOH} nm (log ε)	289 (4.50), 257 (4.36), 227 (4.67)	292 (4.62), 254 (4.44), 226 (4.82)	324 (4.42), 302 (4.41), 250 (4.45), 223 (4.61), 203 (4.71)	321 (4.51), 303 (4.48), 248 (4.48), 225 (4.67), 202 (4.73)	329 (4.49), 300 (4.42), 266 (4.44), 225 (4.68), 209 (4.69)

TABLE II. ^{1}H-NMR Spectral Data for Bl-I through Bl-V (**1**—**5**) in DMSO-d_6 (δ_{ppm}, 500 MHz)

	Bl-I	Bl-II	Bl-III	Bl-IV	Bl-V	
1-Ac	2.55	2.55	2.52	2.51	2.44	3H, s
2-Ac	2.07	2.10	2.05	2.06	2.01	3H, s
4-Ac	2.19	2.23	2.16	2.16		3H, s
7,8-Ac	2.35	2.35				6H, s
4′-Ac	2.31		2.30			3H, s
H-6	7.83	7.82	7.11	7.09	7.06	1H, s
H-9	7.89	7.88	7.14	7.12	7.07	1H, s
H-2′,6′	7.28[a]	7.05 (8.8)	7.25 (8.5)	6.99 (8.5)	7.06 (8.5)	2H, d
H-3′,5′	7.28[a]	6.88 (8.8)	7.22 (8.5)	6.84 (8.5)	6.80 (8.5)	2H, d
OH		9.74	9.38, 9.88	9.34, 9.67, 9.81	9.22, 9.47, 9.59, 9.81	each 1H, s

a) The signal was observed as a singlet. Figures in parentheses are coupling constants in hertz (Hz).

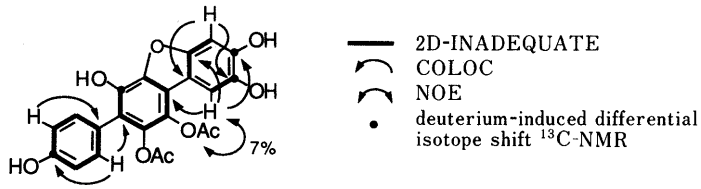


Fig. 2. NMR Spectral Data Summary for Bl-V (CD_3OD)

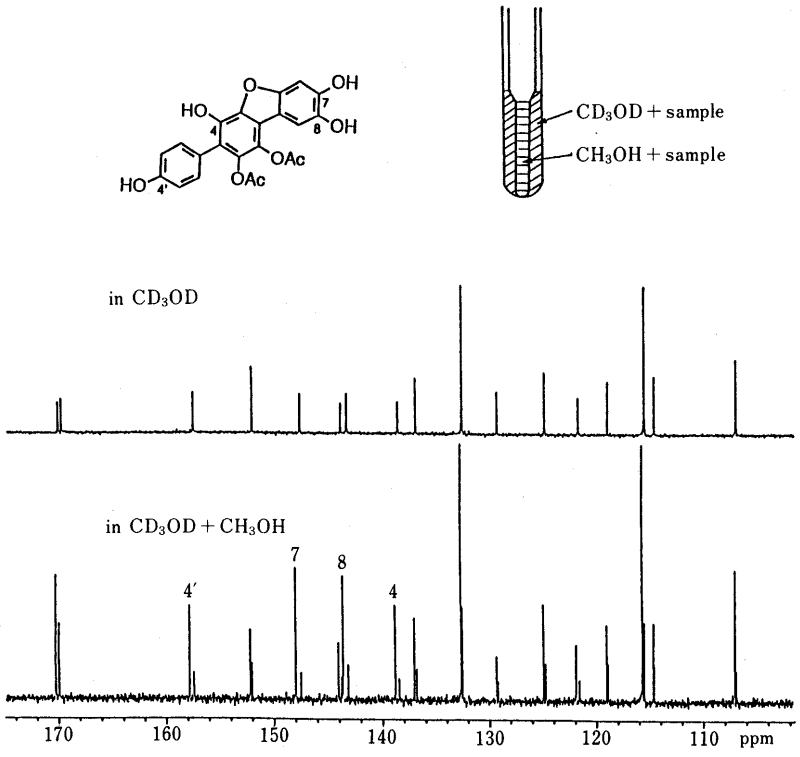


Fig. 3. Deuterium-Induced Differential Isotope Shift ^{13}C-NMR Spectrum of Bl-V (125 MHz)

tetrasubstituted benzene rings. These spectral data were in quite good agreement with those of cycloleucomelone-leucoperacetate recently isolated from the same mushroom by Steglich and co-workers.[4] Thus, the structure of Bl-I was concluded to be **1**.

The structures of Bl-II through Bl-IV (**2**—**4**) were elucidated by comparison of their ^{1}H-NMR spectra with that of **1**. Namely, unambiguous acetylation shifts were observed for the signals in the aromatic region as shown in Table II, which allowed us to determine the location of the hydroxyl and acetoxyl groups in the terphenyl nucleus. The structures were, thus, elucidated to be **2**—**4**. Bl-IV had also been previously isolated from the same mushroom.[4]

The structure of Bl-V (**5**) was not unequivocally determined from the ^{1}H-NMR spectral comparison. However, with the aid of two dimensional (2D) NMR experiments (^{13}C–^{1}H correlation spectroscopy (COSY), correlation spectroscopy *via* long-range coupling (COLOC), incredible natural abundance double quantum transfer experiment (INADEQUATE)), together with the nuclear Overhauser

effect (NOE) difference and deuterium-induced differential isotope shift ^{13}C-NMR spectra, the positions of hydroxyl and acetoxyl groups could be determined. The spectral data for **5** are summarized in Fig. 2. The NOE spectrum suggested that one of two acetoxyl groups was located on C-1 and the other was on C-2 or C-4. The carbon–proton correlations were established by analysis of the ^{13}C–^{1}H COSY spectrum, and the deuterium-induced differential isotope shift ^{13}C-NMR spectrum clarified the signals of the carbons (C-4, C-7, C-8, C-4′) bearing a hydroxyl group. In the NMR spectrum (Fig. 3), the signals due to carbons bearing a deuterium-substituted hydroxyl group were observed with an upfield shift (δ 0.37—0.50) as compared with those bearing a hydroxyl group.[5] The signals of the protons and carbons except for C-1, C-2, C-4 and C-4′ were thus assigned through the COLOC spectrum as shown in Table III, which revealed long range couplings (J_{CH}) between H-6/C-8, H-6/C-9a, H-9/C-7, H-9/C-5a, H-9/C-9b, H-2′ (6′)/C-3, H-2′ (6′)/C-4′ and H-3′ (5′)/C-1′. Finally, the carbon–carbon correlations were established on the basis of the 2D INADEQUATE spectrum, and hence the position of the other acetyl group was deduced to be at C-2. Consequently, the structure of Bl-V was concluded to be **5**.

Biological Activity The assay of 5-lipoxygenase inhibitory activity was performed according to the method of Amano *et al.*[6] with a slight modification. In this procedure, polymorphonuclear leukocytes (PMNLs) prepared from guinea pigs after stimulation with 2% casein were used as an enzyme source for 5-lipoxygenase. The 50%-inhibitory concentration was calculated from the difference of the production of 5-hydroxyeicosatetraenoic acid (5-HETE) from arachidonic acid in the presence and absence of a test material. AA-861 and BW-755C were used as controls.

Inhibitory effects of Bl-I through Bl-V (**1**—**5**) on 5-lipoxygenase are listed in Table IV. Among them, **3** displayed potent inhibitory activity, and its IC_{50} value (0.35 μM) was comparable to that of AA-861, a promising therapeutic drug for asthma.[7] Therefore, further biological studies seem worthwhile to evaluate the physiological action of **3**.

TABLE III. ^{13}C-NMR Spectral Data for Bl-V (**5**) in CD_3OD[a]

Position	δ (ppm)	Position	δ (ppM)
1	126.9 (s)	9	107.3 (d)
2	137.4 (s)	9a	115.0 (s)
3	122.2 (s)	9b	119.4 (s)
4	139.0 (s)	1′	125.2 (s)
4a	144.3 (s)	2′,6′	132.9 (d)
5a	152.5 (s)	3′,5′	115.9 (d)
6	99.4 (d)	4′	158.0 (s)
7	148.2 (s)	$\underline{C}H_3CO$	20.2 (q), 20.4 (q)
8	143.8 (s)	$CH_3\underline{C}O$	170.2 (s), 170.5 (s)

a) The spectrum was recorded at 125 MHz.

TABLE IV. Inhibitory Effects of Bl-I through Bl-V (**1**—**5**) on 5-Lipoxygenase

Compound	IC_{50} (μM)
Bl-I	1.18
Bl-II	1.04
Bl-III	0.35
Bl-IV	1.29
Bl-V	4.95
AA-861	0.26
BW-755C	27.9

Experimental

Melting points were determined on a Yanagimoto micro hot plate and are uncorrected. IR spectra were recorded on a JASCO A-100S IR spectrometer with polystyrene calibration at 1601 cm^{-1}. UV spectra were measured on a Hitachi U-3200 spectrophotometer. EI and HR MS were taken on a JEOL JMS DX-303. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL JNM GX-500 (500 and 125 MHz, respectively) using tetramethylsilane (TMS) as an internal standard. Chemical shifts are shown in δ (ppm) and multiplicities are indicated as follows: singlet = s, doublet = d. Coupling constants (J) are shown in hertz (Hz). TLC analyses were performed on Kieselgel 60F_{254} (Merck) and spots were detected under UV irradiation and by heating on a hot plate after spraying 50% sulfric acid reagent.

Isolation Procedure The fruiting bodies (5.0 kg) of *B. leucomelas*, collected at Miyagi prefecture in Oct. 1987, were extracted twice with MeOH (6.0 l) at room temperature for 2 d. After concentration of the extract, the residual aqueous suspension was diluted with H_2O and then extracted twice with EtOAc (3.0 l) and *n*-BuOH (1.5 l) successively. The EtOAc and *n*-BuOH layers were evaporated under reduced pressure to afford gummy residues (26.1 g and 4.7 g, respectively). The EtOAc-soluble material (20.0 g) was subjected to silica gel column chromatography (200 g: 3.5 cm i.d. × 38 cm) and eluted with *n*-hexane–EtOAc, $CHCl_3$–EtOAc and $CHCl_3$–MeOH mixtures. The $CHCl_3$–EtOAc (3 : 1—1 : 1) eluates were subsequently chromatographed on silica gel to give Bl-I (**1**, 175.8 mg). Bl-II through Bl-V (**2**—**5**) were obtained in the yields of 79.9 mg, 2.04 g, 1.96 g and 551.2 mg, respectively, from the fractions eluted with increasing amounts of MeOH in $CHCl_3$ after purification by successive column chromatography on silica gel. Physico-chemical properties and spectral data for Bl-I through Bl-V (**1**—**5**) are summarized in Tables I, II and III.

Acetylation of Bl-II through Bl-V (2—5) A solution of Bl-II (**2**, 13.0 mg) in pyridine (0.3 ml) was treated with Ac_2O (0.2 ml). After standing overnight at room temperature, the reaction mixture was poured into ice water (5 ml), then extracted twice with EtOAc (10 ml). The combined EtOAc layer was subsequently washed with 0.5 N HCl (5 ml), saturated aqueous $NaHCO_3$ (5 ml), water (10 ml) and saturated aqueous NaCl (10 ml), and then dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was chromatographed on silica gel to give the acetate (6.9 mg) as colorless needles, the data for which were identical with those of Bl-I (**1**). The acetates of Bl-III through Bl-V (**3**—**5**) were obtained similarly, and were also identical with Bl-I (**1**).

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