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The Structures of Pyridovericin and Pyridomacrolidin, New Metabolites from the Entomopathogenic Fungus, *Beauveria bassiana*

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Entomopathogenic fungi seem to be interesting as a source of biologically active compounds such as an insecticides[1)] and immunosuppressants[2,3)], because all insect orders are susceptible to fungal diseases. With the goal of finding novel bioactive compounds from fungi, we investigated metabolite diversity using an HPLC screening program. During the screening process, we isolated two novel metabolites named pyridovericin (**1**) and pyridomacrolidin (**2**) (Fig. 1) from the mycelial cake of the entomopathogenic fungus, *Beauveria bassiana* EPF-5[4)]. In this paper, we describe the physico-chemical properties and structural determination of **1** and **2**.

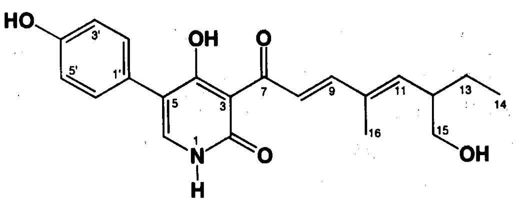
Structure of Pyridovericin (1)

The physico-chemical properties of **1** and **2** are summarized in Table 1. The molecular formula of **1** was determined to be $C_{21}H_{23}NO_5$ [Found *m/z* 369.1580 (M^+), Calcd for $C_{21}H_{23}NO_5$ 369.1576] by high-resolution EI-MS measurement. The IR spectrum (KBr) suggested the presence of the hydroxy group (3403 cm^{-1}), amide carbonyl (1661 cm^{-1}) and hydrogen bonded carbonyl (1611 cm^{-1}), respectively.

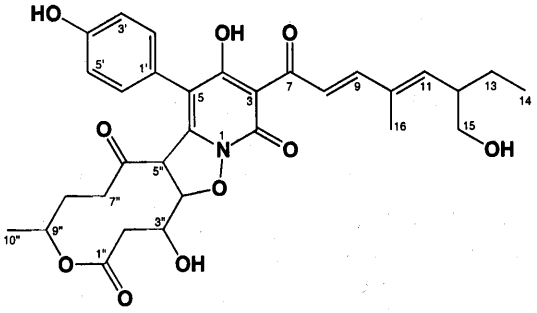
The ^{13}C NMR spectrum of **1** accounts for the presence of 21 carbon signals. The analyses of ^1H NMR and DEPT spectra indicated the presence of two methyls (δ_C 11.6 and 12.7), one methylene (δ_C 24.0), one oxygenated methylene (δ_C 64.0), one methine (δ_C 43.5), eight olefinic methines (δ_C 114.9 × 2, 123.1, 130.0 × 2, 140.5, 147.4 and 149.3) and eight quaternary carbons including six olefinic carbons (δ_C 105.9, 112.7, 123.4, 134.5, 156.7 and 176.9) and two carbonyl carbons (δ_C 161.7 and 193.7). The ^1H NMR spectrum showed 23 proton signals including two methyls (δ_H 0.82, t and δ_H 1.85, s), one methylene (δ_H 1.22, m and δ_H 1.59, m), one oxygenated methylene (δ_H 3.37, 2H, m), one methine (δ_H 2.53, m), three olefinic protons (δ_H 5.95, d, δ_H 7.51, d and δ_H 8.00, d), one aromatic proton (δ_H 7.54, s) and *p*-disubstituted benzene protons (δ_H 6.78, 2H, d and δ_H 7.27, 2H, d). Furthermore, the existence of four exchangeable protons: one aliphatic hydroxy proton (δ_H 4.56, t), one phenolic hydroxy proton (δ_H 9.45, s), one NH proton (δ_H 11.58, br.s) and one hydrogen bonded phenolic hydroxy proton (δ_H 17.54, br.s) was demonstrated. The chemical shift data of ^1H and ^{13}C NMR (DMSO-d_6) of **1** are shown in Table 2.

The ^1H-^1H COSY, ^1H-^{13}C COSY and HMBC spectra of **1** revealed the presence of two partial structures A and B, a 4-hydroxy-5-(*p*-hydroxyphenyl) pyridone moiety and a 4-methyl-6-hydroxymethyl-octa-2,4-dienoyl moiety, respectively, as shown in Fig. 2. The presence of the *p*-hydroxyphenyl moiety deduced from the appearance of proton signals of typical AA′BB′ multiplets and two carbon signals arising from the chemically equivalent pairs of carbons 2′-C, 6′-C and 3′-C, 5′-C was evidenced by the correlations from 4′-OH (δ_H 9.45) to 4′-C (δ_C 156.7) and 3′ (or 5′)-C (δ_C 114.9), from 3′ (or 5′)-H (δ_H 6.78) to 4′-C, and from 2′ (or 6′)-H

Fig. 1. Structures of pyridovericin (**1**) and pyridomacrolidin (**2**).



Pyridovericin (**1**)



Pyridomacrolidin (**2**)

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Table 1. Physico-chemical properties of pyridovericin (**1**) and pyridomacrolidin (**2**).

	Pyridovericin (**1**)	Pyridomacrolidin (**2**)
Appearance	Pale yellow powder	Pale yellow powder
MP	203 ~ 206 °C (dec.)	192 ~ 194 °C (dec.)
$[\alpha]_D^{15}$ (*c* 0.1, MeOH)	-20.3°	+19.4°
Molecular formula	$C_{21}H_{23}NO_5$	$C_{31}H_{35}NO_{10}$
Molecular weight	369	581
FAB-MS (*m/z*)	370 $(M+H)^+$ 368 $(M-H)^-$	582 $(M+H)^+$ 580 $(M-H)^-$
HR-MS (*m/z*)	EI	FAB
Found:	369.1580 $(M)^+$	582.2329 $(M+H)^+$
Calcd:	369.1576 for $C_{21}H_{23}NO_5$	582.2339 for $C_{31}H_{36}NO_{10}$
UV λ_{max}^{MeOH} nm (ε)	248 (16,400), 336 (15,900)	238 (14,500), 342 (14,600)
IR ν_{max}^{KBr} (cm^{-1})	3403, 2963, 2934, 1661, 1611, 1578 1522, 1474, 1462, 1325, 1258, 1223 1034, 992	3399, 2961, 2930, 1723, 1643, 1613 1518, 1441, 1433, 1400, 1343, 1267 1227, 1173, 1092, 1038, 984
TLC (Rf value)[a]	0.34	0.44
Solubility Soluble:	MeOH, EtOAc, DMSO, acetone	MeOH, EtOAc, DMSO, acetone, $CHCl_3$
Insoluble:	H_2O, n-hexane	H_2O, n-hexane

[a] Silica gel TLC (Merck Art. 5715): $CHCl_3$ - MeOH (8 : 1).

(δ_H 7.27) to 4'-C and 5-C (δ_C 112.7). The location at 4-OH (δ_H 17.54) was assigned from the correlation to three carbons, 4-C (δ_C 176.9), 3-C (δ_C 105.9) and 5-C (δ_C 112.7). Furthermore, the appearance of correlations of a singlet olefinic proton 6-H (δ_H 7.54) to an amide carbonyl 2-C (δ_C 161.7), 4-C, 5-C and 1'-C (δ_C 123.4) indicated the presence of a pyridone ring in partial structure A. The linkage of *p*-hydroxyphenyl group to the 5-C position of pyridone moiety was established by correlations from 2' (or 6')-H to 5-C and from 6-H to 1'-C, as shown in Fig. 2. The ^{1}H-^{1}H COSY spectrum of **1** revealed two proton sequences [$-^8CH=^9CH-$ and $=^{11}CH-^{12}CH(^{15}CH_2OH)-^{13}CH_2-^{14}CH_3$] in partial structure B. In the HMBC spectrum, a methyl proton 16-CH_3 (δ_H 1.85) showed correlations with three olefinic carbons 9-C (δ_C 149.3), 10-C (δ_C 134.5) and 11-C (δ_C 147.4). Both olefinic protons, 8-H (δ_H 8.00) and 9-H (δ_H 7.51) were correlated to a carbonyl carbon 7-C (δ_C 193.7). The terminal olefinic methine proton 11-H (δ_H 5.95) was correlated to an olefinic carbons 10-C and 9-C. The configuration of the two double bonds (8-C to 9-C and 10-C to 11-C) were elucidated as *E* from its coupling constant ($J_{8,9}=15.6$ Hz) and observation of NOE between 9-H and 11-H, and 8-H and 16-CH_3, respectively. These data deduced (2*E*,4*E*)-4-methyl-6-hydroxymethyl-octa-2,4-dienoyl moiety for the partial structure B. The connectivity of the carbonyl carbon 7-C to 3-C on a pyridone ring was deduced from the fact that a phenolic 4-OH (δ_H 17.54) should be hydrogen bonded to the carbonyl oxygen (IR; 1611 cm^{-1}). Thus, the planner structure of **1** was determined to be a 3-[(2*E*,4*E*)-4-methyl-6-hydroxymethyl-octa-2,4-dienoyl]-4-hydroxy-5-(*p*-hydroxyphenyl)-2(1*H*) pyridone, as shown in Fig. 1.

Structure of Pyridomacrolidin (2)

The molecular formula of **2** was established to be $C_{31}H_{35}NO_{10}$ by high-resolution FAB-MS [Found *m/z* 582.2329 $(M+H)^+$, calcd for $C_{31}H_{36}NO_{10}$ 582.2339]. The UV spectrum of **2** in MeOH exhibited an absorption maxima at 238 nm (ε 14,500) and 342 nm (ε 14,600), suggesting that the compound contained the same chromophore with **1**. The IR spectrum of **2** showed a newly appeared absorption of ester carbonyl (1723 cm^{-1}) in addition to a hydroxy group (3399 cm^{-1}), amide carbonyl (1643 cm^{-1}) and hydrogen bonded carbonyl (1613 cm^{-1}).

Comparative studies of ^{1}H and ^{13}C NMR spectral data (see Table 2) of **1** and **2** indicated that **2** possesses the same structure as **1**, except for the signals of an amide proton and olefinic proton at 6-C observed in **1**, as shown in Fig. 3. The *E*-configuration of two double bonds, 8-C

Table 2. ^{1}H and ^{13}C NMR chemical shift assignments of pyridovericin (**1**) and pyridomacrolidin (**2**)[a].

	^{13}C (δ)		^{1}H (δ)	
Position	**1**	**2**	**1**	**2**
NH-1			11.58 (1H, br.s)[b]	
C-2	161.7	153.1		
C-3	105.9	105.6		
C-4	176.9	173.7		
C-4-OH			17.54 (1H, br.s)	16.71 (1H, s)
C-5	112.7	106.5		
C-6	140.5	145.0	7.54 (1H, s)	
C-7	193.7	193.1		
C-8	123.1	122.6	8.00 (1H, d, J=15.6 Hz)	7.90 (1H, d, J=15.1 Hz)
C-9	149.3	149.9	7.51 (1H, d, J=15.6 Hz)	7.53 (1H, d, J=15.1 Hz)
C-10	134.5	134.4		
C-11	147.4	148.1	5.95 (1H, d, J=9.8 Hz)	5.98 (1H, d, J=10.3 Hz)
C-12	43.5	43.6	2.53 (1H, m)	2.54 (1H, m)
C-13	24.0	23.9	1.22 (1H, m)	1.24 (1H, m)
			1.59 (1H, m)	1.58 (1H, m)
C-14	11.6	11.6	0.82 (3H, t, J=7.6 Hz)	0.83 (3H, t, J=7.6 Hz)
C-15	64.0	64.0	3.37 (2H, m)	3.37 (2H, m)
C-15-OH			4.56 (1H, t, J=5.4 Hz)	4.56 (1H, t, J=5.4 Hz)
C-16	12.7	12.7	1.85 (3H, s)	1.86 (3H, s)
C-1'	123.4	121.5		
C-2',6'	130.0	131.6	7.27 (2H, d, J=8.3 Hz)	7.09 (2H, d, J=8.8 Hz)
C-3',5'	114.9	115.7	6.78 (2H, d, J=8.3 Hz)	6.88 (2H, d, J=8.8 Hz)
C-4'	156.7	157.7		
C-4'-OH			9.45 (1H, s)	9.72 (1H, s)
C-1"		168.4		
C-2"		40.7		2.47 (1H, dd, J=13.2, 5.4 Hz)
				2.75 (1H, dd, J=13.2, 2.2 Hz)
C-3"		67.5		4.29 (1H, m)
C-3"-OH				6.06 (1H, d, J=4.4 Hz)
C-4"		87.8		5.03 (1H, d, J=5.9 Hz)
C-5"		54.7		5.23 (1H, d, J=5.9 Hz)
C-6"		207.4		
C-7"		39.8		1.36 (1H, m), 1.58 (1H, m)
C-8"		32.8		1.58 (1H, m), 1.77 (1H, m)
C-9"		71.4		4.71 (1H, m)
C-10"		18.5		1.03 (3H, d, J=5.9 Hz)

[a] ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) were measured in DMSO-d_6 at 30°C.
[b] Proton number, multiplicity and coupling constants in Hz are indicated in parentheses.

and 10-C were evidenced by NOE data (9-H/11-H and 8-H/16-CH3), and a coupling constant ($J_{8,9}=15.1$ Hz). The remaining partial structure C ($C_{10}H_{14}O_5$) in **2** was clarified from HMBC experiments among three proton sequences [-$^{2''}$CH$_2$-$^{3''}$CH(OH)-, -O-$^{4''}$CH-$^{5''}$CH- and -$^{7''}$CH$_2$-$^{8''}$CH$_2$-$^{9''}$CH($^{10''}$CH$_3$)-O-] assigned from the ^{1}H-^{1}H COSY spectrum. Observation of the correlations from 2″-H$_2$ (δ_H 2.47 and 2.75) to 1″-C (δ_C 168.4), from 3″-H (δ_H 4.29) to 4″-C (δ_C 87.8), from 3″-OH (δ_H 6.06) to 4″-C, from 4″-H (δ_H 5.03) to 2″-C (δ_C 40.7), 3″-C (δ_C 67.5) and 6″-C (δ_C 207.4), from 5″-H (δ_H 5.23) to 3″-C and 6″-C, from 7″-H$_2$ (δ_H 1.36 and 1.58) to 6″-C, from 8″-H$_2$ (δ_H 1.58 and 1.77) to 6″-C, and from 9″-H (δ_H 4.71) to 1″-C enabled the deduction of a 10-membered macrocyclic structure for C, as shown in Fig. 3. Although the oxygenated methine 4″-H (δ_H 5.03, $J_{4'',5''}=5.9$ Hz) showed no correlation with 3″-H (δ_H 4.29), the observation of NOE between 3″-H and 4″-H suggests that the dihedral angle for both protons should be *ca.* 90°. The connectivity between partial structures A and C in **2** was determined from the following HMBC data. Appearance of the correlations from 5″-H to 6-C (δ_C

Fig. 2. ^{1}H-^{1}H COSY and HMBC experiments of pyridovericin (**1**).

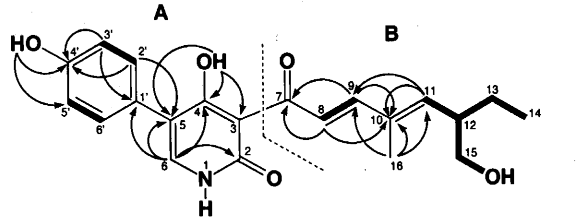
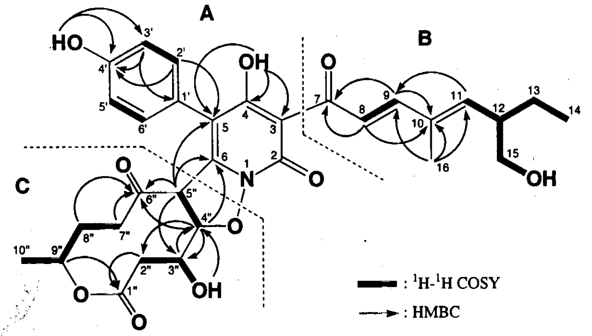


Fig. 3. ^{1}H-^{1}H COSY and HMBC experiments of pyridomacrolidin (**2**).



145.0) and 5-C (δ_C 106.5), and from 4″-H to 6-C indicated clearly that 5″-C should be connected to 6-C in the pyridone ring. Taking into consideration the above-described HMBC data and degree of unsaturation, an oxygenated carbon 4″-C (δ_C 87.8) should be connected to an amide nitrogen atom on the pyridone ring *via* an oxygen atom. Thus, compound **2** possesses an unique structure by which a 10-membered macrocyclic lactone skeleton was linked to a pyridone ring of **1**.

Although the *p*-hydroxyphenyl pyridone related compounds, tenellin, bassianin and ilicicolin H, have been reported[5,6] from entomopathogenic fungi, *Beauveria tenella*, *Beauveria bassiana* and *Cylindrocladium ilicicola*, respectively, this is the first report of isolation of a novel pyridone compound with a 10-membered macrocyclic lactone.

Experimental

^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were measured on a JEOL JNM EX-400 spectrometer at 30°C in DMSO-d_6. FAB-MS spectrum was measured on a JEOL AX-505W mass spectrometer with a matrix of glycerol, 3-nitrobenzyl alcohol and polyethylenglycol. EI-MS spectrum was measured on a JEOL JMS-DX300 mass spectrometer. Infrared spectra (KBr) and Ultraviolet spectra were taken on a Shimadzu FT IR-4200 spectrophotometer and Shimadzu UV-3100 spectrophotometer, respectively. Optical rotation was measured in MeOH on a JASCO DIP-370 digital polarimeter.

References

1) Roberts, D. W.: Toxins of entomopathogenic fungi. *In* Microbial Control of Pests and Plant Diseases 1970~1980. *Ed.*, H. D. Burges, pp. 441~464, Academic Press, London, New York, 1981

2) Fujita, T.; K. Inoue, S. Yamamoto, T. Ikumoto, S. Sasaki, R. Toyama, K. Chiba, Y. Hoshino & T. Okumoto: Fungal metabolites. Part 11. A potent immunosuppressive activity found in *Isaria sinclairii* metabolite. J. Antibiotics 47: 208~215, 1994

3) Iijima, M.; T. Masuda, H. Nakamura, H. Naganawa, S. Kurasawa, Y. Okami, M. Ishizuka, T. Takeuchi & Y. Iitaka: Metacytofilin, a novel immunomodulator produced by *Metarhizium* sp. TA2759. J. Antibiotics 45: 1553~1556, 1992

4) Takahashi, S.; N. Kakinuma, K. Uchida, R. Hashimoto, T. Yanagisawa & A. Nakagawa: Pyridovericin and pyridomacrolidin: novel metabolites from entomopathogenic fungi, *Beauveria bassiana*. J. Antibiotics 51: 596~598, 1998

5) Wat, C. K.; A. G. McInnes, D. G. Smith, J. L. C. Wright & L. C. Vining: The yellow pigments of *Beauveria* species. Structures of tenellin and bassianin. Can. J. Chem. 55: 4090~4098, 1977

6) Matsumoto, M. & H. Minato: Structure of ilicicolin H, an antifungal antibiotic. Tetrahedron Lett.: 3827~3830, 1976