STUDIES ON THE METABOLITES OF *PENICILLIUM VULPINUM*

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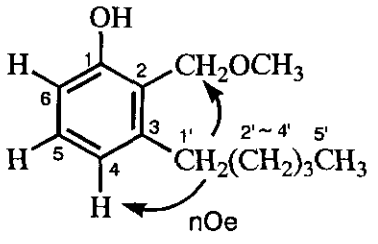
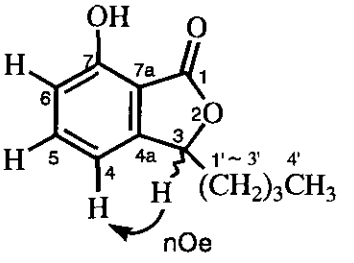
Abstract —— Two new metabolites, 2-methoxymethyl-3-pentylphenol (**1**) and 3-butyl-7-hydroxyphthalide (**2**), were isolated from the culture broth of *Penicillium vulpinum* along with four known phenols, gentisyl alcohol (**3**), 2-methylhydroquinone (**4**), 3-hydroxybenzyl alcohol (**5**), 6-methylsalicylic acid (**6**), and epoformin (**7**). The structures of new metabolites were determined by analyses of their MS and NMR spectra. It was found that the cytotoxic activity of the culture broth of *P. vulpinum* was mainly due to epoformin (**7**) and 2-methylhydroquinone (**4**).

In the course of our studies on the isolation of biologically active substances from fungi metabolites, we found that the culture broth of *Penicillium vulpinum* [1] exhibited strong cytotoxic activity against leukemia cells (L1210) in tissue culture. In this paper, we would like to report the isolation and cytotoxic activities of six phenols including two new compounds (**1** and **2**) and epoformin (**7**)[2] from the culture broth.

Compound (**1**) was obtained as an oil. Its MS spectrum showed the molecular ion peak at *m/z* 208 corresponding to the molecular formula $C_{13}H_{20}O_2$. The ^1H NMR spectrum of **1** indicated the presence of n-pentyl group [0.90 (3H, t, J = 6.6 Hz, CH_3), 1.28 - 1.38 (4H, br m, H-3' and H-4'), 1.44 - 1.56 (2H, br m, H-2'), 2.55 (2H, t, J = 7.3 Hz, H-1')], a methoxymethyl group [3.46 (3H, s, OCH_3), 4.74 (2H, s, OCH_2)], 1,2,3-trisubstituted benzene ring [6.69 (1H, d, J = 8.1 Hz, H-6), 6.74 (1H, d, J = 8.1 Hz, H-4), 7.11 (1H, t, J = 8.1 Hz, H-5)] and a phenolic hydroxyl group [7.87 (1H, s)].



(1)



(2)

The ^{13}C NMR spectrum also suggested the presence of these functional groups. (see Table 1)
The substitution pattern on the benzene ring was confirmed by the nOe experiment on **1**. Irradiation at δ 2.55 (H-1') produced significant enhancement of oxymethylene proton (4.74) and aromatic proton (H-4) signals. Thus, the structure of **1** was determined to be 2-methoxymethyl-3-pentylphenol.
Compound (**2**) was obtained as colorless crystals. mp 49-50°, $[\alpha]_D$ -45.5° (c = 0.8, $CHCl_3$). Its HRMS spectrum exhibited the molecular ion peak at *m/z* 206.0986 corresponding to the molecular formula $C_{12}H_{14}O_3$ (calcd 206.0943). The ^1H NMR spectrum of **2** showed the presence of n-butyl group [0.92 (3H, t, *J* = 6.6 Hz, CH_3), 1.29 - 1.53 (4H, br m, H-2' and H-3'), 1.73 - 1.84 and 1.98 - 2.18 (1H each, br m, H-1')], an oxymethine group [5.49 (1H, dd, *J* = 4.4, 8.1 Hz, H-3)], 1,2,3-trisubstituted benzene ring [6.90 (1H, d, *J* = 8.1 Hz, H-6), 6.92 (1H, d, *J* = 8.1 Hz, H-4), 7.54 (1H, t, *J* = 8.1 Hz, H-5)] and a phenolic hydroxyl group [7.80 (1H, s)]. The ^1H NMR decoupling experiment on **2** indicated that the n-butyl group should be attached to the oxymethine group. The ^{13}C NMR and the IR spectra of **2** suggested the presence of γ-lactone ring [172.2 (C-1), 82.9 (C-3), IR (KBr): 1727 cm^{-1}] conjugated with the benzene ring. Finally, the position of hydroxyl group could be assigned at C-7 by the result of nOe experiments on the methyl ether of **2**. Irradiation of H-3 signal at δ 5.38 produced significant enhancement of H-4 signal (6.94) while irradiation of methoxyl proton signal at δ 3.99 produced the enhancement of H-6 signal (6.91). Thus, the structure of **2** was determined to be 3-butyl-7-hydroxyphthalide.
The other compounds isolated from the culture broth of *P. vulpinum* were assigned to be gentisyl alcohol (**3**), 2-methylhydroquinone (**4**), 3-hydroxybenzyl alcohol (**5**), 6-methylsalicylic acid (**6**) and

Table 1 ^1H and ^{13}C NMR data for 1 and 2

position	^1H NMR (400 MHz, δ, $CDCl_3$)		^{13}C NMR (100 MHz, δ, $CDCl_3$)	
	(1)	(2)	(1)	(2)
1			157.0	172.2
2			119.7	
3		5.49 dd (4.4, 8.1)	141.3	82.9
4	6.74 d (8.1)	6.92 d (8.1)	121.2	115.3
4a				150.4
5	7.11 t (8.1)	7.54 t (8.1)	128.9	136.8
6	6.69 d (8.1)	6.90 d (8.1)	114.5	113.0
7				156.6
7a				111.2
1'	2.55 t (7.3)	1.73 - 1.84 br m (1H) 1.98 - 2.18 br m (1H)	33.2	34.3
2'	1.44 - 1.56 br m	1.29 - 1.53 br m	31.7	26.8
3'	1.28 - 1.38 br m		22.5	22.4
4'		0.92 t (6.6)	31.1	13.8
5'	0.90 t (6.6)		14.0	
CH_2O	4.74 s		70.1	
CH_3O	3.46 s		58.2	
OH	7.87 s	7.87 s		

epoformin (**7**), respectively, from their spectral data. As shown in Figure 1, compounds (**3**), (**4**) and epoformin (**7**) exhibited relatively strong cytotoxic activities against leukemia cells (L 1210) in tissue culture compared with the other compounds (**1**), (**2**), (**5**) and (**6**). Thus, the cytotoxic activity of the culture broth of *P. vulpinum* was mainly ascribed to epoformin (**7**) and **4**.

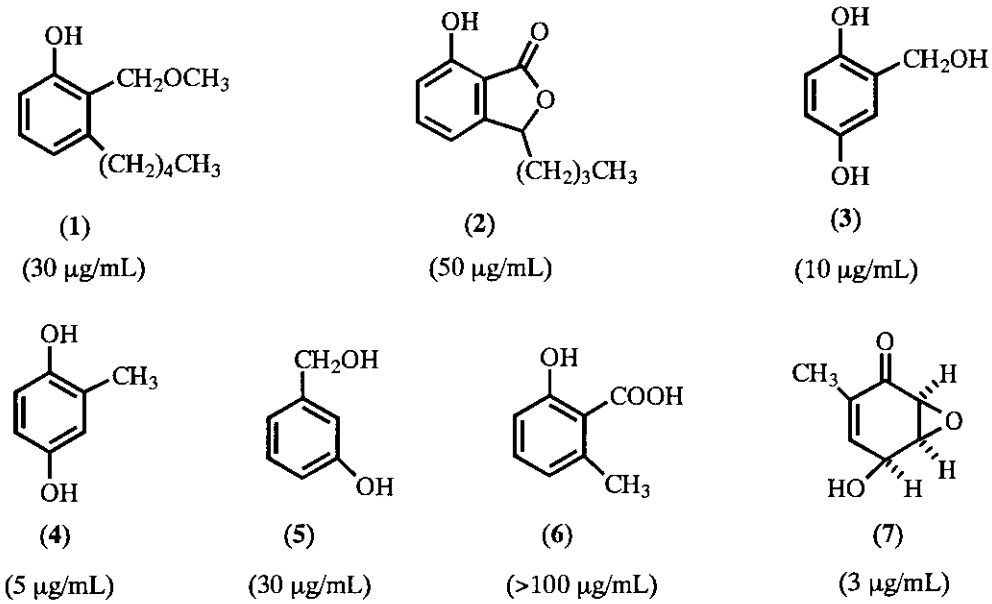


Figure 1 Growth inhibitory effect (IC_{50}) of the metabolites of *P. vulpinum* against leukemia cells (L 1210)

EXPERIMENTAL

The ^1H and ^{13}C NMR spectra were measured on a JEOL GSX-400 spectrometer in $CDCl_3$ containing TMS as an internal standard. The MS spectra were recorded on a Hitachi RMU-6M instrument. Optical rotation was measured on a JASCO DIP-370 polarimeter.

Isolation of Compounds (1-7)

Penicillium vulpinum was grown in a potato-dextrose medium (10 L) under shaking at 24°C for 3 days and the culture broth was centrifuged and then filtered to remove mycelia. The filtrate was fractionated on a HP-20 (Nippon Rensui®) column eluted successively with H_2O, 40% MeOH, 70% MeOH, MeOH and acetone. The 40% MeOH fr. (6.0 g) was subjected to silica gel column chromatography eluted successively with hexane - EtOAc (4 : 1, 3 : 1, 2 : 1, 1 : 1 and 1 : 2) and EtOAc. The hexane - EtOAc (1 : 2) fr. (20 mg) was further purified by HPLC [Senshu pak silica, hexane - EtOAc (1 : 2)] to give **3** (5 mg). The 70% MeOH fr. (0.97 g) was chromatographed on a silica gel column eluted with stepwise gradients of hexane - EtOAc (7 : 1, 5 : 1, 3 : 1, 1 : 1, 1 : 2 and EtOAc). The hexane - EtOAc (1 : 1) fr. (102 mg) was recrystallized from MeOH to give **4** (12 mg) and then the mother liquor was concentrated and purified by

HPLC [Shodex SIL-5E, hexane - EtOAc (2 : 1) to give **7** (5 mg) and **5** (38 mg). The MeOH fr. (0.66 g) was chromatographed on a silica gel column eluted with stepwise gradients of hexane - EtOAc (20 : 1, 10 : 1, 7 : 1, 5 : 1, 3 : 1, 1 : 1, 1 : 2). The hexane - EtOAc (10 : 1) fr. (36 mg) was further purified by reverse phase HPLC (Senshu pak ODS, 80% MeOH) to give **1** (3 mg) and **2** (16 mg). The hexane - EtOAc (5 : 1) fr. (86 mg) was purified by HPLC [Shodex SIL-5E, hexane - EtOAc (5 : 1) to give **6** (15 mg).

Assay of Cytotoxicity

The effects of isolated compounds on the growth of L1210 cells were investigated as follows. Cells were suspended in RPMI-1640 medium (NISSUI) containing 1% glutamine and 15% calf serum. Aliquots (2 mL) of cell suspension (approximately 10 x 10^4 cells/mL) were transferred into vials. After addition of a compound to be tested at the concentration indicated, the cells were incubated under an atmosphere containing 5% CO_2 at 37°C for 3 days and then viable cell number were counted. The cytotoxicity was decided by comparing the viable cell number with that of the control.

ACKNOWLEDGMENT

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REFERENCE AND NOTE

1. The culture for this experiment is preserved in the Laboratory of Biology, College of Pharmacy, Nihon University, Narashinodai, Funabashi, Chiba 274-8555, Japan
2. I. Yamamoto, E. Mizuta, T. Henmi, T. Yamamoto, and S. Yamatodani, *Chem. Abstr.*, 1974, **80**, 106812h.

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