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New Brominated and Halogen-less Derivatives and Structure-activity Relationship of Azaphilones Inhibiting gp120-CD4 Binding

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Novel brominated and halogen-less azaphilone (oxoisochromane) derivatives, 5-bromoochrephilone and dechloroisochromophilone IV, and known derivatives, dechloroisochromophilone III and isorotiorin, were isolated from the culture broth of a producing organism of isochromophilones I and II (azaphilones inhibiting gp120-CD4 binding), *Penicillium multicolor* FO-2338, fermented in a medium containing potassium bromide. Nineteen azaphilone-related compounds isolated from the above strain and from other fungi were tested for the inhibition of gp120-CD4 binding and the structure-activity relationship is discussed. Consequently, 5-bromoochrephilone is the strongest inhibitor (IC_{50}, 2.5 μM). A halogen atom at C-5, a proton at C-8 and a diene structure in C-3 side chain of 6-oxoisochromane ring are necessary for gp120-CD4 binding.

Fungal metabolites having an oxoisochromane ring system, which are called "azaphilones" after high affinity to ammonia[1], have been found to possess various bioactivities. In the course of screening of microbial metabolites for the inhibition of gp120-CD4 binding, we discovered the inhibitors isochromophilones I (**1**) and II (**2**) having a 6-oxoisochromane ring, which were isolated from a culture broth of *Penicillium multicolor* FO-2338[2~4] together with the known azaphilones ochrephilone (**3**)[5], sclerotiorin (**4**)[6] and rubrorotiorin (**5**)[6]. **1**, 5-chloro derivative of ochrephilone, exhibited a strong inhibitory activity against gp120-CD4 binding but **3** showed no inhibition. These results suggested that a chlorine atom at C-5 of **1** was very important for the inhibition of gp120-CD4 binding. Thus, we attempted the preparation of brominated derivatives by a fermentative process. When the isochromophilone producer *P. multicolor* FO-2338 was fermented in a production medium containing 3.0% potassium bromide, a new brominated derivative and several other azaphilone-related compounds were produced in the culture broth. In this paper, we wish to report the isolation and the structures of a 5-bromo derivative and other azaphilone-related compounds, and the structure-activity relationship among azaphilone-related compounds including those isolated from other fungi for the inhibition of gp120-CD4 binding.

Fermentation and Isolation

The production of azaphilones by *P. multicolor* FO-2338 was carried out using Erlenmeyer flasks and a jar fermentor containing a production medium supplemented with 3.0% potassium bromide as described in the Experimental section.

The isolation procedures for azaphilone-related compounds are summarized in Fig. 1. **3**, **6**, **7** and **10** were isolated from the cultured broth obtained using Erlenmeyer flasks. **8** and **9** in addition to **3** and **10** were isolated from the cultured broth using a jar fermentor.

Structure Elucidation

6 was obtained as a yellow powder. The molecular formula was determined to be $C_{19}H_{26}O_4$ based on its HR-FAB-MS. The ^{1}H- and ^{13}C-NMR spectra indicated the presence of a 3,5-dimethyl-1,3-heptadiene moiety which is also contained in isochromophilones I (**1**) and II (**2**). In the ^{1}H-NMR, two olefin signals at δ 5.65 (s) and 5.72 (s), a methyl signal at δ 1.48 (s), oxymethylene

Fig. 1. Isolation procedures for azaphilones.

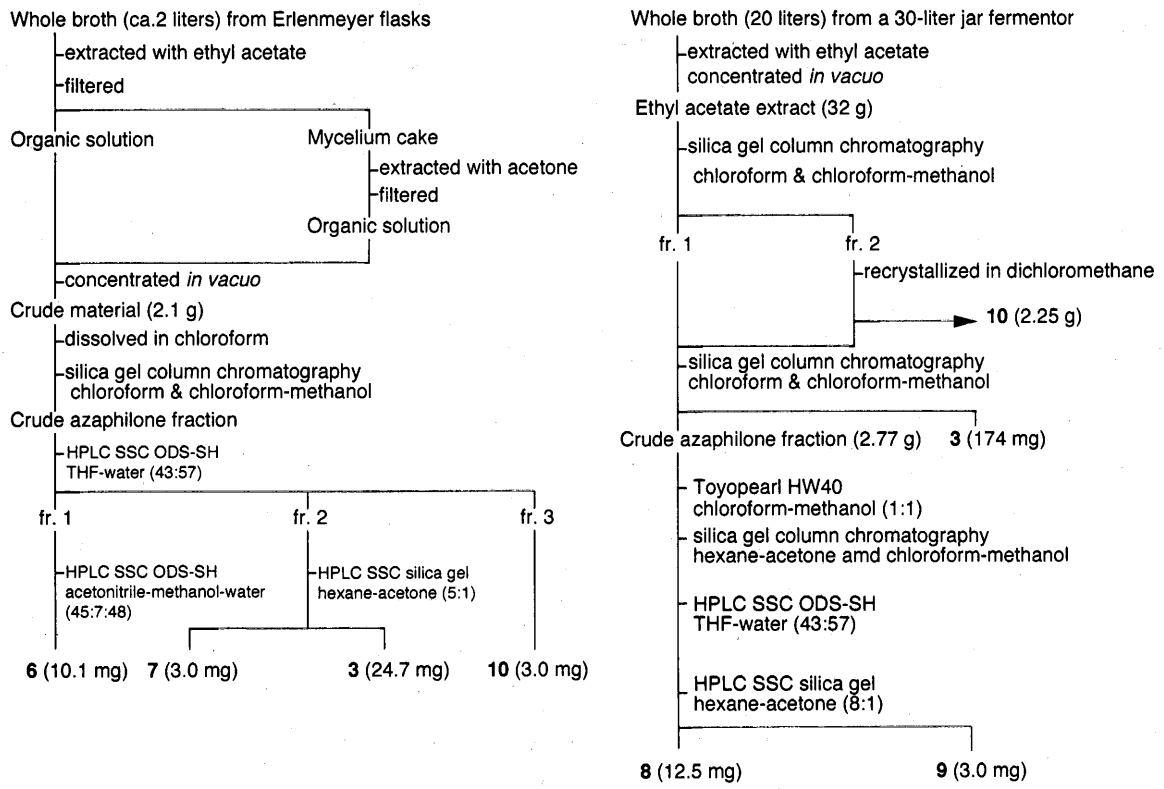
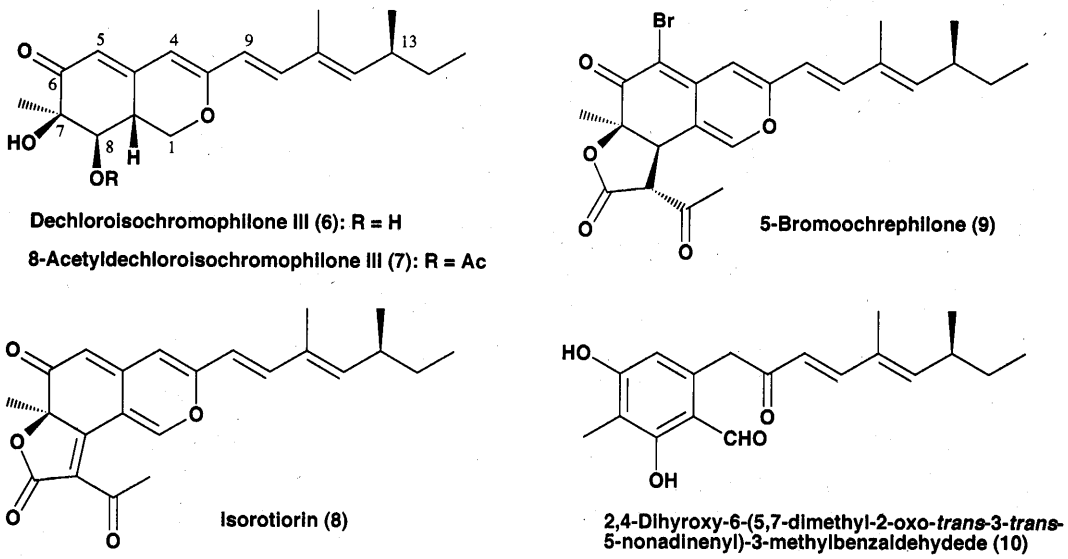


Fig. 2. The structures of azaphilones and related compounds obtained by fermentation in the presence of potassium bromide.



signals at δ 3.78 (dd) and 4.82 (dd) and methine signals at δ 3.00 (m) and 3.45 (d) were observed in addition to the signals of 3,5-dimethyl-1,3-heptadiene. From the above data and the 2D NMR study, **6** was identified to be dechloroisochromophilone III[7].

7 was also obtained as a yellow powder. The molecular formula was determined to be $C_{19}H_{26}O_4$ based on its HR-FAB-MS. The molecular weight was forty-two mass units larger than that of **6**. In the IR spectrum, the absorptions at 1740 and 1630 cm^{-1} indicated the presence of a carbonyl group and an α,β-unsaturated carbonyl group, respectively. The ^{1}H-NMR of **7** was

Table 1. ^{13}C- and ^{1}H-NMR spectral data of **6** and **7**.

Number	6 δC	6 δH	7 δH
1	69.2 t	3.78 1H dd *J*=13.5, 10.9Hz	3.80 1H dd *J*=13.5, 10.9Hz
		4.82 1H dd *J*=10.9, 5.6Hz	4.37 1H dd *J*=10.9, 5.1Hz
3	162.3 s		
4	104.6 d	5.65 1H s	5.67 1H s
4a	152.5 s		
5	115.7 d	5.72 1H d *J*=1.6Hz	5.77 1H d *J*=2.0Hz
6	196.8 s		
7	74.8 s		
8	74.6 d	3.45 1H d *J*=9.2Hz	5.00 1H d *J*=9.6Hz
8a	37.1 d	3.00 1H m	3.35 1H m
9	119.4 d	5.89 1H d *J*=15.5Hz	5.89 1H d *J*=15.5Hz
10	141.0 d	6.94 1H d *J*=15.5Hz	6.89 1H d *J*=15.5Hz
11	132.5 s		
12	146.6 d	5.57 1H d *J*=9.5Hz	5.55 1H d *J*=9.6Hz
13	35.3 d	2.24 1H m	2.45 1H m
14	30.6 t	1.33 2H m	1.33 2H m
15	12.3 q	0.82 3H t *J*=7.5Hz	0.84 3H t *J*=7.5Hz
7-CH_3	21.0 q	1.48 3H s	1.36 3H s
11-CH_3	12.8 q	1.78 3H d*J*=1.0Hz	1.78 3H d *J*=1.0Hz
13-CH_3	20.7 q	0.98 3H d *J*=6.9Hz	0.97 3H d *J*=6.7Hz
$COCH_3$			2.22 3H s

δ=ppm in $CDCl_3$.

Table 2. ^{13}C- and ^{1}H-NMR spectral data of **8** and **9**.

Number	8 δ_C	8 δ_H	9 δ_H
1	153.1 d	8.84 1H s	7.42 1H s
2	156.3 s		
4	108.2 d	6.16 1H s	6.56 1H s
4a	123.1 s		
5	105.3 d	6.34 1H s	
6	189.9 s		
7	87.5 s		
8	165.6 s		3.83 1H d *J*=12.0Hz
8a	110.7 s		
9	115.4 d	5.97 1H d *J*=15.8Hz	6.05 1H d *J*=15.8Hz
10	144.1 d	7.04 1H d *J*=15.8Hz	7.04 1H d *J*=15.8Hz
11	131.8 s		
12	148.3 d	5.67 1H d *J*=9.9Hz	5.68 1H d *J*=9.9Hz
13	35.5 d	2.48 1H m	2.45 1H m
14	30.0 t	1.35 2H m	1.35 2H m
15	11.9 q	0.85 3H t *J*=7.5Hz	0.85 3H t *J*=7.5Hz
7-CH_3	26.4 q	1.69 3H s	1.62 3H s
11-CH_3	12.4 q	1.81 3H s	1.83 3H s
13-CH_3	20.1 q	1.00 3H d *J*=6.7Hz	0.98 3H d *J*=8.6Hz
2'	168.3 s		
3'	142.3 s		3.80 1H d *J*=12.2Hz
4'	194.4 s		
5'	30.0 q	2.59 3H s	2.44 3H s

δ=ppm in $CDCl_3$.

very similar to that of **6** (Table 1). However, a methyl signal appeared at δ 2.22, and an oxymethylene signal (8-H) was shifted downfield to δ 5.00 by acetylation. From the above data, the structure of **7** was determined to be the new compound dechloroisochromophilone IV.

8 was obtained as a red powder. The molecular formula was determined to be $C_{23}H_{24}O_5$ by HR-FAB-MS, and the molecular weight (380) was two mass units less than that of **3**. The ^{1}H- and ^{13}C-NMR spectra of **8** were very similar to those of **3**[4]. However, two methine proton signals (8-, 3'-*H* in **3**) were not observed. In the ^{13}C-NMR spectrum, olefin carbon signals were observed at δ 110.7

and 142.3 instead of the two methine carbon signals of **3**. From these results, the structure of **8** was identified to be isorotiorin[8].

9 was obtained as a yellow powder. The molecular ion peaks at *m/z* 461 and 463 (1 : 1) in the EI-MS suggested that **9** possessed a bromine atom. The molecular formula of **9** was determined to be $C_{23}H_{25}O_5Br$ by HR-EI-MS. The UV and IR spectra of **9** were very similar to those of **1**[2,4].

The ^{1}H-NMR data were also closely similar to those of **8** (Table 2) and identical with those of **1**[4]. These data indicated that **9** has a 6-oxoisochromane skeleton as does **1** and that a bromine atom should be attached to C-5. From these results, the structure of **9** was determined to be the new compound 5-bromoochrephilone.

10 was obtained as colorless needles. The molecular formula was determined to be $C_{19}H_{24}O_4$ from HR-FAB-MS. In the IR spectrum, the absorptions at 1680 and 1625 cm^{-1} indicated the presence of two carbonyl groups. In the ^{1}H-NMR, the signals of four methyls, two methylenes, a methine, four olefin protons, one aldehyde proton and two phenolic protons were observed. The ^{1}H- and ^{13}C-NMR and ^{1}H-^{1}H COSY data revealed the presence of a 3,5-dimethyl-1,3-heptadiene residue. The remainder of the structure was established by HMBC correlations as shown in Fig. 3. The 3,5-dimethyl-1,3-heptadiene residue was attached to a ketone carbonyl group. From these data, the structure of **10** was identical with 2,4-dihyroxy-6-(5,7-dimethyl-2-oxo-*trans*-3-*trans*-5-nonadienyl)-3-methylbenzaldehyde[9].

Stereochemistry

The stereochemistry of **6**, **7** and **8** was examined by CD spectroscopy, coupling constants and NOE experiments. The CD curve (Fig. 4A) and ^3J values of 8-H/8a-H and 8a-H/1-Hα (9.2 and 10.9 Hz, respectively) of **7** were closely similar to those of **6**, indicating that both **6** and **7** have the same configuration. NOEs were observed between 8-H and 1-Hα, and 8a-H and 1-Hβ, respectively. These results indicated that the conformations between 8-H and 8a-H, and 8a-H and 1-Hα were diaxial.

The CD curve of **8** was similar to that of **4** (Fig. 4B), so the stereochemistry of **8** was 7*R* and 13*S*[10].

Inhibitory Activities of Azaphilones against gp120-CD4 Binding

Figure 5 shows the structures of azaphilones which were examined for the inhibition of gp120-CD4 binding

Fig. 3. HMBC correlations of **10**.

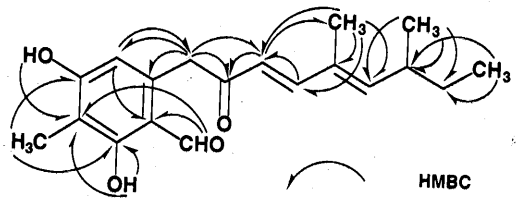


Fig. 4. CD spectra of **6** and **7** (A), and **8** and **4** (B).

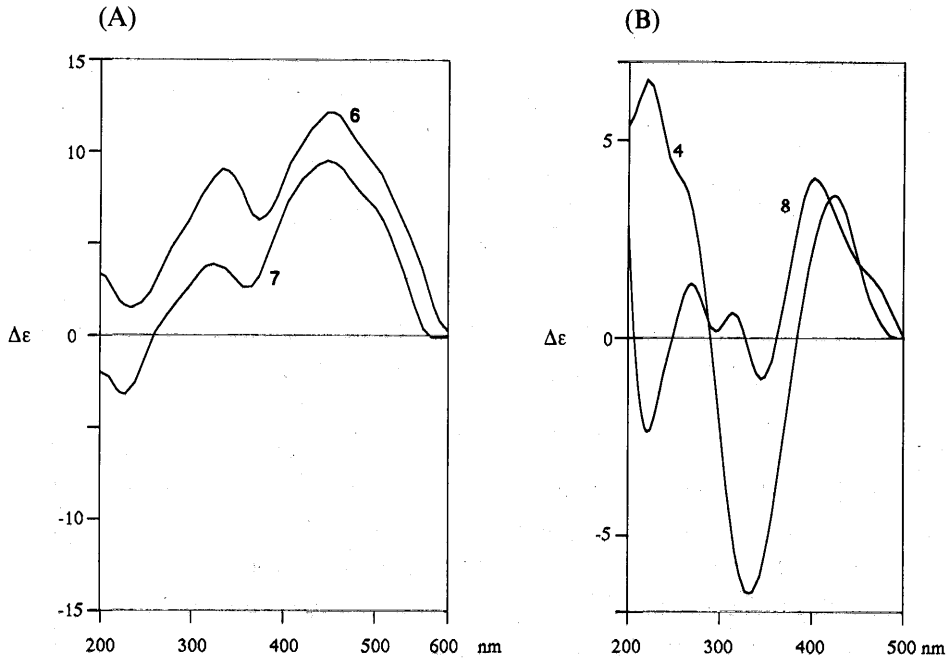
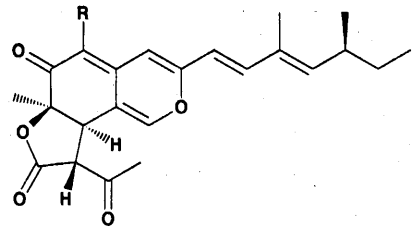
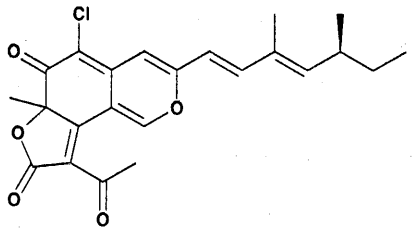


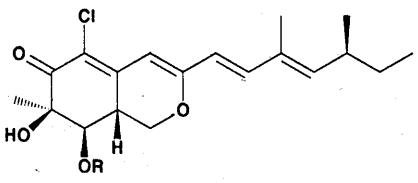
Fig. 5. The structures of azaphilones which were examined by gp120-CD4 binding assay.



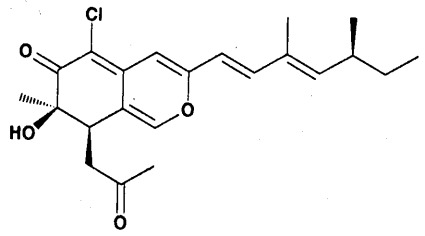
Isochromophilone I (1): R=Cl
Ochrephilone (3): R=H



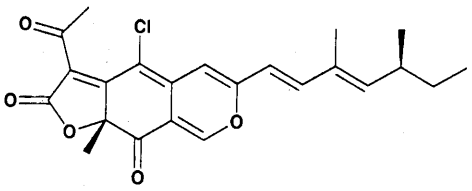
Rubrorotiorin (5)



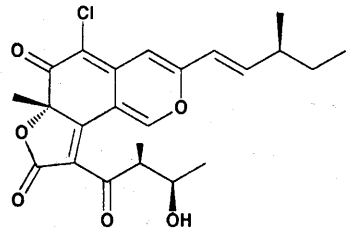
Isochromophilone III (14): R=H
Isochromophilone IV (15): R=Ac



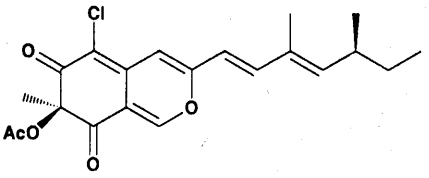
Isochromophilone II (2)



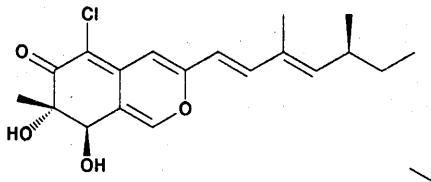
Rotiorin (11)



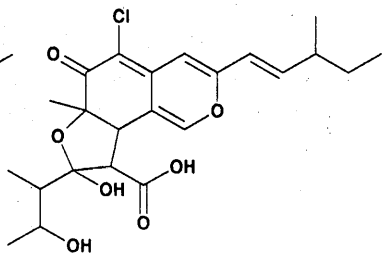
Chaetoviridin A (16)



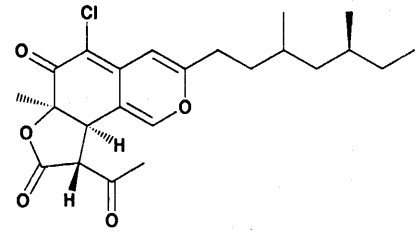
Sclerotiorin (4)



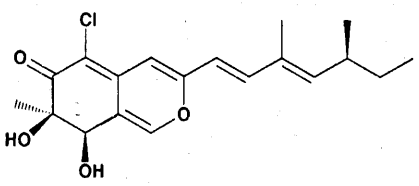
Luteusin A (12)



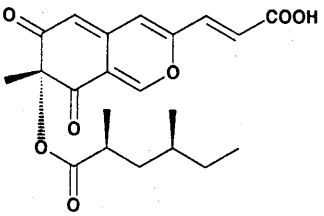
Chaetoviridin B (17)



Tetrahydroisochromophilone (19)



Isochromophilone V (13)



Lunatoic acid (18)

by the ELISA method. Table 3 shows the IC_{50} values of azaphilones against gp120-CD4 binding. Among them, the novel 5-bromo-derivative, **9**, showed the most potent inhibition with an IC_{50} value of 2.5 μM. A monoamine oxidase inhibitor, luteusin A (TL-1, **12**)[11] and acyl-CoA: cholesterol acyltransferase inhibitors, isochromophilones V (**13**), III (**14**) and IV (**15**)[12] which have a 5-chloro-8-hydroxy-6-oxoisochromane skeleton, weakly inhibited the binding, but 8-oxoderivatives, **4** and rotiorin (**11**), and dechloroderivatives, **3**, **6**, **7** and **8**, were inactive. **16**[1], **17**[1] and **18**[13] which possess a short side chain at C-3 in the isochromane skeleton, and tetrahydroiso-

Table 3. Inhibitory activities of azaphilones against gp120-CD4 binding.

Inhibitor	IC_{50} (μM)
Isochromophilone I (**1**)	6.6
Isochromophilone II (**2**)	3.9
Ochrephilone (**3**)	114
Sclerotiorin (**4**)	>250
Rubrorotiorin (**5**)	>240
Dechloroisochromophilone III (**6**)	>300
8-Acetyldechloroisochromophilone III (**7**)	>270
Isorotiorin (**8**)	>260
5-Bromoochrephilone (**9**)	2.5
Rotiorin (**11**)	>240
Luteusin A (**12**)	9.4
Isochromophilone V (**13**)	14.6
Isochromophilone III (**14**)	48
Isochromophilone IV (**15**)	96
Chaetoviridin A (**16**)	>230
Chaetoviridin B (**17**)	140
Lunatoic acid (**18**)	>260
Tetrahydroisochromophilone I (**19**)	>260

chromophilone I (**19**), were also inactive. These results indicate that the halogen atom at C-5 and the orientation from C-8 to C-1 in the isochromane ring of azaphilones in addition to the diene structure in C-3 side chain are very important for the inhibition of gp120-CD4 binding.

Discussion

The previous report that a chlorine atom at C-5 in isochromophilone I is necessary for gp120-CD4 binding induced an attempt to prepare 5-brominated derivatives[2]. The fermentation of the producing organism in the presence of potassium bromide provided 5-bromoochrephilone (**9**) in addition to the byproducts **6**, **7**, **8** and **10**. Among 18 azaphilone-related compounds tested for gp120-CD4 binding inhibition, **9** was the strongest inhibitor (IC_{50}: 2.5 μM). The above results also indicated that a halogen atom at C-5, a proton at C-8 and a diene structure in C-3 side chain of 6-oxoisochromane ring are necessary for inhibition of gp120-CD4 binding.

Azaphilones were well-known to react with amines and to exhibit various biological activities[7,12~17]. For example, **4** which is very reactive with amines, strongly inhibits phospholipase A2[17]. However, it did not inhibit gp120-CD4 binding as shown in Table 3. These facts suggest that the mechanisms of inhibition of gp120-CD4 binding is not related to the reactivity with amines of gp120 or CD4.

A novel dechloroazaphilone derivative, **7**, two known dechloroazaphilone derivatives, **6** and **8**, and an octaketide compound, **10**, were also isolated from the culture broth of the isochromophilone producer *P. multicolor* FO-2338 fermented in the presence of potassium bromide. **10** was reported as an intermediate in the total synthesis of **4**[9]. It is considered that **10** is also an intermediate of the biosynthesis of azaphilone compounds and that the addition of potassium bromide represses the biosynthesis from **10** to **4** to provide the above dechloroazaphilones (**6**, **7** and **8**), the intermediate **4**, and 5-bromoochrephilone (**9**) as possible shunt products.

Experimental

General Procedures

The UV spectra were recorded on a Beckman model DU640 spectrophotometer. IR spectra were recorded on a Horiba FT-210 diffraction infrared spectrometer. FAB-MS spectra were obtained with a JEOL model JMS-AX505 HA spectrometer. ^{1}H-NMR (270 MHz) and ^{13}C-NMR (67.5 MHz) spectra were obtained on a JEOL 270-EX spectrometer. Optical rotation and CD spectra were measured with a Jasco DIP-370 polarimeter and a Jasco DIP-370 polarimeter.

Assay for Inhibition of gp120-CD4 Binding

The inhibitory activities against gp120-CD4 binding were determined by enzyme-linked immunosorbent assay (ELISA) using recombinant soluble CD4 (sCD4) and recombinant gp120 as described previously[3].

Fermentation

The production of azaphilones was carried out using a flask and a jar fermentor, as follows. A loopful of mycelia from a slant culture of *P. multicolor* FO-2338 was transferred to each of twenty 500-ml Erlenmeyer flasks containing 100 ml of a production medium. The flasks were incubated at 27°C on a rotary shaker at 200 rpm for 4 days. On the other hand, with a jar fermentor, a loopful of mycelia from a slant culture of *P. multicolor* FO-2338 was transferred into a 500-ml Erlenmeyer flask containing 100 ml of a seed medium consisting of glucose 2.0%, yeast extract 0.2%, polypeptone 0.3%, $MgSO_4 \cdot 7H_2O$ 0.05%, KH_2PO_4 0.1% and agar 0.1% (adjusted to pH 5.8 before sterilization). The flask was incubated at 27°C on a rotary shaker at 200 rpm for 3 days to provide a seed culture. The seed culture (400 ml) was transferred to a 30-liter jar

fermentor containing 20 liters of a production medium. Fermentation was carried out at 27°C for 4 days. The production medium consisted of sucrose 2.0%, glucose 1.0%, corn steep powder 1.0%, meat extract 0.5%, yeast extract 0.5%, KH_2PO_4 0.1%, $CaCO_3$ 0.3%, KBr 3.0%, trace metal solution (containing in g/liter $FeSO_4 \cdot 7H_2O$ 1.0, $MnCl_2 \cdot 4H_2O$ 1.0, $ZnSO_4 \cdot 7H_2O$ 1.0, $CuSO_4 \cdot 5H_2O$ 1.0, $CoCl_2 \cdot 2H_2O$ 1.0) 1.0% and agar 0.3%.

Isolation

The isolation procedures are summarized in Fig. 1. Azaphilone and related compounds were monitored with their UV absorptions. The culture broths of Erlenmeyer flasks were combined and extracted with equal volume of ethyl acetate, and then filtered. The mycelium residue was reextracted with 1 liters of acetone. The organic solvent layers were combined and concentrated *in vacuo* to give crude material (2.1 g). The crude material was dissolved in chloroform and chromatographed on a silica gel column (4 × 33 cm) eluted with chloroform and chloroform - methanol (98 : 2, 95 : 5 and 90 : 10) to yield a crude azaphilone fraction. The fraction was subjected to HPLC (ODS-SH, 10 mm i.d. × 250 mm, Senshu Scientific Co., Ltd.; THF - water (43 : 57); flow-rate, 3.0 ml/minute detection UV at 330 nm), to give three fractions. The first fraction was purified with HPLC (ODS-SH, 10 mm i.d. × 250 mm; acetonitrile - methanol - water (45 : 7 : 48); flow-rate, 3.0 ml/minute; detection UV at 330 nm), to give **6** (10.1 mg). The second fraction was purified with HPLC (SSC silica gel, 10 mm i.d. × 250 mm, Senshu Scientific Co., Ltd.; *n*-hexane - acetone (5 : 1); flow-rate, 3.0 ml/minute detection UV at 330 nm), to give **7** (3.0 mg) and **3** (24.7 mg). The third fraction was crystallized with chloroform to give **10** (3.0 mg). On the other hand, 20 liters of cultured broth of *P. multicolor* FO-2338 in a 30-liter jar fermentor was extracted with 18 liters of ethyl acetate. The organic layer was concentrated *in vacuo* to give crude material (32 g). It was dissolved in chloroform and chromatographed on a silica gel column (7 × 20 cm) eluted with chloroform and chloroform - methanol (98 : 2) to yield fractions 1 (3.10 g) and 2 (8.57 g). Fraction 2 was recrystallized in dichloromethane to give **10** (2.25 g). The mother liquor and Fraction 1 were combined and rechromatographed on a silica gel column, eluted with dichloromethane - ethanol (98 : 2) to give crude azaphilone fraction (2.77 g) and **3** (174 mg). The crude azaphilone fraction was chromatographed on a Toyopearl HW-40 eluted with chloroform - methanol (1 : 1), a silica gel column (2.8 × 21 cm) eluted with chloroform - methanol (98 : 2). Finally, **8** (12.5 mg) and **9** (3.0 mg) were obtained after purification by HPLC (ODS-SH, 10 mm i.d. × 250 mm; THF - water (45 : 57); flow-rate, 3.0 ml/minute detection UV at 330 nm, and SSC silica gel, 10 mm i.d. × 250 mm; *n*-hexane - acetone (8 : 1); flow-rate, 3.0 ml/minute detection UV at 330 nm).

Dechloroisochromophilone III (**6**): Yellow powder; $[\alpha]_D^{23}$ −298° (*c* 0.1, EtOH), EI-MS, *m/z* 318 $(M)^+$; FAB-MS, *m/z* 319 $(M+H)^+$; HR-FAB-MS, found 319.1892, calcd. 319.1909 for $C_{19}H_{27}O_4$; Molecular formula, $C_{19}H_{26}O_4$; UV $\lambda_{max}^{CH_3OH}$ nm (ε), 265 (5,500), and 374 (20,700); IR ν_{max}^{KBr} cm^{-1}, 3430 (O–H), 2960 (C–H), 2925 (C–H), 1645 (C=O), and 1580 (C=C); ^{1}H- and ^{13}C-NMR ($CDCl_3$), see Table 1.

Dechloroisochromophilone IV (**7**): Yellow powder; $[\alpha]_D^{23}$ −260° (*c* 0.1, EtOH); EI-MS, *m/z* 360 $(M)^+$; FAB-MS, *m/z* 361 $(M+H)^+$; HR-EI-MS, found 360.1947, calcd. 360.1937 for $C_{21}H_{28}O_5$; UV $\lambda_{max}^{CH_3OH}$ nm (ε), 246 (5,100); and 372 (21,300); IR ν_{max}^{KBr} cm^{-1}, 3440 (O–H), 2965 (C–H), 2930 (C–H), 1740 (C=O), 1630 (C=O), and 1580 (C=C); ^{1}H-NMR ($CDCl_3$), see Table 1.

Isorotiorin (**8**): Red powder; $[\alpha]_D^{23}$ +458° (*c* 0.1, EtOH); FAB-MS, *m/z* 381 $(M+H)^+$; HR-EI-MS, found 381.1718, calcd. 381.1702 for $C_{23}H_{24}O_5$; UV $\lambda_{max}^{CH_3OH}$ nm (ε), 282 (11,100); 416 (13,800), and 557 (2,930); IR ν_{max}^{KBr} cm^{-1}, 2960 (C–H), 2925 (C–H), 1765 (C=O), 1685 (C=O), 1635 (C=O), 1620 (C=C), and 1530 (C=C); ^{1}H- and ^{13}C-NMR ($CDCl_3$), see Table 2.

5-Bromoochrephilone (**9**): Yellow powder; $[\alpha]_D^{23}$ +152° (*c* 0.05, EtOH); FAB-MS, *m/z* 461 $(M+H)^+$; HR-FAB-MS, found 461.0976, calcd. 461.0964 for $C_{23}H_{26}O_5{}^{79}Br$; Molecular formula, $C_{23}H_{25}O_5Br$; UV $\lambda_{max}^{CH_3OH}$ nm (ε), 257 (13,400), 273 (12,600), 335 (9,400), 358 (10,400), and 412 (11,000); IR ν_{max}^{KBr} cm^{-1}, 2965 (C–H), 1780 (C=O), 1720 (C=O), 1630 (C=O), and 1560 (C=C); ^{1}H-NMR ($CDCl_3$), see Table 2.

2,4-Dihyroxy-6-(5,7-dimethyl-2-oxo-*trans*-3-*trans*-5-nonadienyl)-3-methylbenzaldehyde (**10**): Colorless needles; $[\alpha]_D^{23}$ +51° (*c* 1.0, $CHCl_3$); FAB-MS, *m/z* 317 $(M+H)^+$; HR-EI-MS, found 316.1680, calcd. 316.1675 for $C_{19}H_{25}O_4$; Molecular formula, $C_{19}H_{24}O_4$; UV $\lambda_{max}^{CH_3OH}$ nm (ε), 290 (45,200); IR ν_{max}^{KBr} cm^{-1}, 3460 (O–H), 2965 (C–H), 2925 (C–H), 1680 (C=O), 1625 (C=O), 1595 (C=C), and 1580 (C=C); ^{1}H-NMR (δ = ppm in $CDCl_3$), 12.64 (1H s, 2-O*H*), 9.85 (1H s, 1-C*H*O), 7.34 (1H d *J* = 15.7 Hz, 4′-H), 6.55 (1H s, 4-O*H*), 6.18 (1H d *J* = 15.7 Hz, 3′-H), 6.16 (1H s, 5-H), 5.57 (1H d *J* = 9.9 Hz, 6′-H), 4.07 (2H s, 1′-H), 2.48 (1H m, 7′-H), 2.05 (3H s, 3-CH_3), 1.79 (3H s, 5′-CH_3), 1.40 (2H m, 8′-H), 1.00 (3H d *J* = 6.6 Hz, 7′-CH_3) , 0.85 (3H t, *J* = 7.2 Hz, 9′-H);

^{13}C-NMR (δ = ppm in $CDCl_3$), 197.2 (s, C-2'), 192.5 (d, 1-*C*HO), 164.3 (s, C-2), 161.3 (s, C-4), 151.9 (d, C-4'), 151.8 (d, C-6'), 137.3 (s, C-6), 131.8, (s, C-5'), 122.4 (s, C-3'), 112.9 (s, C-3), 111.1 (d, C-5), 110.6 (s, C-1), 43.7 (t, C-1'), 35.2 (d, C-7'), 29.8 (t, C-8'), 12.4 (q, 5'-*C*H_3), 12.4 (q, 7'-*C*H_3), 11.9 (q, C-9'), 6.9 (q, 3-*C*H_3).

Reduction of **1**

1 (13.14 mg) was dissolved in 10 ml of ethanol and treated with 5% Pd-C (15 mg) under hydrogen gas at room temperature for 40 minutes. The reaction mixture was filtered, and the filtrate was evaporated to dryness. The residue was dissolved in dichloromethane and chromatographed on a silica gel column (1.2 × 8.0 cm) using chloroform - methanol (49 : 1) as the developing solvent. Finally, 9, 10, 11, 12-tetrahydroisochromophilone (**19**, 5.32 mg) was obtained by HPLC [column: SSC Silica gel, 10 mm i.d. × 250 mm, mobile phase: *n*-hexane - acetone (9 : 1), flow-rate: 3.0 ml/minute, detection: UV at 330 nm].

9, 10, 11, 12-Tetrahydroisochromophilone (**19**): Colorless powder; FAB-MS, *m/z* 421 $(M+H)^+$; HRFAB-MS, found 421.1778, calcd. 421.1738 for $C_{23}H_{30}O_5Cl$; ^{1}H-NMR (270 MHz, δ in $CDCl_3$), 7.45 (1H, s, 1-H), 6.48 (1H s, 3-H), 3.78 (1H, d, J = 12.2 Hz, 3'-H), 3.70 (1H, d, J = 12.2 Hz, 8-H), 2.46 (3H, s, 5'-H), 1.59 (3H, s, 7-CH_3), 0.98 (3H, d, 11-CH_3), 0.90 (3H, d, 13-CH_3), 0.83 (3H, t, J = 7.4 Hz, 15-H).

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