Asperinines A and B, Dimeric Tetrahydroanthracene Derivatives from *Aspergillus ruber*

Kunizo ARAI,* Yasuji AOKI, and Yuzuru YAMAMOTO

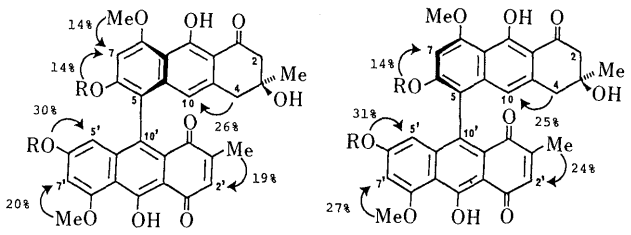
Faculty of Pharmaceutical Sciences, Kanazawa University, Takara-machi 13-1, Kanazawa 920, Japan. *Received August 25, 1988*

The isolation and structures of two new dimeric tetrahydroanthracene derivatives, named asperinines A (1) and B (2), are described. These compounds are the atropisomers of asperflavin(5)-viocristin(10′).

Keywords *Aspergillus ruber*; asperinine A; asperinine B; asperflavin; viocristin; tetrahydroanthracene; 1,4-anthraquinone

During the investigation of secondary metabolites from *Aspergillus ruber* IFO 6004, we isolated two new dimeric hydroanthracene derivatives which consist of viocristin and asperflavin, and named them asperinine A (**1**) and asperinine B (**2**), respectively.

This fungus also produces many other kinds of metabolites, such as asperflavin (**3**) and viocristin (**4**), together with six 9,10-anthraquinone derivatives[1] [erythroglaucin (**5**), physcion (**6**), catenarin (**7**), rubrocristin (**8**), ω-hydroxyemodin-5-methylether (**9**) and ω-hydroxyrubrocristin (**10**)], four echinulin analogues[1] [echinulin (**11**), neoechinulin A (**12**), L-alanyl-2-(1,1-dimethylallyl)-L-tryptophan anhydride (**13**) and L-alanyl-L-tryptophan anhydride (**14**)], and five auroglaucin analogues[1] [auroglaucin (**15**), isodihydroauroglaucin (**16**), isotetrahydroauroglaucin (**17**), aspergin (**18**) and flavoglaucin (**19**)].

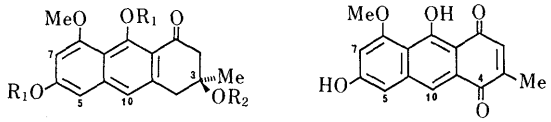


1 : R=H
1a : R=Me
1b : R=Ac

2 : R=H
2a : R=Me
2b : R=Ac

arrows indicate signal enhancement in percent in the NOE experiment

Fig. 1



3 : R_1=R_2=H
3a : R_1=Me, R_2=H
3b : R_1=Me, R_2=Bz

4

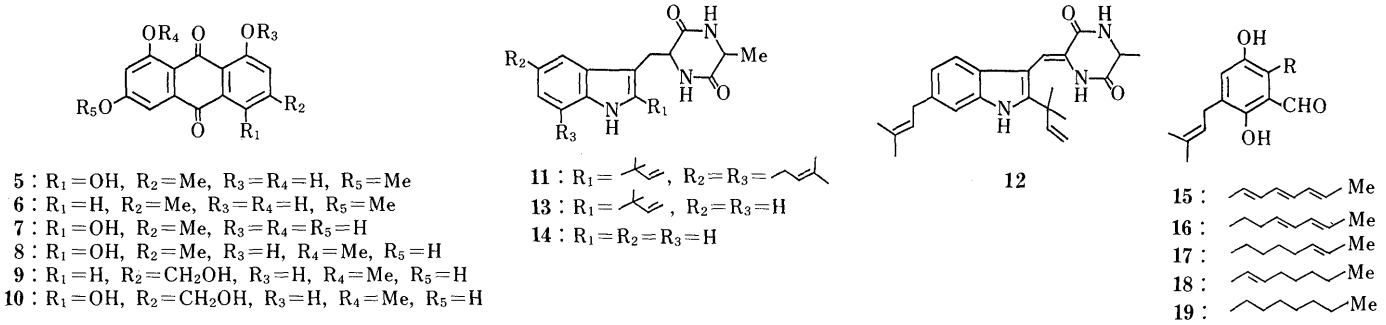
Fig. 2

Asperflavin (**3**) is the major metabolite in the culture broth of this fungus. It was first isolated from *A. flavus* and its structure was determined by Grove in 1972,[2] though the configuration at C-3 remained obscure. Endo and Naoki have determined the configuration at C-3 of torosachryson (an isomer of asperflavin) using the exciton chirality method.[3] Following this method, dimethylasperflavin (**3a**) was converted to a monobenzoate derivative (**3b**), which showed negative first and positive second Cotton effects in the circular dichroism (CD) spectrum, thus revealing the absolute configuration at C-3 in asperflavin as *S*.

Viocristin (**4**) was isolated from *A. cristatus* by Laatsch and Anke in 1982 as the first natural product bearing at 1,4-anthraquinone skeleton.[4]

Asperinines A (**1**) and B (**2**) showed different *Rf* values on thin-layer chromatography (TLC) and opposite CD curves to each other, whereas their ultraviolet (UV) spectra, infrared (IR) spectra and mass spectra (MS) were almost identical to each other. In the proton nuclear magnetic resonance (^{1}H-NMR) spectra, differences in the chemical shifts of some signals were observed, though the overall patterns were very similar (Table I).

On treatment with methyl iodide in dimethyl sulfoxide (DMSO), asperinine A (**1**) gave a 6,6′-dimethyl derivative (**1a**). Similarly, a diacetyl derivative (**1b**) was obtained on treatment with acetic anhydride. On the other hand, treatment of asperinine A (**1**) with acetic anhydride and pyridine yielded a pentaacetyl derivative (**20**). Dimethylasperinine A (**1a**) gave, on acetylation with acetic anhydride and pyridine, the triacetyl derivative (**21**). Asperinine B (**2**) gave



5 : R_1=OH, R_2=Me, R_3=R_4=H, R_5=Me
6 : R_1=H, R_2=Me, R_3=R_4=H, R_5=Me
7 : R_1=OH, R_2=Me, R_3=R_4=R_5=H
8 : R_1=OH, R_2=Me, R_3=H, R_4=Me, R_5=H
9 : R_1=H, R_2=CH_2OH, R_3=H, R_4=Me, R_5=H
10 : R_1=OH, R_2=CH_2OH, R_3=H, R_4=Me, R_5=H

11 : R_1=, R_2=R_3=
13 : R_1=, R_2=R_3=H
14 : R_1=R_2=R_3=H

12

15 :
16 :
17 :
18 :
19 :

Fig. 3

© 1989 Pharmaceutical Society of Japan

NII-Electronic Library Service

TABLE I. NMR Data for Asperinines and Their Derivatives

	Asperflavin (3) (DMSO-d_6)	Viocristin (4) (DMSO-d_6)	Asperinine A (1) (DMSO-d_6)	Asperinine B (2) (DMSO-d_6)
2-H	2.92 (m)	—	2.50—2.88 (m)	2.50—2.87 (m)
4-H	2.71 (m)	—		
10-H	6.78 (s)	—	6.01 (m)	5.98 (m)
7-H	6.43 (d, 2 Hz)	—	6.66 (s)	6.65 (s)
3-Me	1.26 (s)	—	1.14 (s)	1.14 (s)
9-OH	14.97 (s)	—	15.88 (s)	15.88 (s)
(6-OH)	10.25 (s)	—	9.67 (br s)	9.67 (br s)
8-OMe	3.85 (s)	—	3.95 (s)	3.95 (s)
5-H	6.55 (d, 2 Hz)	—	—	—
3′-H	—	2.08 (d, 1.5 Hz)	1.88 (d, 1 Hz)	1.89 (d, 1 Hz)
2′-H	—	6.97 (m)	7.00 (m)	7.00 (m)
5′-H	—	6.98 (d, 2 Hz)	6.23 (d, 2 Hz)	6.20 (d, 2 Hz)
7′-H	—	6.70 (d, 2 Hz)	6.67 (d, 2 Hz)	6.66 (d, 2 Hz)
8′-OMe	—	3.91 (s)	3.93 (s)	3.92 (s)
(6′-OH)	—	10.75 (s)	10.35 (br s)	10.34 (br s)
9′-OH	—	14.90 (s)	15.00 (s)	15.00 (s)
10′-H	—	7.74 (s)	—	—

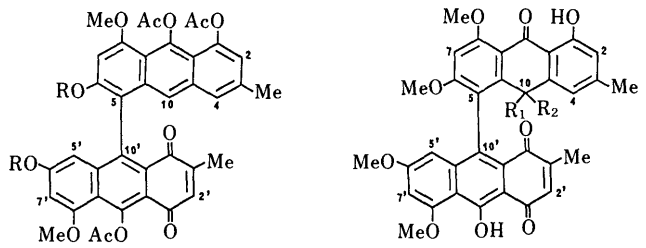
	Dimethyl asperinine A (1a) ($CDCl_3$)	Dimethyl asperinine B (2a) ($CDCl_3$)	Diacetyl asperinine A (1b) ($CDCl_3$)	Diacetyl asperinine B (2b) ($CDCl_3$)
2-H	2.75 (m)	2.76 (m)	2.45—3.00 (m)	2.45—3.00 (m)
4-H				
10-H	6.11 (m)	6.08 (m)	6.30 (s)	6.27 (s)
7-H	6.72 (s)	6.73 (s)	7.04 (s)	7.03 (s)
3-Me	1.31 (s)	1.28 (s)	1.16 (s)	1.16 (s)
9-OH	15.93 (s)	15.93 (s)	14.86 (s)	14.86 (s)
(6-OH) (OMe)	3.75 (s)	(OMe) 3.78 (s)	(OAc) 1.76 (s)	(OAc) 1.76 (s)
8-OMe	4.14 (s)	4.15 (s)	3.97 (s)	3.97 (s)
5-H	—	—	—	—
3′-H	1.97 (d, 1 Hz)	1.97 (d, 1 Hz)	1.90 (d, 1 Hz)	1.87 (d, 1 Hz)
2′-H	6.84 (m)	6.84 (m)	6.90 (m)	6.90 (m)
5′-H	6.24 (d, 2 Hz)	6.21 (d, 2 Hz)	6.39 (d, 2 Hz)	6.42 (d, 2 Hz)
7′-H	6.63 (d, 2 Hz)	6.64 (d, 2 Hz)	7.12 (d, 2 Hz)	7.14 (d, 2 Hz)
8′-OMe	4.05 (s)	4.05 (s)	3.97 (s)	3.97 (s)
(6′-OH) (OMe)	3.51 (s)	(OMe) 3.48 (s)	(OAc) 2.17 (s)	(OAc) 2.16 (s)
9′-OH	15.27 (s)	15.30 (s)	15.53 (s)	15.52 (s)
10′-H	—	—	—	—

	20 (DMSO-d_6, at 100 °C)	**21** (DMSO-d_6, at 60 °C)	**22** ($CDCl_3$)	**23** ($CDCl_3$)
2-H	7.00 (d, 1.5 Hz)	6.78 (br s)	6.66 (d, 1 Hz)	7.19 (d, 1 Hz)
4-H	7.42 (d, 1.5 Hz)	7.18 (br s)	6.31 (d, 1 Hz)	6.84 (d, 1 Hz)
10-H	7.50 (s)	7.23 (s)	3.54 (s)	—
7-H	6.87 (s)	6.79 (s)	6.61 (s)	6.94 (s)
3-Me	1.70 (s)	2.30 (s)	2.20 (s)	2.27 (s)
1-OH	—	—	13.41 (s)	13.11 (s)
OMe	4.00 (s)	3.36, 3.95 (each s)	3.74, 4.15 (each s)	3.74, 4.20 (each s)
3′-Me	1.84 (d, 1.5 Hz)	1.87 (d, 1.5 Hz)	2.03 (d, 0.5 Hz)	1.96 (d, 1 Hz)
2′-H	6.82 (m)	6.67 (m)	6.87 (m)	7.00 (m)
5′-H	6.55 (d, 2 Hz)	6.28 (d, 2.5 Hz)	6.31 (d, 2 Hz)	6.24 (d, 2 Hz)
7′-H	7.10 (d, 2 Hz)	6.55 (d, 2.5 Hz)	6.66 (d, 2 Hz)	6.63 (d, 2.5 Hz)
OMe	4.03 (s)	3.95, 4.07 (each s)	3.57, 4.06 (each s)	3.58, 4.05 (each s)
9′-OH	—	—	15.87 (s)	15.99 (s)
Ac	2.10, 2.32, 2.41, 2.48, 2.50	2.38, 2.42, 2.58	—	—

the corresponding derivatives (**2a**, **2b**, **20** and **21**) when subjected to the same procedures as used for **1**.

Reductive cleavage of asperinine A (**1**) with sodium bisulfite gave *S*-(+)-asperflavin ($[\alpha]_D$ +5.1 °) and viocristin (**4**). Asperinine B (**2**) also afforded the same products **3** ($[\alpha]_D$ +5.3 °) and **4**, on similar reduction, thus indicating that both compounds possess an *S*-asperflavin-viocristin structure. This was supported by their MS, which showed two prominent peaks at *m/z* 288 and *m/z* 284 corresponding to asperflavin (**3**) and viocristin (**4**), respectively. The strong

NII-Electronic Library Service



20 : R=Ac
21 : R=Me

22 : $R_1=R_2=H$
23 : R_1, R_2= O=

Fig. 4

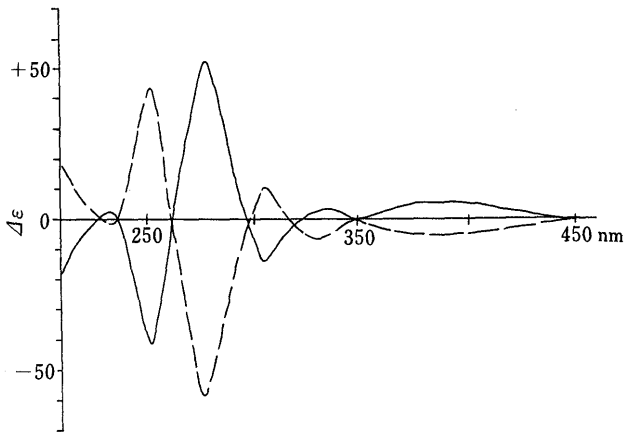


Fig. 5. CD Spectra of Asperinines A (**1**) and B (**2**)
(**1**), —; (**2**), ---.

and opposite Cotton effects in the CD spectra of asperinines A (**1**) and B (**2**) suggested that they are atropisomers caused by restricted rotation around the biphenyl linkage (Fig. 5). Further, the non-identity of asperinines A and B in terms of *Rf* values and ^{1}H-NMR spectra indicated that they are diastereoisomers.

In the ^{1}H-NMR spectra of asperinines A and B, the signals of all protons in the asperflavin moiety are similar to those of the torosachryson moiety in torosanin,[5] in which the linked position was determined to be at C-5 of torosachryson. In a comparison of the ^{1}H-NMR spectra of asperinines A and B with those of asperflavin and viocristin, the signals of H-10 of the asperflavin moiety and H-5′ of the viocristin moiety in asperinines were observed at unusually high field. This phenomenon can be ascribed to the naphthalenoid ring current effect manifested by the twisted dimeric structures. So, these two protons should be located close to the connecting bond between aromatic moieties.

Treatment of 6,6′-dimethylasperinines A (**1a**) and B (**2a**) with hydrochloric acid and acetic acid gave the anhydro derivative (**22**), which afforded an anthraquinone derivative (**23**) on flushing with oxygen gas in an alkaline solution. Further, nuclear Overhauser effect (NOE) enhancements in **1a** and **2a** were observed at the H-10 singlet on irradiation of H-4, and at the H-7 singlet on irradiation of 6-OMe or 8-OMe. These chemical conversions, and the NOE examination of dimethylasperinines A (**1a**) and B (**2a**) confirmed that the linkage position in the asperflavin moiety to viocristin is at C-5 (Fig. 1). On the other hand, the linkage position in the viocristin moiety to asperflavin was concluded to be at C-10′ from the following evidence. The ^{1}H-NMR spectra of asperinines A and B both showed the presence of *meta*-coupling ($J=2$ Hz) in the viocristin moiety, which means that C-5′ and C-7′ of the viocristin moiety are not participating in binding to asperflavin. Further, on acetylation of 6,6′-dimethylasperinine A, only one singlet peak (δ 6.11), which corresponds to the C-10 proton of the asperflavin moiety in asperinine A, was shifted remarkably downfield by 1.07 ppm, but no signal was affected on acetylation of the 9′-hydroxy group of the viocristin moiety. These results suggest that C-10′ of the viocristin moiety participates in the juncture. From these results, asperinines A (**1**) and B (**2**) were both determined to have the structure of asperflavin(5)-viocristin(10′).

Recently, Natori *et al.* determined the absolute configuration of the 9–9′ bond of chaetochromin A to be *S* based on X-ray analysis. This result was in agreement with the result obtained by the application of the exciton chirality method (the CD curve of chaetochromin A exhibit positive first and negative second Cotton effects).[6] The CD curve of asperinine A (**1**) exhibits strong positive first [$\Delta\varepsilon$ +56.6 (277 nm)] and negative second [$\Delta\varepsilon$ −44.7 (252 nm)] Cotton effects. These are due to the coupling between the ^1B_b transitions of the two naphthalene chromophores, and this phenomenon shows that the long axes of the viocristin and asperflavin moieties are twisted in a clockwise manner. In contrast, the CD spectrum of asperinine B (**2**) shows an opposite curve to that of asperinine A (**1**) and indicated the *R*-configuration of **2**.

Many compounds possessing a tetrahydroanthracene moiety in their molecule have been isolated. One such group is dimeric hydroanthracenes, such as flavomannins,[7-9] phlegmacines[9,10] and singueanols.[3] Another group is the compounds in which one tetrahydroanthracene moiety has been converted to an anthrone or a 9,10-anthraquinone structure, such as torosanin, anhydrophlegmacins[5,9] and flavomannin-quinone-6,6′-dimethylether.[8]

Asperinines A (**1**) and B (**2**) are compounds of a new type, consisting of tetrahydroanthracene and 1,4-anthraquinone moieties.

Experimental

All melting points were taken on a Yanagimoto micro melting point apparatus and are uncorrected. The ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL 100 spectrometer with tetramethylsilane as an internal standard. MS were taken on a Hitachi M-80 type spectrometer. UV and IR spectra were measured with Hitachi 323 type UV and JASCO A-102 IR spectrophotometers, respectively. The $[\alpha]_D$ values were measured with a JASCO DIP-140 digital polarimeter. The CD spectra were recorded on a JASCO J-20 spectropolarimeter. Kiesel gel $60F_{254}$ (Merck) precoated plates were used for TLC. Preparative TLC (PLC) was carried on Kieselgel $60PF_{254}$ (Merck) plates. Column chromatography was carried out with Wako gel C-200.

Isolation of Metabolites from *Aspergillus ruber* *A. ruber* IFO 6004 was cultivated stationarily on a malt extract medium (glucose, 120 g; malt extract, 120 g; polypeptone 18 g; tap water, 6 l) for 3 weeks at 27 °C. The dried mycelia were extracted with petroleum ether (bp 30—70 °C) and then ethylether. The hexane-soluble fraction of the petroleum ether extracts was separated by silica-gel column chromatography with a mixed solvent of hexane–benzene (13 : 7, v/v) to give aspergin (**18**) (38 mg), mp 72—73 °C, flavoglaucin (**19**) (86 mg), mp 105.5—109 °C, auroglaucin (**15**) (7 mg), mp 151—152 °C, isotetrahydroauroglaucin (**17**) (18 mg), mp 86—87 °C, and isodehydroauroglaucin (**16**) (23 mg), mp 110—112 °C. The hexane-insoluble portion was also chromatographed with hexane–benzene (3 : 1, v/v) to give erythroglaucin (**5**)(16 mg), mp 210—214 °C and physcion (**6**) (26 mg), mp 208.5—210 °C. The ether extract was chromatographed using

NII-Electronic Library Service

chloroform–ethyl acetate (3:1, v/v) as an eluting solvent to afford catenarin (**7**) (16 mg), mp 245 °C, viocristin (**4**) (33 mg), mp >300 °C, rubrocristin (**8**) (21 mg), mp >300 °C, echinulin (**11**) (60 mg), mp 241—244 °C, and asperflavin (**3**) (57 mg), mp 220—230 °C (dec.).

The culture broth was extracted with ethyl acetate and the extract was chromatographed with chloroform–ethyl acetate (3:1, v/v) to separate several fractions. From the first eluate, erythroglaucin (**5**) (5 mg), physcion (**6**) (8 mg), catenarin (**7**) (4 mg), viocristin (**4**) (18 mg) and rubrocristin (**8**) (7 mg) were isolated by rechromatography. From the second fraction, neoechinulin A (**12**) (17 mg), mp 267—271 °C (dec.), was obtained. Asperflavin (**3**) (230 mg) was isolated from the third fraction. The fourth fraction was further chromatographed on Sephadex LH-20 using methanol to give ω-hydroxyemodin-5-methylether (**9**) (6 mg), mp 276—279 °C, and ω-hydroxyrubrocristin (**10**) (3 mg), mp >300 °C. The fifth fraction was reseparated by PLC using chloroform–ethyl acetate (1:1, v/v) as a solvent to give a mixture of asperinines A (**1**) and B (**2**), and L-alanyl-2-(1,1-dimethylallyl)-L-tryptophan anhydride (**13**) (15 mg), mp 291—294 °C. A mixture of asperinines A (**1**) and B (**2**) was rechromatographed on PLC using chloroform–acetone (4:1, v/v) to afford asperinine A (**1**) (24 mg), mp >300 °C (lower band), and asperinine B (**2**) (35 g), mp >300 °C (upper band). Further elution with ethyl acetate gave L-alanyl-L-tryptophan anhydride (**14**) (1 mg), mp 290—291 °C (dec.).

ω-Hydroxyrubrocristin (10) ^{1}H-NMR (DMSO-d_6) δ: 3.88 (3H, s, OMe), 4.57 (2H, s, CH_2OH), 5.43 (1H, br s, OH), 6.80 and 7.20 (each 1H, d, J=2 Hz), 7.29 (1H, s, aromatic-H), 11.20 and 13.52 (each 1H, s, OH). MS m/z: 316 (M^+), 255, 171. *Anal.* Calcd for $C_{16}H_{12}O_7$: C, 60.76; H, 3.82. Found: C, 60.63; H, 3.85.

Asperflavin (3) MS m/z: 288 (M^+), 270, 255, 230, 212. *Anal.* Calcd for $C_{16}H_{16}O_5$: C, 66.77; H, 5.55. Found: C, 66.85; H, 5.59. IR (KBr) cm^{-1}: 3490, 3350, 1632, 1582. UV (EtOH) nm: 230, 270, 317, 335, 395. $[\alpha]_D$ +6.7° (c=0.44, MeOH).

Viocristin (4) MS m/z: 284 (M^+), 266, 255, 238, 210. *Anal.* Calcd for $C_{16}H_{12}O_5$: C, 67.60; H, 4.44. Found: C, 67.90; H, 4.44. IR (KBr) cm^{-1}: 3400, 1665, 1635, 1615, 1590, 1518. UV (EtOH) nm: 253, 281 (sh), 340, 487, 417, 530.

Asperinine A (1) MS m/z: 570 (M^+), 288, 284, 270. *Anal.* Calcd for $C_{32}H_{26}O_{10}\cdot 1/2H_2O$: C, 66.33; H, 4.69. Found: C, 66.40; H, 4.71. IR (KBr) cm^{-1}: 3440, 1660 (sh), 1642 (sh), 1610 (sh), 1597. UV (MeOH) nm (ε): 254 (71000), 272.5 (53800), 342 (14200), 364 (10400), 404 (13100), 518 (9790). CD (MeOH) $\Delta\varepsilon$ (nm): −44.7 (252), 0 (263), +56.6 (277), 0 (296), −14.9 (305), 0 (324), +3.25 (335), 0 (345). $[\alpha]_D$ −482° (c=0.028, EtOH).

Asperinine B (2) *Anal.* Calcd for $C_{32}H_{26}O_{10}\cdot 1/2H_2O$: C, 66.33; H, 4.69. Found: C, 66.36; H, 4.75. The MS, IR and UV spectra are almost superimposable on those of asperinine A. CD (MeOH) $\Delta\varepsilon$ (nm): +45.2 (252), 0 (263), −60.2 (277), 0 (298), +11.5 (305), 0 (317), −6.26 (332), 0 (345). $[\alpha]_D$ +250° (c=0.024, EtOH).

Dimethylasperflavin Monobenzoate (3b) Following Endo and Naoki's method,[3] dimethylasperflavin (**3a**) (20 mg) was dissolved in pyridine (1 ml), and benzoyl chloride (0.5 ml) was added. The mixture was left to stand for 14 h at room temperature and then poured into water. The precipitate was subjected to PLC chloroform to give a monobenzoate (**3b**) as yellow needles, mp 149—150 °C (ref. 148—149 °C) (35 mg). ^{1}H-NMR ($CDCl_3$) δ: 1.81 (3H, s, Me), 2.88 (1H, d, J=17 Hz, CH_2), 3.29 (1H, d, J=17 Hz, CH_2), 3.31 (1H, d, J=17 Hz, CH_2), 3.88, 3.89 and 3.93 (each 3H, s), 4.04 (1H, d, J=17 Hz, CH_2), 6.43 and 6.58 (each 1H, d, J=2.4 Hz), 7.26—7.39 (3H, m), 7.71—7.81 (2H, m). MS m/z: 420 (M^+). *Anal.* Calcd for $C_{25}H_{24}O_6$: C, 71.41; H, 5.75. Found: C, 71.67; H, 5.77. CD (MeOH) $\Delta\varepsilon$ (nm): −23.5 (264), +65.3 (228).

6,6′-Dimethylasperinine A (1a) Asperinine A (**1**) (103 mg) was dissolved in DMSO (15 ml) and then methyl iodide (1 ml) and 2 N sodium hydroxide (2 ml) were added. The mixture was reacted for 5 min at room temperature, then poured into water and extracted with ethyl acetate. The product was recrystallized from chloroform–methanol to yield a dimethyl derivative (**1a**) (72 mg) as dark red leaflets, mp >300 °C. *Anal.* Calcd for $C_{34}H_{30}O_{10}$: C, 68.22; H, 5.05. Found: C, 67.56; H, 5.05. MS m/z: 598 (M^+), 580, 567, 549. IR (KBr) cm^{-1}: 3450, 1656, 1646, 1588. UV (MeOH) nm (ε): 254 (71300), 274 (56200), 340 (13600), 406 (13500), 496 (sh) (9770), 512 (10000). CD (MeOH) $\Delta\varepsilon$ (nm): −67.9 (252), 0 (262), +77.1 (276), 0 (297), −13.7 (304), 0 (318), +4.27 (330), 0 (340), −2.13 (345). $[\alpha]_D$ −305° (c=0.04, EtOH).

6,6′-Dimethylasperinine B (2a) A similar treatment of asperinine B (**2**) gave a dimethyl derivative (**2a**) as dark red prisms, mp 293—294 °C (dec.). *Anal.* Calcd for $C_{34}H_{30}O_{10}$: C, 68.22; H, 5.05. Found: C, 68.08; H, 5.00. The MS, IR and UV spectra of **2a** are closely similar to those of dimethylasperinine A (**1a**), but the sign of the optical rotation is opposite. CD (MeOH) $\Delta\varepsilon$ (nm): +60.0 (252), 0 (262), −72.7 (276), 0 (298), +9.3 (304), 0 (314), −6.23 (330), 0 (340), +2.14 (345). $[\alpha]_D$ +117° (c=0.052, EtOH).

Acetylation of Asperinines A and B with Acetic Anhydride Asperinine A (**1**) (10 mg) was treated with acetic anhydride (2 ml) at 60 °C for 5 min. The product was recrystallized from methanol to give the diacetate (**1b**), mp >300 °C (9 mg) as red needles. *Anal.* Calcd for $C_{36}H_{30}O_{12}$: C, 63.66; H, 4.62. Found: C, 63.66; H, 4.62. MS m/z: 654 (M^+), 612, 595, 570, 553. IR (KBr) cm^{-1}: 3450, 1764, 1660, 1640, 1620, 1600, 1585. $[\alpha]_D$ −350° (c=0.03, EtOH).

A similar treatment of asperinine B (**2**) also gave an acetate (**2b**) which shows IR and MS spectra identical with those of **1b**, though the optical rotation is opposite. $[\alpha]_D$ +154° (c=0.035, EtOH).

Acetylation of Asperinines A and B with Acetic Anhydride and Pyridine Asperinine A (**1**) (20 mg) was treated with acetic anhydride (1 ml) and pyridine (2 ml) for 72 h at room temperature. The product was recrystallized from methanol to give a pentaacetyl anthracene derivative (**20**), mp 237—245 °C (dec.), as a yellow powder. *Anal.* Calcd for $C_{42}H_{34}O_{14}$: C, 66.14; H, 4.46. Found: C, 65.81; H, 4.15. MS m/z: 762 (M^+), 720, 678, 594, 552. IR (KBr) cm^{-1}: 3452, 1764, 1660, 1640, 1618, 1578.

Asperinine B (**2**) also afforded a pentaacetyl anthracene derivative which is identical with **20** in terms of the spectral data (NMR, MS, IR and UV).

Acetylation of Dimethylasperinines A and B with Acetic Acid and Pyridine Dimethylasperinine A (**1a**) (20 mg) was treated with acetic anhydride (0.5 ml) and pyridine (1 ml) for 72 h at room temperature. The mixture was separated by PLC (chloroform–ethyl acetate (1:2, v/v)) and from the upper band, a triacetate (**21**), mp 225—234 °C (dec.), was obtained as a red powder. *Anal.* Calcd for $C_{40}H_{34}O_{12}$: C, 67.99; H, 4.82. Found: C, 67.47; H, 4.62. MS m/z: 706 (M^+), 664, 622, 580. IR (KBr) cm^{-1}: 1764, 1660, 1610, 1562.

Dimethylasperinine B (**2a**) also gave a triacetate which was identical with **21** in terms of the spectra data (NMR, MS, IR and UV).

Reductive Cleavage of Asperinines A and B Following Shibata's method,[11] sodium bisulfite (3 g) was added to a solution of asperinine A (**1**) (100 mg) in 1 N sodium carbonate (10 ml) and warmed. After the color of the solution had changed to pale yellow, the mixture was cooled, acidified, and extracted with ethyl acetate. The organic layer was evaporated to dryness and redissolved in methanol (5 ml). Aqueous 10% ferric chloride solution was added to the above solution and reacted for 15 min at room temperature. The mixture was extracted with chloroform and the extract was separated by PLC (ethyl acetate–chloroform (1:1, v/v)) to give viocristin (**4**) (21 mg) and (+)-asperflavin (**3**) (17 mg), $[\alpha]_D$ +5.1° (c=0.24, MeOH).

Asperinine B (**2**) also gave (+)-asperflavin ($[\alpha]_D$ +5.3°) and viocristin when subjected to the same procedure.

Dimethylanhydroasperinines A and B Concentrated hydrochloric acid (1 ml) was added to a solution of dimethylasperinine A (**1a**) (50 mg) in acetic acid (10 ml). The solution was left to stand for 5 min at 60 °C and then cooled to room temperature. The reacted product was recrystallized from chloroform–ethyl acetate to give the anhydro derivative (**22**) (24 mg) as a red-purple powder. *Anal.* Calcd for $C_{34}H_{28}O_9\cdot 1/2H_2O$: C, 69.27; H, 4.92. Found: C, 69.18; H, 4.74. MS m/z: 580 (M^+). IR (KBr) cm^{-1}: 1660 (sh), 1645, 1635.

Dimethylasperinine B (**2a**) also yielded an dimethylanhydroasperinine B, which was identical with **22**.

Oxidation of Dimethylanhydroasperinines A and B Following to Cameron *et al.*'s method,[12] dimethylanhydroasperinine A (**22**) (59 mg) was dissolved in a solution of chloroform (5 ml), DMSO (10 ml), and 0.1 N sodium hydroxide (1 ml) and then oxygen gas was passed into this solution for 1 min. It was acidified and extracted with chloroform. The extract was purified by PLC (ethyl acetate–chloroform (1:5, v/v)) and recrystallized from chloroform–methanol to give an oxo derivative (**23**) (28 mg) as red crystals, mp >300 °C. CI-MS m/z: 595 (M^++1), 299. *Anal.* Calcd for $C_{34}H_{26}O_{10}\cdot H_2O$: C, 66.66; H, 4.58. Found: C, 66.54; H, 4.13. IR (KBr) cm^{-1}: 1660 (sh), 1640 (sh), 1633, 1603.

Dimethylanhydroasperinine B also gave an oxo derivative, which showed the same NMR, IR and MS spectra as those of **23**.

Acknowledgement The authors wish to thank Professor Y. Tsuda, Kanazawa University, for his helfpul discussion, and are also grateful to Mr. Y. Itatani and Misses K. Handa and E. Fukui of this faculty for elemental analyses and MS spectral measurements.

NII-Electronic Library Service

References

1) W. B. Turner, "Fungal Metabolites," Academic Press, Inc. Ltd. London, 1971; W. B. Turner and D. C. Aldridge, "Fungal Metabolites II," Academic Press, Inc. Ltd. London, 1983.
2) J. F. Grove, *J. Chem. Soc., Perkin Trans. 1*, **1972**, 2406.
3) M. Endo and H. Nåoki, *Tetrahedron*, **36**, 2449 (1980).
4) H. Laatsh and H. Anke, *Justus Liebigs Ann. Chem.*, **1982**, 2406.
5) S. Kitanaka and M. Takido, *Phytochemistry*, **21**, 2103 (1982).
6) K. Koyama, S. Natori and Y. Iitaka, *Chem. Pharm. Bull.*, **35**, 4049 (1987).
7) J. Atherton, B. W. Bycroft, J. C. Roberts, P. Roffey and M. E. Wilcox, *J. Chem. Soc. C*, **1968**, 2560.
8) W. Steglich, E. Töpfer-Petersen and W. Reininger, *Phytochemistry*, **11**, 3299 (1972).
9) W. Steglich and E. Töpfer-Petersen, *Z. Naturforsch*, **27b**, 1286 (1972); *idem, ibid.*, **28c**, 354 (1973).
10) S. Takahashi, S. Kitanaka, M. Takido, U. Sankawa and S. Shibata, *Phytochemistry*, **16**, 999 (1977).
11) N. Takeda, S. Seo, Y. Ogihara, U. Sankawa, I. Iitaka, I. Kitagawa and S. Shibata, *Tetrahedron*, **29**, 3703 (1973).
12) D. W. Cameron, J. S. Edmonds and W. D. Raverty, *Aust. J. Chem.*, **29**, 1535 (1976).

NII-Electronic Library Service