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New mycotoxins from the scale insect fungus *Aschersonia coffeae* Henn. BCC 28712

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ABSTRACT

Nine new mycotoxins; five xanthones **1**–**5**, hydroxanthone **6**, and three anthraquinones **7**–**9**, together with nine known compounds; sterigmatocystin (**10**), demethylsterigmatocystin (**11**), dihydrodemethylsterigmatocystin (**12**), sterigmatin (**13**), austocystin F (**14**), averufin (**15**), aflatoxin B1, paeciloquinone A, and zeorin, were isolated from the scale insect fungus *Aschersonia coffeae* Henn. BCC 28712. The structures of these compounds were elucidated using NMR spectroscopic and MS spectrometric analyses. Compounds **1**–**3** and **6**–**9** displayed cytotoxic activity while the xanthone **2** and anthraquinones **8** and **9** also showed antimalarial activity.

1. Introduction

Aschersonia is a member of entomogenic fungi that attacks scale insects and whitefly and has been studied for the use in biocontrol.[1–3] As a consequence, the study of their bioactive compounds is of great interest. Several biologically active secondary metabolites from this genus have been reported. Examples are; dustanin and the 3β-acetoxyl derivative from *Aschersonia tubulata* BCC 1785 exhibited antimycobacterial activity,[4] ascherxanthone A from *Aschersonia* sp. BCC 8401, 17(21)-hopane-6α,12β-diol, and aschernaphthopyrone A from *Aschersonia apraphysata* BCC 11964 showed antiplasmodial activity,[5,6] ascherxanthone B from *Aschersonia luteola* BCC 8774 revealed antifungal activity,[7] and destruxins A4 and A5 from an *Aschersonia* species exhibited insecticidal activity.[8] As part of a research program on the discovery of novel bioactive compounds from Thai microorganisms, we investigated the constituents of the insect pathogenic fungus *Aschersonia coffeae* Henn. BCC 28712, guided by its antimalarial (*Plasmidium falciparum*) activity (IC_{50}=4.39 μg/mL) and cytotoxic activity against human breast cancer (MCF-7, IC_{50}=10.39 μg/mL), oral human epidermoid carcinoma (KB, IC_{50}=9.42 μg/mL), and human small-cell lung cancer (NCI-H187, IC_{50}=2.89 μg/mL) cell lines. The study led to the isolation and structural elucidation of nine new compounds, which are five xanthones (**1**–**5**), one hydroxanthone (**6**) and three anthraquinones (**7**–**9**), together with nine known compounds, sterigmatocystin (**10**),[9] demethylsterigmatocystin (**11**),[10] dihydrodemethylsterigmatocystin (**12**),[11] sterigmatin (**13**),[10] austocystin F (**14**),[12] averufin (**15**),[13,14] aflatoxin B1,[15] paeciloquinone A,[16] and zeorin.[17] Many xanthones and anthraquinones were recognized as mycotoxins and were mostly found in many species of fungi in the genus *Aspergillus*,[18,19] interestingly, they have now been reported for the first time from fungus in the genus *Aschersonia*. The biological activities of these compounds were also evaluated.

2. Results and discussion

Compounds **4**, **5**, **8**, **9**, averufin (**15**), and aflatoxin B1 were obtained from both culture broth and mycelia extract of BCC 28712. Compounds **1**, **2**, **3**, **7**, sterigmatocystin (**10**), demethylsterigmatocystin (**11**), sterigmatin (**13**), austocystin F (**14**), paeciloquinone A, and zeorin were obtained from the crude extract of the mycelia, while compounds **6** and dihydrodemethylsterigmatocystin (**12**) were present only in the culture broth extract.

Compound **1** was obtained as a yellow solid. The molecular formula was established by HRMS (ESITOF), in combination with ^{13}C NMR spectroscopy as $C_{18}H_{12}O_6$. The IR spectrum showed an absorption band at 3369 cm^{-1} for a hydroxyl group and the characteristic absorption bands of a γ-pyrone unit at 1652 and 1631 cm^{-1}. The ^1H NMR spectroscopic data were closely related to that of sterigmatocystin (**10**).[9] The spectrum showed signals for one chelated phenolic proton, three protons of a 1,2,3-trisubstitued benzene ring, one singlet aromatic proton, one cis-double bond, two vicinal methine protons, and one methoxyl

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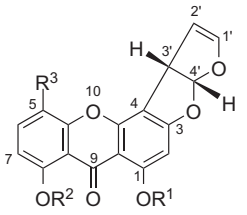
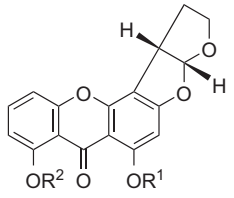
group. Correlations from H-5 to C-8a/C-10a, from H-6 to -10a, from H-7 to C-8a/C-9, and from H-2 to C-9a in HMBC spectrum revealed the xanthone moiety. The presence of methoxyl group at C-8 was indicated by an HMBC correlation between the methoxyl protons and their corresponding carbon. The NOESY correlation between the methoxyl protons and H-7 also supported the position of methoxyl group at C-8 of the xanthone unit. The hydroxyl group was assigned to C-1 on the basis of its chemical shift. The bishydrofuran part was established on the COSY correlations from H-1′ to H-2′, H-2′ to H-3′, and H-3′ to H-4′ together with HMBC correlations from H-1′ to C-2′/C-3′/C-4′, from H-2′ to C-1′/C-3′/C-4′, from H-3′ to C-1′/C-2′/C-4′/C-3/C-4, and from H-4′ to C-1′/C-3′/C-3/C-4. The cross peak between H-3′ and H-4′ in NOESY spectrum indicated a cis ring fusion of the bishydrofuran unit. The attachment of the bishydrofuran part to C-3 and C-4 of the xanthone unit was confirmed by the correlations between H-3′ and C-4a in the HMBC spectrum and the correlation between H-2 and hydroxyl proton in the NOESY spectrum. Therefore, compound **1** was determined as a new 8-*O*-methyldemethylsterigmatocystin. For confirmation, methyl ether derivatives of compound **1** and sterigmatocystin (**10**) were prepared, which showed identical ^{1}H NMR spectra. Their optical rotations were almost identical to that of the reported monomethyl ether derivative of sterigmatocystin (**16**),[20] which indicated the same absolute configuration of compound **1** and sterigmatocystin.[21]

Compound **2** was obtained as a yellow solid, possessing the molecular formula $C_{18}H_{12}O_7$ as deduced from HRMS (ESITOF), in combination with ^{13}C NMR spectroscopy. The ^{1}H NMR spectrum was similar to that of sterigmatocystin (**10**)[9] except for the presence of an additional hydroxyl signal at δ 8.28 and a different pattern in the aromatic region. Compound **2** had two *ortho*-coupled protons together with one singlet proton, whereas sterigmatocystin showed signals for a 1,2,3-trisubstituted benzene ring in addition to a similar singlet proton. The HMBC correlations from OH-8 to C-8/C-7/C8a supported the location of the chelated hydroxyl group at C-8. The assignment of an additional hydroxyl group at C-5 was established on the basis of its chemical shift. The cross peak between methoxyl protons and H-2 in the NOESY spectrum confirmed the attachment of the bishydrofuran moiety at C-3 and C-4 of the xanthone unit. Compound **2** was therefore assigned as a new 5-hydroxysterigmatocystin. The almost identical rotations of compound **2** and sterigmatocystin[9] indicated the same configuration of these two compounds.

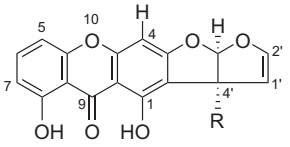
Compound **3** with the molecular formula $C_{23}H_{20}O_{10}$ from HRMS (ESITOF) was obtained as a yellow solid. The ^{1}H NMR spectrum indicated that compound **3** was closely related to sterigmatocystin (**10**).[9] Analysis of the ^{13}C and 2D NMR spectra revealed the similar structural features of these two compounds except for the replacement of the methoxyl group at C-1 with a sugar unit. The correlations from the anomeric proton H-1″ to H-2″, H-2″ to H-3″,



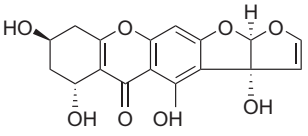
1, $R^1 = R^3 = H$, $R^2 = Me$
2, $R^1 = Me$, $R^2 = H$, $R^3 = OH$
3, $R^2 = R^3 = H$, R^1 =
10, $R^1 = Me$, $R^2 = R^3 = H$, Sterigmatocystin
11, $R^1 = R^2 = R^3 = H$, Demethylsterigmatocystin
16, $R^1 = R^2 = Me$, $R^3 = H$, *O*-Methylsterigmatocystin



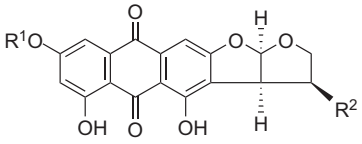
4, $R^1 = H$, $R^2 = Me$
12, $R^1 = R^2 = H$, Dihydrodemethylsterigmatocystin



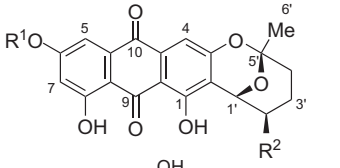
5, R = OMe
13, R = H, Sterigmatin
14, R = OH, Austocystin F



6



7, R^1 = , $R^2 = H$
8, R^1 = , $R^2 = OH$
17, $R^1 = H$, Vericolorin B, $R^2 = H$



9, R^1 = , $R^2 = OH$
15, $R^1 = R^2 = H$, Averufin
18, $R^1 = R^2 = OH$, nidurufin

H-3″ to H-4″, and H-4″ to H-5″ in the COSY spectrum established the sugar moiety. The equatorial position of H-4″ was deduced on the basis of small coupling constants of both axial Ha-5″ and equatorial Hb-5″ with H-4″ (J=3.0 and 3.7 Hz). The large coupling constant between H-3″ and H-2″ (J=7.2 Hz) and small coupling constant between H-2″ and H-1″ (J=4.1 Hz) indicated the axial orientations of H-3″ and H-2″ and the equatorial orientation of H-1″, respectively. The enhancement of Ha-5″ when irradiated at H-3″ in NOE experiment also supported the axial positions of these two protons. The locations of the hydroxyl and methoxyl groups on the sugar ring were confirmed by HMBC correlations of these protons to their corresponding carbons. These results indicated that the sugar unit was 4-*O*-methyl-β-arabinopyranose. The position of attachment of the sugar moiety at C-1 of the xanthone unit was assigned on the basis of HMBC correlation between the anomeric proton H-1″ and C-1. The correlation between H-2 and C-1 in the HMBC spectrum also supported the linkage of the bishydrofuran part at C-3 and C-4 of the xanthone unit. Comparison of the specific rotation of the aqueous layer of its acid hydrolysate ($[\alpha]_D^{25}$ +137.0, *c* 0.05, water) with that of 4-*O*-methyl-L-arabinopyranose ($[\alpha]_D^{27}$ +132, *c* 1.0, water)[22] established the L configuration of 4-*O*-methyl-β-arabinopyranose. However, other unidentified products were obtained in the organic layer instead of the aglycone unit, demethylsterigmatocystin. The same result was also observed for the acid hydrolysis of demethylsterigmatocystin (**11**) itself. Therefore, compound **3** was determined as a new demethylsterigmatocystin-1-(4-*O*-methyl)-β-L-arabinopyranose. Selected NOESY and HMBC correlations for compound **3** are presented in Fig. 1.

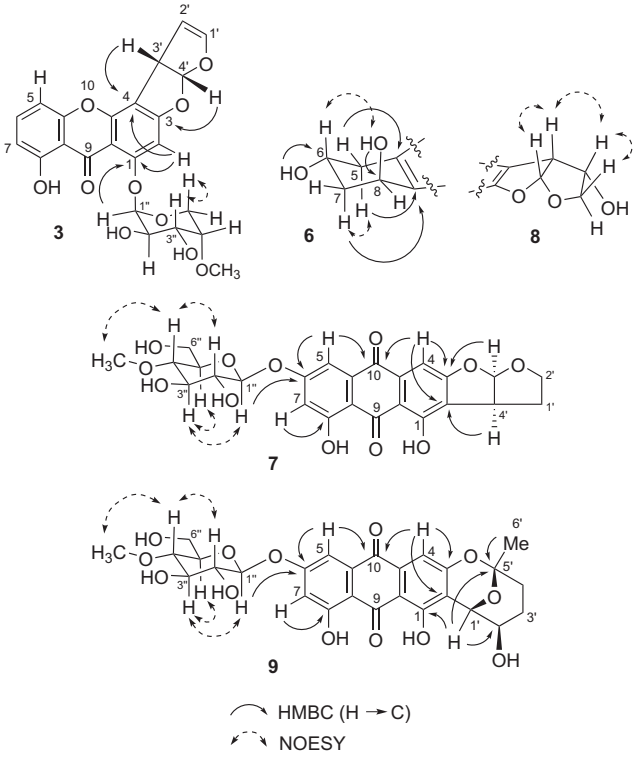


Fig. 1. Selected NOESY correlations of compounds **3** and **6**–**9**.

Compound **4** was obtained as a yellow solid. The HRMS (ESI-TOF), in combination with ^{13}C NMR spectroscopy, gave the molecular formula as $C_{18}H_{14}O_6$. The similarity of the ^{1}H NMR spectrum of compound **4** to that of dihydrosterigmatocystin[11] suggested that the structures of both compounds were closely related. Analysis of the ^{13}C NMR and 2D NMR spectroscopic data revealed differences only at the position of the methoxyl group on the xanthone unit. HMBC correlations from the methoxyl protons to C-8/C-7 and from the hydroxyl proton to C-1/C-2/C-9a established the attachment of the methoxyl and hydroxyl groups at C-8 and C-1, respectively. The cross peak between methoxyl protons and H-7 and the hydroxyl proton and H-2 also supported the assignment of these two groups. Compound **4** was, therefore, determined as a new 8-*O*-methyldihydrodemethylsterigmatocystin. The same absolute configuration of compound **4** and dihydrosterigmatocystin[11] was indicated by the almost identical optical rotations of these two compounds.

Compound **5** was obtained as a yellow solid, and the molecular formula was deduced as $C_{18}H_{12}O_7$ by HRMS (ESITOF), in combination with ^{13}C NMR spectroscopy. The ^{1}H NMR spectrum was similar to that of austocystin F (**14**)[12] except for the presence of an additional methoxyl absorption at δ 3.4 instead of the hydroxyl signal. Assignment of the methoxyl group at C-4′ was established by the HMBC correlation between the *O*-methyl protons and their corresponding carbon (C-4′). The cross peak from *O*-methyl protons to H-1′ and H-3′ in NOESY spectrum also supported the position of the methoxyl group at C-4′ as well as the cis ring fusion of the bishydrofuran ring system. The attachment of the bishydrofuran moiety at C-2 and C-3 of the xanthone unit, which revealed the linear structure of this compound, was deduced by the HMBC correlation between H-4 and C-4a. Compound **5** was, therefore, assigned as a new 4′-*O*-methylaustocystin F. The same absolute configuration of Compound **5** and austocystin F[23] was indicated by the almost identical optical rotations of these two compounds.

Compound **6** was obtained as a red brown solid. Its molecular formula was determined by HRMS (ESITOF) in combination with ^{13}C NMR spectroscopy as $C_{17}H_{14}O_8$. Comparison of the ^{1}H NMR spectrum with that of austocystin F (**14**)[12] showed a very good correlation for the bishydrofuran moiety. For the xanthone part, the spectrum revealed a lack of signals for a 1,2,3-trisubstitued benzene ring and the presence of additional signals for two methylene groups, two oxy-methine protons, and two hydroxyl protons. COSY correlations from H-5 to H-6, H-6 to H-7, and H-7 to H-8 together with HMBC correlations from H-5 to C-10a/C-8a and H-7 to C-8a established the cyclohexene part attached to the γ-pyrone ring. The positions of the hydroxyl groups at C-6 and C-8 on cyclohexene were assigned on the basis of their chemical shifts together with the HMBC correlations between hydroxyl protons and their corresponding carbons. The linear structure of this compound was supported by the correlation between H-4 and C-4a in the HMBC spectrum. The large coupling constant between axial Ha-5 and H-6 (J=8.3 Hz) and the small coupling constant between axial Ha-7 and H-8 (J=4.1 Hz) deduced the axial and equatorial orientations of H-6 and H-8, respectively, which indicated the trans relationship between these two protons. The NOESY correlations from H-6 to OH-8 and from H-3′ to OH-4′ also supported the trans relationship between OH-6 and OH-8 and the cis relationship between H-3′ and OH-4′, respectively. However, due to the shortage of sample, the absolute configuration was not determined. Therefore, compound **6** was only identified as a new austocystin J. Selected NOESY correlations for compound **6** are presented in Fig. 1.

Compound **7** was obtained as a yellow solid. The HRMS (ESITOF), in combination with ^{13}C NMR spectroscopy, gave the molecular formula as $C_{25}H_{24}O_{12}$. The ^{1}H and ^{13}C NMR spectroscopic data showed a good correlation to those of versicolorin B (**17**)[24] except for the presence of an additional sugar unit. The axial orientation of protons 1″–5″of the sugar moiety were established on the basis of large vicinal trans-coupling constants ($J_{1'',2''}$=7.8 Hz, $J_{2'',3''}$=8.0 Hz, $J_{3'',4''}$=9.4 Hz, and $J_{4'',5''}$=9.4 Hz). The HMBC correlation from *O*-methyl protons to C-4″ indicated the position of the methoxyl group at C-4″. The sugar unit was then assigned as 4-*O*-methyl-β-glucopyranose. Attachment of the sugar unit at C-6 of the

anthaquinone part was deduced by the correlation between the anomeric proton H-1″ and C-6 in the HMBC spectrum. Comparison of the specific rotations of the aqueous layer of its hydrolysate ($[\alpha]_D^{25}$ +54.0, *c* 0.02, MeOH) with that of 4-*O*-methyl-D-glucopyranose ($[\alpha]_D^{20}$ +80, *c* 1.30, MeOH)[25] established the D configuration of 4-*O*-methyl-β-glucopyranose. The ^{1}H NMR spectroscopic data as well as optical rotation of the aglycone unit, obtained by acid hydrolysis of compound **7**, were in good agreement with those of veriscolorin B.[24] Compound **7** was, therefore, assigned as a new versicolorin B-6-(4-*O*-methyl)-β-D-glucopyranose. Selected NOESY and HMBC correlations for compound **7** are presented in Fig. 1.

Compound **8** with the molecular formula $C_{25}H_{24}O_{13}$ from HRMS (ESITOF), in combination with ^{13}C NMR spectroscopy, was obtained as a yellow solid. The ^{1}H NMR spectrum was similar to that of compound **7** except for the presence of an additional hydroxyl signal at δ 5.67, one methine proton at δ 4.44 and the absence of two methylene protons at δ 2.18. Assignment of an additional hydroxyl group at C-1′ was established on the basis of its chemical shift. The 2D NMR spectroscopic data of the sugar moiety were in good agreement with those of the sugar unit of compound **7**. The attachment of the sugar unit at C-6 was confirmed by the correlation between the anomeric proton H-1″ and C-6 in the HMBC spectrum. However, due to the small quantity available, acid hydrolysis of compound **8** was not performed. Compound **8** was, consequently, assigned as a new 1′-hydroxyversicolorin B-6-(4-*O*-methyl)-β-glucopyranose. Selected NOESY and HMBC correlations for compound **8** are presented in Fig. 1.

Compound **9** with the molecular formula $C_{27}H_{28}O_{13}$ from HRMS (ESITOF) was obtained as an orange solid. The ^{1}H, ^{13}C, and 2D NMR spectroscopic data revealed the closely related structure of compound **9** and nidurufin (**18**)[14] except for the presence of a sugar unit instead of one hydroxyl group. For the sugar moiety, the large vicinal trans-coupling constants ($J_{1'',2''}$=8.1 Hz, $J_{2'',3''}$=8.5 Hz, $J_{3'',4''}$=9.2 Hz, and $J_{4'',5''}$=9.2 Hz) proved the axial orientation of protons 1″–5″. Assignment of the methoxyl group on the sugar unit was deduced by an HMBC correlation of the *O*-methyl protons to C-4″. These results indicated that the sugar unit was 4-*O*-methyl-β-glucopyranose. The position of attachment of the sugar unit at C-6 was established on the basis of an HMBC correlation between the anomeric proton and C-6. The D-configuration of 4-*O*-methyl-β-glucopyranose was established by comparing the specific rotations of the aqueous layer of its hydrolysate ($[\alpha]_D^{25}$ +58.2, *c* 0.10, MeOH) with that of 4-*O*-methyl-D-glucopyranose ($[\alpha]_D^{20}$ +80, *c* 1.30, MeOH).[25] The ^{1}H NMR spectroscopic data and the optical rotation of the aglycone unit, obtained by acid hydrolysis of compound **9**, were identical to those of nidurufin.[13,14] Therefore, compound **9** was determined as a new nidurufin-6-(4-*O*-methyl)-β-D-glucopyranose. Selected NOESY and HMBC correlations for compound **9** are presented in Fig. 1.

The structures of nine known compounds were elucidated on the basis of their HRMS and NMR spectroscopic data, which were identical in all respects to those of sterigmatocystin (**10**),[9] demethylsterigmatocystin (**11**),[10] dihydrodemethylsterigmatocystin (**12**),[11] sterigmatin (**13**),[10] austocystin F (**14**),[12] averufin (**15**),[13,14] aflatoxin B1,[15] paeciloquinone A,[16] and zeorin.[17]

Eight new compounds, **1**–**3**, and **5**–**9**, were subjected to biological assays for antimalarial (*P. falciparum* K1) activity and cytotoxicity against KB, MCF-7, NCI-H187, and Vero cells (Table 1). Compound **4** was not tested due to the sample shortage. Compounds **2**, **8**, and **9** exhibited moderate antimalarial activity (IC_{50} 1.60–8.54 μg/mL). Compounds **1**, **2**, and **3** showed strong to moderate cytotoxic activity against both NCI-H187 and Vero cells (IC_{50} 0.34–12.93 μg/mL), while compounds **6** and **9** were active against only NCI-H187 cells (IC_{50} 20.28 and 5.12 μg/mL, respectively). Only compounds **7** and **8** exhibited weak cytotoxicity to KB cells (IC_{50} 35.62 and 37.58 μg/mL, respectively).

Table 1
Biological activities of compounds **1**–**3** and **5**–**9**

Compound	Anti-malaria, IC_{50} (μg/mL)	Cytotoxicity, IC_{50} (μg/mL)			
		KB cells	MCF-7 cells	NCI-H187 cells	Vero cells
Compound **1**	>10[a]	>50[a]	>50[a]	1.17[a]	0.34[a]
Compound **2**	8.54	>50	>50	12.93	1.40
Compound **3**	>10	>50	>50	2.86	0.33
Compound **5**	>10[a]	>50[a]	>50[a]	>50[a]	>50[a]
Compound **6**	>10	>50	>50	20.28	>50
Compound **7**	>10	35.62	>50	>50	> 50
Compound **8**	3.88	37.58	>50	8.06	>50
Compound **9**	1.60	>50	>50	5.12	>50
Doxorubicin[b]	—	0.50	8.57	0.06	—
Ellipticine[b]	—	0.74	—	1.31	1.29
Dihydroartemisinin[c]	0.0005	—	—	—	—

[a] Partially soluble in 100% DMSO.
[b] Cytotoxicity controls.
[c] Antimalarial control.

3. Conclusion

In conclusion, nine new mycotoxins; five xanthones **1**–**5**, hydroxanthone **6**, and three anthraquinones **7**–**9**, together with nine known compounds; sterigmatocystin (**10**), demethylsterigmatocystin (**11**), dihydrodemethylsterigmatocystin (**12**), sterigmatin (**13**), austocystin F (**14**), averufin (**15**), alfatoxin B1, paeciloquinone A, and zeorin, were isolated from the insect fungus *A. coffeae* Henn. BCC 28712. Compounds **2**, **8**, and **9** exhibited antimalarial and cytotoxic activities. Compounds **1**, **3**, **6**, and **7** displayed only cytotoxic activity, while compound **5** was inactive in all biological tests.

4. Experimental

4.1. General procedures

Melting points were measured using an electrothermal IA9100 digital melting point apparatus and are uncorrected. Optical rotation measurements were obtained using a JASCO P-1030 digital polarimeter. UV and FT-IR spectra were recorded on a Varian Cary 1E UV–vis spectrophotometer and a Bruker Alpha spectrometer. NMR spectra were recorded on Bruker AV400 and Bruker AV500D spectrometers. ESITOF MS data were obtained on Micromass LCT and Bruker micrOTOF mass spectrometers. Preparative thin layer chromatography was performed on silica gel 60 GF_{254} (Merck). Column chromatography was performed on silica gel 60 (70–230 mesh ASTM, Merck).

4.2. Fungal material

The fungus *A. coffeae* Henn. was isolated from a Homoptera scale insect, collected from the underside of dicotyledonous leaf at Phu Khiao Wildlife sanctuary, Chaiyaphoom Province, Thailand. The specimen was identified by Mrs. Suchada Mongkolsamrit, BIOTEC. This fungus was deposited at the BIOTEC Culture Collection Laboratory (BCC) as BCC 28712 on December 3, 2007.

4.3. Fermentation and isolation

A. coffeae Henn. BCC 28712 was maintained on potato dextrose agar at 25 °C, the agar was cut into pieces (1×1 cm) and inoculated into 4×250 mL Erlenmeyer flasks containing 25 mL of potato dextrose broth (PDB, potato starch 4.0 g, dextrose 20.0 g/L). After incubation at 25 °C for 7 days on a rotary shaker (200 rpm), each primary culture was transferred into 1 L Erlenmeyer flask

containing 250 mL of the same liquid medium (PDB) and incubated under the same conditions for 4 days. Each 25 mL portion of the secondary culture was transferred into 40×1 L Erlenmeyer flasks containing 250 mL of an M102 medium (composition: sucrose (30.0 g), malt extract (20.0 g), bacto-peptone (2.0 g), yeast extract (1.0 g), KCl (0.5 g), $MgSO_4 \cdot 7H_2O$ (0.5 g), and KH_2PO_4 (0.5 g), in 1000 mL of distilled water), and fermentation was carried out under shaking conditions at 250 rpm, 25 °C for 15 days.

After filtration of the culture, the cells were macerated in MeOH (1 L) for 3 days and then in CH_2Cl_2 (1 L) for 3 days. The MeOH and the CH_2Cl_2 extracts were combined and evaporated under reduce pressure. Water (200 mL) was added, and the mixture was extracted with *n*-hexane (3×200 mL), followed by EtOAc (3×200 mL). The dark brown gum (4.38 g), obtained from EtOAc extraction of the mycelium, was fractionated using silica gel column (5×20 cm), step gradient elution with 20–100% EtOAc/*n*-hexane, to provide 7 fractions (1–7). After further purification by silica gel column chromatography, sterigmatin (1.7 mg) and compound **5** (0.305 g) were obtained from fractions 1 and 2, respectively. Fraction 3 was subjected to silica gel column chromatography (4×18 cm), using 10% MeOH/CH_2Cl_2 as an eluent, to furnish compound **5** (0.106 g), sterigmatocystin (67.2 mg), averufin (53.1 mg), and zeorin (0.36 mg). Trituration of fraction 4 with MeOH followed by filtration afforded more of sterigmatocystin (0.568 g) as a yellow solid. Further purification of fraction 5 by preparative HPLC using reverse phase column (SunFire C18 OBD, 5 mm, 19×150 μm, step gradient elution with 45–60% MeCN/H_2O) yielded compounds **2** (18.7 mg), **3** (39.4 mg), sterigmatocystin (32.9 mg), demethylsterigmatocystin (32.0 mg), and austocystin F (9.0 mg). Aflatoxin B1 (94.4 mg) and additional amount of compounds **3** (41.9 mg) and demethylsterigmatocystin (31.9 mg) were obtained from fraction 6 after further purification by preparative HPLC (step gradient elution with 45–70% MeCN/H_2O). Fraction 7 was subjected to silica gel chromatography (4.0×18 cm), using 10% MeOH/CH_2Cl_2 as an eluent, to give 4 fractions (7-1–7-4). More of compound **3** (12.9 mg) and demethylsterigmatocystin (6.9 mg) were obtained from fractions 7-1 and 7-2, respectively. Fractions 7-3 and 7-4 were further purified by preparative HPLC (step gradient elution with 20–50% MeCN/H_2O) to afford compounds **7** (6.1 mg), **8** (1.4 mg), **9** (15.7 mg), austocystin F (0.9 mg), and paeciloquinone A (6.4 mg).

Purification of the crude *n*-hexane extract (0.162 g) by silica gel column chromatography (4.0×18 cm), step gradient elution with 0–5% MeOH/CH_2Cl_2, followed by preparative thin layer chromatography provided compounds **1** (1.7 mg), **4** (1.7 mg), **5** (12.7 mg), sterigmatocystin (6.8 mg), demethylsterigmatocystin (7.3 mg), and zeorin (28.5 mg).

The culture broth was extracted with EtOAc (3×10 L) then evaporated to dryness, leaving a dark brown gum (0.807 g). The crude extract was fractionated using Sephadex LH 20 (3.5×50 cm), eluted with 100% MeOH, and then further purified by preparative HPLC (step gradient elution with 20–60% MeCN/H_2O) to give compounds **4** (4.2 mg), **5** (2.0 mg), **6** (9.3 mg), **8** (2.0 mg), **9** (13.3 mg), dihydrodemethylsterigmatocystin (14.5 mg), alfatoxin B1 (18.0 mg), and averufin (1.0 mg).

*4.3.1. Compound **1**.* Yellow solid; $[\alpha]_D^{26}$ −301.4 (*c* 0.21, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 238 (4.39), 256 (4.21), 324 (4.11) nm; IR (ATR) ν_{max} 3369, 2924, 2853, 1652, 1631, 1603, 1479, 1261, 1229, 1104 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 4.05 (3H, s, OCH_3-8), 4.77 (1H, d, *J*=7.0 Hz, H-3′), 5.43 (1H, s, H-2′), 6.36 (1H, s, H-2), 6.50 (1H, s, H-1′), 6.80 (1H, d, *J*=7.0 Hz, H-4′), 6.84 (1H, d, *J*=8.4 Hz, H-7), 7.05 (1H, d, *J*=8.4 Hz, H-5), 7.63 (1H, t, *J*=8.4 Hz, H-6), 13.51 (1H, s, OH-1); ^{13}C NMR (100 MHz, $CDCl_3$) δ 47.8 (C-3′), 56.7 (OCH_3-8), 94.4 (C-2), 102.8 (C-2′), 104.4 (C-4), 104.8 (C-9a), 106.3 (C-7), 109.9 (C-5), 111.2 (C-8a), 113.2 (C-4′), 135.6 (C-6), 145.6 (C-1′), 151.5 (C-4a), 157.8 (C-10a), 161.1 (C-8), 165.0 (C-3), 165.2 (C-1), 181.5 (C-9); HRMS (ESITOF) *m*/*z* 325.0705 $[M+H]^+$ (calcd for: $C_{18}H_{13}O_6$, 325.0707).

*4.3.2. Compound **2**.* Yellow solid; $[\alpha]_D^{24}$ −360.5 (*c* 0.32, acetone); UV (EtOH) λ_{max} (log ε) 247 (4.06), 277 (3.66), 328 (3.73) nm; IR (ATR) ν_{max} 3321, 3107, 2947, 1653, 1627, 1586, 1489, 1296, 1232, 1129, 1096 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) δ 3.96 (3H, s, OCH_3-1), 4.97 (1H, dt, *J*=2.4, 7.1 Hz, H-3′), 5.56 (1H, t, *J*=2.4 Hz, H-2′), 6.58 (1H, d, *J*=8.8 Hz, H-7), 6.60 (1H, s, H-2), 6.63 (1H, t, *J*=2.4 Hz, H-1′), 6.92 (1H, d, *J*=7.1 Hz, H-4′), 7.22 (1H, d, *J*=8.8 Hz, H-6), 8.28 (1H, s, OH-5), 12.67 (1H, s, OH-8); ^{13}C NMR (125 MHz, acetone-d_6) δ 47.9 (C-3′), 56.1 (OCH_3-1), 90.6 (C-2), 102.9 (C-2′), 105.6 (C-9a), 107.0 (C-4), 109.1 (C-8a), 109.6 (C-7), 113.6 (C-4′), 123.0 (C-6), 136.2 (C-5), 143.0 (C-10a), 145.0 (C-1′), 153.9 (C-4a), 154.4 (C-8), 163.5 (C-1), 164.7 (C-3), 180.8 (C-9); HRMS (ESITOF) *m*/*z* 363.0484 $[M+Na]^+$ (calcd for: $C_{18}H_{12}O_7Na$, 363.0475).

*4.3.3. Compound **3**.* Pale yellow solid; $[\alpha]_D^{26}$ −183.6 (*c* 0.44, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 231 (4.03), 241 (4.09), 324 (3.75) nm; IR (ATR) ν_{max} 3390, 2929, 1649, 1626, 1612, 1584, 1484, 1460, 1270, 1230, 1044 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.33 (3H, s, OCH_3-4″), 3.49 (1H, dd, *J*=3.7, 10.6 Hz, Ha-5″), 3.54 (1H, dd, *J*=3.0, 10.6 Hz, Hb-5″), 3.80 (1H, d, *J*=8.7 Hz, OH-3″), 4.06 (1H, ddd, *J*=2.2, 7.2, 8.7 Hz, H-3″), 4.29 (2H, obs, H-2″, H-4″), 4.44 (1H, d, *J*=10.1 Hz, OH-2″), 4.75 (1H, d, *J*=7.1 Hz, H-3′), 5.36 (1H, t, *J*=2.4 Hz, H-2′), 5.73 (1H, d, *J*=4.1 Hz, H-1″), 6.44 (1H, t, *J*=2.4 Hz, H-1′), 6.68 (1H, s, H-2), 6.69 (1H, d, *J*=8.4 Hz, H-7), 6.77 (1H, d, *J*=7.1 Hz, H-4′), 6.78 (1H, d, *J*=8.4 Hz, H-5), 7.46 (1H, t, *J*=8.4 Hz, H-6), 12.74 (1H, s, OH-8); ^{13}C NMR (100 MHz, $CDCl_3$) δ 47.9 (C-3′), 59.5 (OCH_3-4″), 70.7 (C-3″), 72.4 (C-4″), 72.5 (C-5″), 86.4 (C-2″), 94.6 (C-2), 102.3 (C-2′), 102.5 (C-1″), 106.1 (C-5), 106.6 (C-9a), 108.3 (C-4), 108.8 (C-8a), 111.5 (C-7), 113.3 (C-4′), 136.3 (C-6), 145.6 (C-1′), 153.5 (C-4a), 155.1 (C-10a), 159.6 (C-1), 162.2 (C-8), 164.7 (C-3), 181.8 (C-9); HRMS (ESITOF) *m*/*z* 457.1138 $[M+H]^+$ (calcd for: $C_{23}H_{21}O_{10}$, 457.1129).

*4.3.4. Compound **4**.* Pale yellow solid; $[\alpha]_D^{26}$ −219.2 (*c* 0.17, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 247 (3.97), 257 (3.94), 325 (3.82) nm; IR (ATR) ν_{max} 3350, 2920, 2852, 1656, 1605, 1480, 1310, 1262, 1246, 1167, 1109, 1086 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 2.27–2.30 (2H, m, H-2′), 3.67–3.72 (1H, m, Ha-1′), 4.04 (3H, s, OCH_3-8), 4.16 (2H, obs, Hb-1′, H-3′), 6.27 (1H, s, H-2), 6.46 (1H, d, *J*=5.6 Hz, H-4′), 6.83 (1H, d, *J*=8.2 Hz, H-7), 7.04 (1H, d, *J*=8.2 Hz, H-5), 7.62 (1H, t, *J*=8.2 Hz, H-6), 13.53 (1H, s, OH-1); ^{13}C NMR (125 MHz, $CDCl_3$) δ 31.6 (C-2′), 43.8 (C-3′), 56.6 (OCH_3-8), 67.7 (C-1′), 93.3 (C-2), 103.2 (C-4), 104.5 (C-9a), 106.1 (C-7), 109.6 (C-5), 111.0 (C-8a), 113.1 (C-4′), 135.2 (C-6), 151.7 (C-4a), 157.6 (C-10a), 160.8 (C-8), 165.0 (C-1), 166.5 (C-3), 181.2 (C-9); HRMS (ESITOF) *m*/*z* 327.0865 $[M+H]^+$ (calcd for: $C_{18}H_{15}O_6$, 327.0863).

*4.3.5. Compound **5**.* Pale yellow solid; $[\alpha]_D^{25}$ −235.5 (*c* 0.50, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 225 (3.69), 250 (3.87), 266 (3.74), 325 (3.58) nm; IR (ATR) ν_{max} 3330, 2925, 2852, 1665, 1636, 1612, 1478, 1454, 1375, 1269, 1233, 1132, 1090 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 3.40 (3H, s, OCH_3-4′), 5.68 (1H, d, *J*=2.8 Hz, H-1′), 6.46 (1H, s, H-4), 6.48 (1H, s, H-3′), 6.61 (1H, d, *J*=2.8 Hz, H-2′), 6.78 (1H, dd, *J*=0.7, 8.3 Hz, H-7), 6.87 (1H, dd, *J*=0.7, 8.3 Hz, H-5), 7.57 (1H, t, *J*=8.3 Hz, H-6), 11.77 (1H, s, OH-8), 12.44 (1H, s, OH-1); ^{13}C NMR (125 MHz, $CDCl_3$) δ 52.7 (OCH_3-4′), 90.8 (C-4), 95.5 (C-4′), 103.6 (C-2), 104.0 (C-1′), 107.0 (C-5), 107.4 (C-8a), 108.9 (C-9a), 111.2 (C-7), 113.7 (C-3′), 137.0 (C-6), 149.2 (C-2′), 156.0 (C-10a), 158.3 (C-1), 158.9 (C-4a), 161.3 (C-8), 167.2 (C-3), 185.0 (C-9); HRMS (ESITOF) *m*/*z* 339.0508 $[M-H]^-$ (calcd for: $C_{18}H_{11}O_7$, 339.0510).

*4.3.6. Compound **6**.* Red brown solid; $[\alpha]_D^{24}$ −133.2 (*c* 0.23, acetone); UV (EtOH) λ_{max} (log ε) 239 (4.28), 256 (4.25), 291 (3.91) nm; IR

(ATR) ν_{max} 3369, 2921, 2849, 1665, 1632, 1612, 1478, 1454, 1382, 1195, 1117 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) δ 1.81 (1H, ddd, J=4.1, 10.3, 13.1 Hz, Ha-7), 2.10 (1H, ddd, J=3.1, 3.1, 13.1 Hz, Hb-7), 2.62 (1H, dd, J=8.3, 17.8 Hz, Ha-5), 3.02 (1H, dd, J=4.9, 17.8 Hz, Hb-5), 4.15 (1H, d, J=3.3 Hz, OH-8), 4.33 (1H, d, J=3.9 Hz, OH-6), 4.39–4.41 (1H, m, H-6), 5.06 (1H, dd, J=3.4, 4.1 Hz, H-8), 5.40 (1H, s, OH-4′), 5.65 (1H, d, J=2.8 Hz, H-1′), 6.35 (1H, s, H-3′), 6.47 (1H, s, H-4), 6.68 (1H, d, J=2.8 Hz, H-2′), 13.21 (1H, s, OH-1); ^{13}C NMR (125 MHz, acetone-d_6) δ 37.1 (C-5), 38.9 (C-7), 60.9 (C-8), 62.0 (C-6), 89.1 (C-4′), 89.5 (C-4), 105.9 (C-9a), 106.5 (C-1′), 112.1 (C-2), 117.8 (C-3′), 118.2 (C-8a), 148.2 (C-2′), 157.7 (C-1), 158.5 (C-4a), 164.9 (C-3), 165.1 (C-10a), 182.0 (C-9); HRMS (ESITOF) m/z 347.0763 $[M+H]^+$ (calcd for: $C_{17}H_{15}O_8$, 347.0761).

4.3.7. Compound **7**. Yellow solid; $[\alpha]_D^{26}$ −133.2 (c 0.03, dioxane); UV (MeOH) λ_{max} (log ε) 221 (4.18), 265 (4.07), 284 (4.15), 318 (3.71), 444 (3.73) nm; IR (ATR) ν_{max} 3346, 2924, 2853, 1734, 1653, 1627, 1457, 1437, 1291, 1194, 1084 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 2.14–2.21 (2H, m, H-1′), 3.06 (1H, t, J=9.1 Hz, H-4″), 3.29 (1H, ddd, J=5.2, 7.8, 8.0 Hz, H-2″), 3.42–3.45 (1H, m, H-3″), 3.46 (3H, s, OCH_3-4″), 3.48–3.53 (3H, m, Ha-2′, H-5″, Ha-6″), 3.64 (1H, dd, J=4.5, 9.7 Hz, Hb-6″), 4.14 (2H, obs, Hb-2′, H-4′), 4.76 (1H, t, J=5.4 Hz, OH-6″), 5.14 (1H, d, J=7.8 Hz, H-1″), 5.34 (1H, d, J=5.5 Hz, OH-3″), 5.55 (1H, d, J=5.0 Hz, OH-2″), 6.56 (1H, d, J=4.82 Hz, H-3′), 6.94 (1H, d, J=2.4 Hz, H-7), 7.14 (1H, s, H-4), 7.23 (1H, d, J=2.4 Hz, H-5), 12.2 (2H, br s, OH-1, OH-8); ^{13}C NMR (125 MHz, DMSO-d_6) δ 30.7 (C-1′), 44.0 (C-4′), 60.1 (OCH_3-4″), 60.6 (C-6″), 67.7 (C-2′), 73.7 (C-2″), 76.3 (C-3″), 76.4 (C-5″), 79.3 (C-4″), 100.1 (C-1″), 102.1 (C-4), 109.5 (C-5), 109.5 (C-7), 110.9 (C-8a), 111.6 (C-9a), 113.9 (C-3′), 120.8 (C-2), 135.1 (C-4a), 136.1 (C-10a), 159.7 (C-1), 164.0 (C-6), 164.4 (C-8), 166.0 (C-3), 181.2 (C-10), 190.0 (C-9); HRMS (ESITOF) m/z 515.1199 $[M-H]^-$ (calcd for: $C_{25}H_{23}O_{12}$, 515.1195).

4.3.8. Compound **8**. Yellow solid; $[\alpha]_D^{24}$ −76.81 (c 0.04, MeOH); UV (MeOH) λ_{max} (log ε) 222 (4.18), 265 (4.12), 285 (4.18), 318 (3.78), 442 (3.80) nm; IR (ATR) ν_{max} 3408, 2924, 2854, 1716, 1628, 1611, 1466, 1388, 1294, 1193, 1077 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 3.06 (1H, t, J=9.1 Hz, H-4″), 3.29–3.31 (1H, m, H-2″), 3.42 (1H, m, H-3″), 3.46 (3H, s, OCH_3-4″), 3.51–3.53 (2H, m, H-5″, Ha-6″), 3.57 (1H, dd, J=1.9, 9.9 Hz, Ha-2′), 3.64 (1H, dd, J=3.4, 9.7 Hz, Hb-6″), 3.90 (1H, d, J=9.9 Hz, Hb-2′), 3.95 (1H, d, J=5.6 Hz, H-4′), 4.44 (1H, br s, H-1′), 4.77 (1H, br s, OH-6″), 5.14 (1H, d, J=7.8 Hz, H-1″), 5.34 (1H, d, J=5.2 Hz, OH-3″), 5.56 (1H, d, J=4.5 Hz, OH-2″), 5.67 (1H, d, J=2.9 Hz, OH-1′), 6.65 (1H, d, J=5.6 Hz, H-3′), 6.94 (1H, d, J=2.1 Hz, H-7), 7.09 (1H, s, H-4), 7.22 (1H, d, J=2.1 Hz, H-5), 12.14 (1H, br s, OH-1), 12.37 (1H,br s, OH-8); ^{13}C NMR (125 MHz, DMSO-d_6) δ 53.8 (C-4′), 60.1 (OCH_3-4″), 60.6 (C-6″), 73.4 (C-1′), 73.7 (C-2″), 75.4 (C-2′), 76.2 (C-5″), 76.4 (C-3″), 79.3 (C-4″), 100.1 (C-1″), 102.3 (C-4), 109.5 (C-5), 109.6 (C-7), 110.9 (C-8a), 111.5 (C-9a), 113.6 (C-3′), 118.0 (C-2), 135.1 (C-10a), 136.2 (C-4a), 160.0 (C-1), 164.1 (C-6), 164.4 (C-8), 166.1 (C-3), 181.1 (C-10), 189.9 (C-9); HRMS (ESITOF) m/z 531.1144 $[M-H]^-$ (calcd for: $C_{25}H_{23}O_{13}$, 531.1145).

4.3.9. Compound **9**. Orange solid; $[\alpha]_D^{24}$ −62.0 (c 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 223 (4.18), 265 (4.11), 288 (4.14), 319 (3.73), 447 (3.80) nm; IR (ATR) ν_{max} 3374, 2929, 1630, 1597, 1572, 1469, 1408, 1300, 1203, 1168, 1079 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 1.54 (2H, m, H-3′), 1.55 (3H, s, H-6′), 1.83 (1H, d, J=13.1 Hz, Ha-4′), 2.17 (1H, ddd, J=6.7, 13.1, 13.1 Hz, Hb-4′), 3.06 (1H, t, J=9.1 Hz, H-4″), 3.29 (1H, ddd, J=5.2, 8.1, 8.5 Hz, H-2″), 3.43–3.45 (1H, m, H-3″), 3.46 (3H, s, OCH_3-4″), 3.51–3.53 (2H, m, H-5″, Ha-6″), 3.64 (1H, dd, J=4.9, 10.2 Hz, Hb-6″), 3.76–3.78 (1H, m, H-2′), 4.77 (1H, t, J=5.6 Hz, OH-6″), 5.06 (1H, s, H-1′), 5.15 (1H, d, J=8.1 Hz, H-1″), 5.34 (1H, d, J=5.5 Hz, OH-3″), 5.41 (1H, d, J=4.1 Hz, OH-2′), 5.55 (1H, d, J=5.1 Hz, OH-2″), 6.95 (1H, d, J=2.4 Hz, H-7), 7.06 (1H, s, H-4), 7.25 (1H, d, J=2.4 Hz, H-5), 12.14 (1H, s, OH-8), 12.43 (1H, s, OH-1); ^{13}C NMR (125 MHz, DMSO-d_6) δ 23.1 (C-3′), 27.7 (C-6′), 30.6 (C-4′), 60.1 (OCH_3-4″), 60.6 (C-6″), 63.9 (C-2′), 71.2 (C-1′), 73.7 (C-2″), 76.2 (C-5″), 76.4 (C-3″), 79.3 (C-4″), 100.1 (C-1″), 102.1 (C-5′), 108.1 (C-4), 109.1 (C-9a), 109.4 (C-5), 109.6 (C-7), 111.1 (C-8a), 115.6 (C-2), 134.0 (C-4a), 135.2 (C-10a), 159.1 (C-1), 160.6 (C-3), 164.1 (C-6), 164.3 (C-8), 181.1 (C-10), 189.7 (C-9); HRMS (ESITOF) m/z 559.1443 $[M-H]^-$ (calcd for: $C_{27}H_{27}O_{13}$, 559.1457).

4.4. Methylation of compound 1 and sterigmatocystin

Compound **1** (2.1 mg, 6.5 mmol) was methylated with MeI (1.0 mL, 16.2 mmol) in K_2CO_3 (2.2 mg, 16.2 mmol) and acetone (2.0 mL) at room temperature for 18 h. The reaction mixture was evaporated then diluted with H_2O (1.0 mL) and extracted with EtOAc (1.0 mL). The organic layer was concentrated under reduced pressure to leave a dark brown solid, which was purified by preparative thin layer chromatography (using 40% EtOAc/*n*-hexane as eluent) to furnish the dimethylated product **16** (2.0 mg, 91% yield, $[\alpha]_D^{25}$ −139.2, c 0.10, $CHCl_3$) as a white solid.

Methylation of sterigmatocystin (**10**, 10.4 mg, 32.1 mmol) was conducted as the same manner to also obtain product **16** (9.0 mg, 83% yield, $[\alpha]_D^{25}$ −135.2, c 0.56, $CHCl_3$).

The ^1H NMR spectra and the optical rotations of these two dimethylated products were identical to those of the reported monomethyl ether derivative of sterigmatocystin.[20,26]

4.5. Hydrolysis of compound 3

Compound **3** (5.0 mg, 10.9 mmol) was hydrolyzed with 3 M aqueous HCl (0.5 mL) in dioxane (0.1 mL) at 90 °C for 12 h. The reaction mixture was then diluted with H_2O (2.0 mL) and extracted with EtOAc (2.0 mL). The aqueous layer was concentrated in vacuo to yield 4-*O*-methyl-L-arabinopyranose (1.2 mg, 67% yield, $[\alpha]_D^{25}$ +137.0, c 0.05, water).

4.6. Hydrolysis of compound 7

Compound **7** (2.8 mg, 5.4 mmol) was hydrolyzed by the method described for compound **3**. The aqueous layer was concentrated in vacuo to yield 4-*O*-methyl-D-glucopyranose (0.5 mg, 52% yield, $[\alpha]_D^{25}$ +54.0, c 0.02, MeOH). The organic layer was evaporated to dryness under reduced pressure to obtain the aglycone unit (1.3 mg, 71% yield, $[\alpha]_D^{25}$ −176.0, c 0.01, dioxane) whose ^1H NMR spectrum and the optical rotation were identical to those of versicolorin B.[24]

4.7. Hydrolysis of compound 9

Compound **9** (5.0 mg, 8.9 mmol) was hydrolyzed as mentioned above. The aqueous layer was concentrated in vacuo to yield 4-*O*-methyl-D-glycopyranose (1.5 mg, 94% yield, $[\alpha]_D^{25}$ +58.2, c 0.10, MeOH). The organic layer was evaporated to dryness under reduced pressure to obtain the aglycone (3.0 mg, 88% yield, $[\alpha]_D^{24}$ +113.0, c 0.01, MeOH) whose ^1H NMR spectrum as well as optical rotation were in good agreement with those of nidurufin.[13,14]

4.8. Biological assays

Assay for activity against *P. falciparum* (K1, multidrug resistant strain) was performed using the microculture radioisotope technique.[27] Cytotoxicity to Vero cells (African green monkey kidney fibroblasts) was performed using the green fluorescent protein (GFP)-based method.[28] Anticancer activities against KB cells (oral human epidermoid carcinoma), MCF-7 cells (human breast cancer),

and NCI-H187 cells (human small-cell lung cancer) were evaluated using the resazurin microplate assay.[29]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tet.2012.07.059.

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