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Cerebrosides and 2-Pyridone Alkaloids from the Halotolerant Fungus *Penicillium chrysogenum* Grown in a Hypersaline Medium

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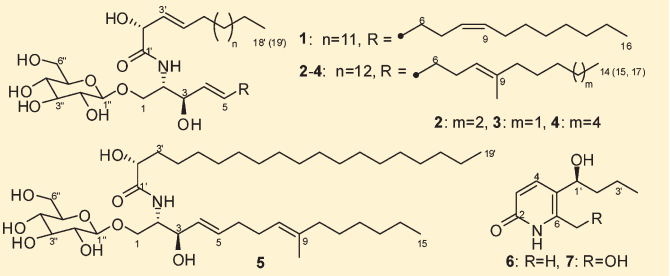
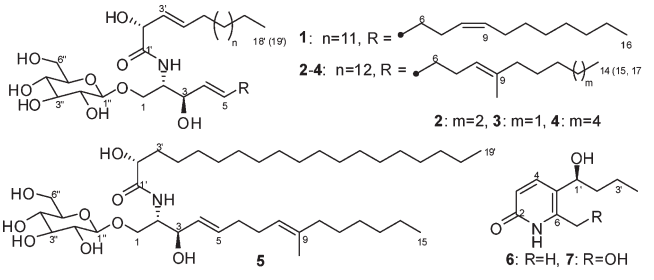
Supporting Information

ABSTRACT: Five new cerebrosides, chrysogesides A−E (**1**−**5**), and two new 2-pyridone alkaloids, chrysogedones A and B (**6** and **7**), were isolated from the fermentation broth of *Penicillium chrysogenum* PXP-55, a halotolerant fungus grown in a hypersaline medium. Among them, chrysogesides B−D (**2**−**4**) are the first cerebrosides that contain an unsaturated C_{19}-fatty acid. Their structures were identified by spectroscopic and chemical methods, including CD spectroscopy as well as the modified Mosher's method. Compound **2** showed antimicrobial activity against *Enterobacter aerogenes* with an MIC value of 1.72 μM.



As part of our ongoing efforts to discover structurally novel and bioactive natural compounds from halotolerant fungi,[1−3] a marine-derived halotolerant fungal strain, PXP-55, identified as *Penicillium chrysogenum*, was isolated from the surface of the roots of the mangrove plant, *Rhizophora stylosa* (Rhizophoraceae), collected in Wenchang, Hainan Province, China. The fungus *P. chrysogenum* has been reported to produce polyketides, prenylated polyketides, and terpenoids such as berkeleydione,[4] deoxyartemisinin,[5] trichodimerol,[6] and xanthohumol 4′-*O*-β-glucopyranoside[7] and the sorbicillinoid alkaloids sorbicillactones A and B.[8] The EtOAc extract of the fermentation broth of *Penicillium chrysogenum* PXP-55 grown at 10% salinity showed cytotoxicity against P388 cells and a distinct HPLC profile from extracts produced at 0% and 3% salinity (Figure S40). Chemical studies of this extract resulted in the isolation and identification of five new cerebrosides, chrysogesides A−E (**1**−**5**), and two new 2-pyridone alkaloids, chrysogedones A and B (**6** and **7**), together with six known compounds, pyrrole-3-carboxylic acid,[9] 3-(hydroxymethyl)-6-[4-(3-methylbut-2-enyloxy) benzyl]piperazine-2,5-dione,[10] 4-(2-hydroxyethyl)phenol,[11] 2-(2-hydroxyphenyl)acetic acid,[12] methyl 2-(4-hydroxyphenyl) acetate,[13] and 2-(4-hydroxyphenyl)acetic acid.[14]



Chrysogeside A (**1**) was obtained as an amorphous, white powder. The molecular formula was determined to be $C_{40}H_{74}NO_9$ according to a HRESIMS peak at *m*/*z* 712.5361 $[M + H]^+$. The NMR spectra and the ESIMS/MS pattern of **1** were almost the same as those of alternaroside C,[3] a compound we isolated from *Alternaria raphani*, except for the presence of a C_{16} sphingosine chain rather than a C_{18} chain. The loss of *m*/*z* 252.2 ($C_{16}H_{30}NO$) and the fragment at *m*/*z* 280.3 ($C_{18}H_{32}O_2$) in the ESIMS/MS spectrum (Figures S24 and S38) supported that the sphingosine and fatty acid units were 2-amino-1,3-dihydroxyhexadec-4,8-diene and 2-hydroxyoctadec-3-enoic acid, respectively. Methanolysis of **1** gave methyl 2*R*-hydroxyoctadec-3-enoate ($[\alpha]^{25}_D$ −56), which was identified by ESIMS and ^{1}H NMR data (*m*/*z* 335 $[M + Na]^+$, Figures S29 and S32) and methyl D-glucopyranosides ($[\alpha]^{25}_D$ +74, ESIMS *m*/*z* 195 $[M + H]^+$).[15] The NMR signals of the anomeric proton and carbon at $\delta_{H/C}$ 4.10 (d, *J* = 7.7)/103.5 (CH) suggested the β-configuration of the glucoside.[3] The values of $\delta_{C\text{-}1-C\text{-}13}$ and $\delta_{C\text{-}1'-C\text{-}15'}$ and the specific rotation ($[\alpha]^{25}_D$ −6) of **1** were close to those of alternaroside C ($[\alpha]^{20}_D$ −4),[3] suggesting that **1** shared the same 2*S*,2′*R*,3*R*- and 3′*E*,4*E*,8*Z*-configurations with alternaroside C. Thus, the structure of **1** was established as (2*R*,3*E*)-2-hydroxy-*N*-[(2*S*,3*R*,4*E*,8*Z*)-1-β-D-glucopyranosyloxy-3-hydroxyhexadec-4,8-dien-2-yl]octadec-3-enamide.

Chrysogesides B−D (**2**−**4**) were also cerebrosides with molecular formulas of $C_{41}H_{75}NO_9$, $C_{40}H_{73}NO_9$, and $C_{43}H_{79}NO_9$ by HRESIMS (*m*/*z* 726.5493 $[M + H]^+$, 712.5346 $[M + H]^+$, and 754.5852 $[M + H]^+$, respectively). The similar NMR spectroscopic data (Table 1 and Table S1)

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Table 1. ^{1}H and ^{13}C NMR Data for 1 and 2 (^{1}H 600 MHz, ^{13}C 150 MHz, DMSO-d_6, TMS, δ ppm)

	1		2	
no.	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	68.6, CH_2	3.95, m; 3.50, dd (11.0, 4.4)	68.7, CH_2	3.95, m; 3.51, dd (11.0, 4.4)
2	52.9, CH	3.78, m	52.9, CH	3.78, m
3	70.5, CH	3.97, m	70.5, CH	3.97, m
4	131.0, CH	5.36, dd (14.3, 6.6)	130.9, CH	5.36, dd (15.6, 6.6)
5	130.7, CH	5.55, dt (14.3, 6.2)	130.9, CH	5.56, dt (15.6, 6.2)
6	32.0, CH_2	1.97, m	27.4, CH_2	1.94, m
7	28.6, CH_2	1.97, m	32.2, CH_2	1.94, m
8	129.5, CH	5.38, "t" like (3.3)	123.5, CH	5.09, br t (5.6)
9	130.2, CH	5.38, "t" like (3.3)	134.8, C	
10	28.7, CH_2	1.97, m	39.5, CH_2	1.94, m
11	31.9, CH_2	1.23, m	27.3, CH_2	1.23, m
12	29.0, CH_2	1.23, m	29.1, CH_2	1.23, m
13	28.9, CH_2	1.23, m	31.4, CH_2	1.26, m
14	31.3, CH_2	1.26, m	22.1, CH_2	1.29, m
15	22.1, CH_2	1.29, m	13.9, CH_3	0.85, t (6.6)
16	13.9, CH_3	0.85, t (6.6)		
CH_3-9			15.7, CH_3	1.54, br s
1′	172.0, C		172.0, C	
2′	71.9, CH	4.30, br t (5.5)	71.9, CH	4.30, br t (4.4)
3′	129.0, CH	5.43, dd (15.4, 5.5)	129.1, CH	5.43, dd (15.4, 5.5)
4′	130.9, CH	5.67, dt (15.4, 6.6)	130.9, CH	5.68, dt (15.4, 6.4)
5′	31.7, CH_2	1.97, m	31.7, CH_2	1.94, m
6′−15′	29.0 × 10, CH_2	1.23, m	29.1 × 10, CH_2	1.23, m
16′	31.3, CH_2	1.26, m	28.8, CH_2	1.23, m
17′	22.1, CH_2	1.29, m	31.4, CH_2	1.26, m
18′	14.0, CH_3	0.85, t (6.6)	22.1, CH_2	1.29, m
19′			13.9, CH_3	0.85, t (6.6)
1″	103.5, CH	4.10, d (7.7)	103.5, CH	4.11, d (7.7)
2″	73.4, CH	2.96, m	73.4, CH	2.96, m
3″	76.5, CH	3.13, m	76.6, CH	3.14, m
4″	70.0, CH	3.03, m	70.0, CH	3.04, m
5″	76.9, CH	3.08, m	76.9, CH	3.08, m
6″	61.0, CH_2	3.66, dd (12.0, 6.6);	61.1, CH_2	3.66, br dd (12.0, 6.6);
		3.43, ddd (11.0, 5.5, 5.4)		3.43, ddd (12.0, 6.6, 5.5)
NH		7.39, d (9.9)		7.39, d (8.8)
OH-3		4.95, d (5.5)		4.95, d (5.5)
OH-2′		5.78, d (4.4)		5.78, d (5.0)
OH-2″		4.99, d (4.6)		4.98, d (4.6)
OH-3″		4.92, d (4.4)		4.92, d (5.0)
OH-4″		4.93, d (4.4)		4.94, d (4.6)
OH-6″		4.52, t (6.6)		4.52, t (6.0)

and the same ESIMS/MS patterns (Figures S26, S27, and S38) of **2**−**4** compared to those of alternaroside B[3] suggested they are analogues. Methanolysis of **2**−**4** afforded the same methyl D-glucopyranosides as found for **1** and the same methyl C_{19}-fatty acid ester that was identified by GC-MS (t_R 16.92 min, m/z 326 $[M]^+$, Figure S37) and further supported by the key fragments at m/z 294.3 ($C_{19}H_{34}O_2$) and 276.3 ($C_{19}H_{32}O$) in the ESIMS/MS spectra of **2**−**4**, indicating that the difference among compounds **2**−**4** is the chain length of the sphingosine unit. Fatty acids with odd carbons are rare in nature, and the methyl C_{19}-fatty acid ester is a new structure that was carefully identified as methyl (2*R*,3*E*)-2-hydroxynonadec-3-enoate by MS and NMR analysis including the large $J_{3',4'}$ (15.4 Hz) and comparison of the $[\alpha]^{25}_D$ (−51) with the specific rotation of (2*R*,3*E*)-hydroxyoctadec-3-enoate. The loss of m/z 252.2 ($C_{16}H_{30}NO$) for **2**, m/z 238.2 ($C_{15}H_{28}NO$) for **3**, and m/z 280.3 ($C_{18}H_{34}NO$) for **4** in the ESIMS/MS spectra indicated that sphingosine units of **2**−**4** were 2-amino-1,3-dihydroxy-9-methylpentadec-4,8-diene, 2-amino-1,3-dihydroxy-9-methyltetradec-4,8-diene, and 2-amino-1,3-dihydroxy-9-methylheptadec-4,8-diene, respectively. This deduction was also confirmed by $^{1}H-^{1}H$ COSY correlations from H-1 to H-8 and from H-13 to H-15 and the key HMBC correlations

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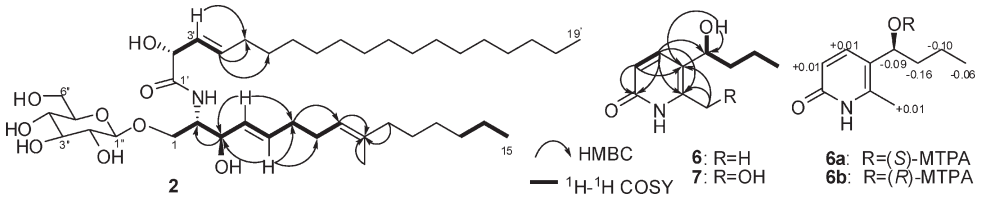

Figure 1. Key ^{1}H−^{1}H COSY and HMBC correlations of **2**, **6**, and **7** and $\Delta\delta$ ($=\delta_S - \delta_R$) values for (*S*)- and (*R*)-MTPA esters of **6**.

from H-8, H-10, and CH_3-9 to the sp^2-quaternary carbon (C-9) (Figure 1). The coincidence of NMR data from C-1 to C-10 and C-1′ to C-4′ and the close specific rotations ($[\alpha]^{25}_D$ −8 for **2**, −5 for **3**, and −4 for **4**) to that of alternaroside B ($[\alpha]^{20}_D$ −9)[3] supported the configurations of all sphingosine units in **2**−**4** as 2′*S*,3′*R*,4′*E*,8′*E*. Therefore, the new structures of **2**−**4** were determined as (2*R*,3*E*)-2-hydroxy-*N*-[(2*S*,3*R*,4*E*,8*E*)-l-β-D-glucopyranosyloxy-3-hydroxy-9-methylpentadec-4,8-dien-2-yl]nonadec-3-enamide (**2**), (2*R*,3*E*)-2-hydroxy-*N*-[(2*S*,3*R*,4*E*,8*E*)-l-β-D-glucopyranosyloxy-3-hydroxy-9-methyltetradec-4,8-dien-2-yl]nonadec-3-enamide (**3**), and (2*R*,3*E*)-2-hydroxy-*N*-[(2*S*, 3*R*,4*E*,8*E*)-l-β-D-glucopyranosyloxy-3-hydroxy-9-methylheptadec-4,8-dien-2-yl]nonadec-3-enamide (**4**).

The molecular formula of chrysogeside E (**5**) was determined to be $C_{41}H_{77}NO_9$ according to its HRESIMS peak at *m*/*z* 728.5646 $[M + H]^+$, with a molecular weight 2 amu greater than **2**. Except for a saturated $-CH_2CH_2-$ unit substitution for the corresponding $-CH{=}CH-$ unit, the NMR spectrum of **5** was almost the same as that of **2**. Besides, compound **5** also showed the same ESIMS/MS pattern at *m*/*z* 710.6 $[M + H - H_2O]^+$, 548.5 $[M + H - 180]^+$, 530.5 $[548.5 - H_2O]^+$, 296.3 $[548.5 - 252.2]^+$, and 278.3 $[296.3 - H_2O]^+$ (Figures S28 and S38), indicating that **5** was the hydrogenated derivative of the double bond of **2** in the fatty acid moiety. Methanolysis of **5** also gave methyl D-glucopyranosides and methyl 2*R*-hydroxynonadecanoate, which was identified by ^{1}H NMR and ESIMS data (*m*/*z* 351 $[M + Na]^+$, Figures S31 and S36) and specific rotation ($[\alpha]^{25}_D$ −50). These data combined with specific rotation ($[\alpha]^{25}_D$ −3) confirmed that the structure of **5** was (2*R*)-2-hydroxy-*N*-[(2*S*,3*R*,4*E*,8*E*)-l-β-D-glucopyranosyloxy-3-hydroxy-9-methylpentadec-4,8-dien-2-yl]nonadecanamide.

Chrysogedone A (**6**) was obtained as a yellow, amorphous powder. Its molecular formula was determined as $C_{10}H_{15}NO_2$ according to its HREIMS peak at *m*/*z* 181.1099 $[M]^+$. Diagnostic IR absorption peaks were observed for a hydroxy group, an amide group, and a 2-pyridone nucleus at 3482, 3392, 1715, 1641, and 1557 cm^{-1}, respectively.[16] Its 1D NMR spectra revealed three sp^2 quaternary carbons, two sp^2 and one sp^3 methines, two sp^3 methylenes, and two methyl groups (Table 2). The two sp^2 methines at $\delta_{H/C}$ 6.20/112.8 and 7.57/144.6 and three sp^2 quaternary carbons at δ_C 161.5, 157.6, and 119.2 suggested a 5,6-disubstituted 2-pyridone nucleus, which was further supported by HMBC correlations from H-3 (δ_H 6.20, d) to C-5 (δ_C 119.2, C) and C-2 (δ_C 161.5, C) and from H-4 (δ_H 7.57, d) to C-6 (δ_C 157.6, C). ^{1}H−^{1}H COSY connections from OH-1′ (δ_H 5.16, br s) to H-4′ (δ_H 0.87, t) through H-1′ (δ_H 4.48, t), H-2′ (δ_H 1.42/1.58, m), and H-3′ (δ_H 1.21/1.33, m) indicated a $CH_3-CH_2-CH_2-CH-OH$ moiety in the molecule. The key HMBC correlations between H-4 and C-1′ and between OH-1′ and C-5 revealed that the C_4-moiety was connected to the 5-position of the 2-pyridone nucleus. According to the HMBC connections of CH_3- with C-5 and C-6, the remaining methyl group was linked to the 6-position of the 2-pyridone nucleus (Figure 1). The absolute configuration of **6** was determined by the modified Mosher's method.[17] When reacted with (*R*)- and (*S*)-MTPA chloride, compound **6** gave the corresponding (*S*)- and (*R*)- MTPA esters **6a** and **6b**, respectively, and the ^{1}H NMR spectra of **6a** and **6b** were assigned according to their ^{1}H−^{1}H COSY correlations. The observed chemical shift differences $\Delta\delta_{S-R}$ (Figure 1) clearly defined the absolute configuration of C-1′ as *S*. Thus, the structure of compound **6** was elucidated as (*S*)-5-(1-hydroxybutyl)-6-methylpyridin-2(1*H*)-one.

Table 2. ^{1}H and ^{13}C NMR Data for 6 and 7 (^{1}H 600 MHz, ^{13}C 150 MHz, DMSO-d_6, TMS, δ ppm)

	6		**7**	
no.	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
2	161.5, C		161.3, C	
3	112.8, CH	6.20, d (9.2)	114.1, CH	6.30, d (9.2)
4	144.6, CH	7.57, d (9.7)	144.7, CH	7.62, d (9.6)
5	119.2, C		120.0, C	
6	157.6, C		158.4, C	
1′	66.2, CH	4.48, t (6.6)	65.5, CH	4.60, t (8.2)
2′	39.2, CH_2	1.58, m; 1.42, m	39.4, CH_2	1.57, m; 1.41, m
3′	18.3, CH_2	1.33, m; 1.21, m	18.3, CH_2	1.33, m; 1.21, m
4′	13.8, CH_3	0.87, t (7.3)	13.9, CH_3	0.86, t (7.3)
6-CH_3 or 6-$\underline{CH_2}$OH	16.6, CH_3	2.22, s	57.1, CH_2	4.27, d (5.0)
1′-OH		5.16, br s		5.20, d (3.7)
2- $CH_2\underline{OH}$				5.53, t (5.9)

The molecular formula of chrysogedone B (**7**) was determined to be $C_{10}H_{15}NO_3$ based on the molecular ion peak at *m*/*z* 197.1041 $[M]^+$ in the HREIMS spectrum, with one oxygen atom more than that of **6**. Its 1D NMR spectra were similar to those of **6** except for the lack of a methyl group, an additional oxygenated methylene group, and downfield shifts for C-3, C-5, and C-6. These data indicated that **7** was the hydroxylated derivative of **6** on the 6-methyl group, which was also supported by similar 2D NMR spectra (Figures S21−23). Compared with **6**, compound **7** displayed a close specific rotation ($[\alpha]^{25}_D$ −13 in **7** vs −17 in **6**) and similar CD Cotton effects at 208 ($\Delta\varepsilon$ −8.3), 233 ($\Delta\varepsilon$ +0.9), and 296 ($\Delta\varepsilon$ −1.2) nm (Figure S41), indicating the same *S*-configuration. Thus, the structure of **7** was elucidated as (*S*)-5-(1-hydroxybutyl)-6-(hydroxymethyl)pyridin-2(1*H*)-one.

The new isolates **1**−**5** were evaluated for cytotoxicity against P388 and HeLa cells with the MTT method,[18] and **6** and **7** were assayed against HL-60 and A549 cells with the MTT and SRB methods,[19] respectively. Their antimicrobial activities against *Staphylococcus aureus*, *Escherichia coli*, *Enterobacter aerogenes*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Candida albicans*

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were also evaluated by an agar dilution method.[20] Compound **2** showed antimicrobial activity against *Enterobacter aerogenes* with an MIC value of 1.72 μM, while none of the compounds had cytotoxic effects on the four cancer cell lines (IC_{50} > 50 μM) or any additional antimicrobial activities (MIC > 150 μM).

EXPERIMENTAL SECTION

General Experimental Procedures. Specific rotations were obtained on a JASCO P-1020 digital polarimeter. UV spectra were recorded on a Waters 2487 absorbance detector. CD spectra were measured on a JASCO J-715 spectropolarimeter. IR spectra were taken on a Nicolet NEXUS 470 spectrophotometer in KBr disks. NMR data were recorded on a JEOL JNM-ECP 600 spectrometer using TMS as internal standard, and chemical shifts were recorded as δ values. ESIMS and EIMS were measured on a Q-TOF ULTIMA GLOBAL GAA076 LC and VG-Auto Spec-3000 mass spectrometer, respectively. The GC-MS system consisted of an Agilent 6890 gas chromatograph and an Agilent 5973 mass selective detector in the electron-ionization mode. Semipreparative HPLC was performed using an ODS column [YMC-pack ODS-A, 10 × 250 mm, 5 μm, 4 mL/min] on a Waters 600 multisolvent delivery system equipped with a photodiode array detector (Waters 996). TLC and column chromatography (CC) were performed on plates precoated with silica gel GF_{254} (10−40 μm) and over silica gel (200−300 mesh, Qingdao Marine Chemical Factory) and Sephadex LH-20 (Amersham Biosciences), respectively. Vacuum-liquid chromatography (VLC) utilized silica gel H (Qingdao Marine Chemical Factory).

Fungal Material. The working strain *Penicillium chrysogenum* PXP-55 was isolated from the surface of the roots of the mangrove plant, *Rhizophora stylosa* (Rhizophoraceae), collected in Wenchang, Hainan Province, China. It was identified according to its morphological characteristics and 18S rRNA sequences (Supporting Information; GenBank GU227344) by Prof. C. X. Fang, China Center for Type Culture Collection. The voucher specimen was deposited in our laboratory at −80 °C. The producing strain was prepared on potato dextrose agar slants and stored at 4 °C.

Fermentation and Extraction. *P. chrysogenum* PXP-55 was incubated on a rotary shaker (180 rpm) at 28 °C for 10 days in 500 mL × 400 conical flasks containing the liquid medium (150 mL/flask) composed of maltose (20 g/L), mannitol (20 g/L), monosodium glutamate (10 g/L), glucose (10 g/L), yeast extract (3 g/L), corn steep liquor (1 g/L), $MgSO_4$ (0.3 g/L), KH_2PO_4 (0.5 g/L), artificial sea salt (100 g/L), and tap water after adjusting its pH to 7.0. The fermented whole broth (60 L) was filtered through cheesecloth to separate into filtrate and mycelia. The filtrate was concentrated in vacuo to about a quarter of its original volume and then extracted three times with EtOAc to give an EtOAc solution, while the mycelia were extracted three times with acetone. The acetone solution was evaporated under reduced pressure to afford an aqueous solution. The aqueous solution was extracted three times with EtOAc to give another EtOAc solution. Both EtOAc solutions were combined and concentrated in vacuo to give an EtOAc extract (40 g).

Purification. The EtOAc extract (40 g) was subjected to VLC on a silica gel column using step gradient elution with MeOH−$CHCl_3$ (0−100%). The collected materials were combined into five fractions based on TLC properties. Fractions 3 (3.5 g) and 4 (2.4 g) were eluted with petroleum ether−acetone (v/v 10:1, 8:1, 6:1, 3:1, 1:1) and purified into 10 subfractions on a silica gel column, respectively. Subfractions 3-3 (506 mg) and 3-6 (314 mg) were separated into three subfractions (3-3-1−3-3-3) and two subfractions (3-6-1 and 3-6-2) by a Sephadex LH-20 column (MeOH−$CHCl_3$, 1:1), respectively. The subfraction 3-3-3 (278 mg) was further separated by HPLC (35% MeOH) to yield pyrrole-3-carboxylic acid (84.6 mg, t_R 19 min), and the subfraction 3-6-1 (232 mg) was separated by HPLC (45% MeOH) to yield **6** (41.7 mg, t_R 8.4 min). Subfractions 4−4 (802 mg)/4−5 (460 mg)/4−10 (438 mg) were separated into subfractions 4-4-1−4-4-4, 4-5-1 and 4-5-2, and 4-10-1−4-10-3 by a Sephadex LH-20 column (MeOH−$CHCl_3$, 1:1), respectively. Subfraction 4-4-2 (148 mg) was further separated by HPLC (30% MeOH) to yield methyl 2-(4-hydroxyphenyl)acetate (5.5 mg, t_R 15.6 min), subfraction 4-4-3 (212 mg) was separated by HPLC (20% MeOH and 0.2% TFA) to yield 4-(2-hydroxyethyl)phenol (6.4 mg, t_R 11.3 min), 2-(2-hydroxyphenyl)acetic acid (10.9 mg, t_R 16.5 min), and 2-(4-hydroxyphenyl)acetic acid (24.3 mg, t_R 18.58 min), and subfraction 4-5-2 (221 mg) was separated by HPLC (30% MeOH) to yield **7** (9.5 mg, t_R 9.9 min). Subfraction 4-10-2 (192 mg) was further separated by HPLC (50% MeOH) to yield 3-(hydroxymethyl)-6-[4-(3-methylbut-2-enyloxy)benzyl]piperazine-2,5-dione (1.6 mg, t_R 13.7 min). Fraction 5 (3.2 g) was purified by Sephadex LH-20 eluted with $CHCl_3$−MeOH (1:1) to yield subfractions 5-1−5-3. Subfraction 5-2 (925 mg) was purified by Sephadex LH-20 eluted with MeOH to afford subfraction 5-2-1 (408 mg), which was further purified by HPLC with 97% MeOH to give **1** (14.2 mg, t_R 9 min), **2** (215 mg, t_R 15 min), **3** (7.4 mg, t_R 7 min), **4** (7.5 mg, t_R 22 min), and **5** (12.5 mg, t_R 19 min).

Chrysogeside A (1): white, amorphous powder; $[\alpha]^{25}_D$ −6 (*c* 0.5, MeOH); IR (KBr) ν_{max} 3380, 2919, 2851, 1649, 1540, 1513, 1459, 1074, 1037 cm^{-1}; ^1H NMR and ^{13}C NMR data, Table 1; HRESIMS *m/z* 712.5361 $[M + H]^+$ (calcd for $C_{40}H_{74}NO_9$, 712.5364); ESIMS/MS *m/z* 712.6 $[M + H]^+$, 694.6 $[M + H - H_2O]^+$, 532.5 $[M + H - 180]^+$, 514.5 $[532.5 - H_2O]^+$, 496.5 $[514.5 - H_2O]^+$, 280.3, 262.2, and 252.2.

Chrysogeside B (2): white, amorphous powder; $[\alpha]^{25}_D$ −8 (*c* 0.5, MeOH); IR (KBr) ν_{max} 3389, 2923, 2853, 1642, 1544, 1464, 1379, 1078, 1040 cm^{-1}; ^1H NMR and ^{13}C NMR data, Table 1; HRESIMS *m/z* 726.5493 $[M + H]^+$ (calcd for $C_{41}H_{76}NO_9$, 726.5520); ESIMS/MS *m/z* 726.5 $[M + H]^+$, 708.5 $[M + H - H_2O]^+$, 546.5 $[M + H - 180]^+$, 528.5 $[546.5 - H_2O]^+$, 510.5 $[528.5 - H_2O]^+$, 294.3, 276.3, and 252.2.

Chrysogeside C (3): white, amorphous powder; $[\alpha]^{25}_D$ −5 (*c* 0.4, MeOH); IR (KBr) ν_{max} 3374, 2923, 2851, 1535, 1523, 1451, 1379, 1071, 1020 cm^{-1}; ^1H NMR and ^{13}C NMR data, Table S1; HRESIMS *m/z* 712.5346 $[M + H]^+$ (calcd for $C_{40}H_{74}NO_9$, 712.5364); ESIMS/MS *m/z* 712.5 $[M + H]^+$, 694.6 $[M + H - H_2O]^+$, 532.5 $[M + H - 180]^+$, 514.5 $[532.5 - H_2O]^+$, 496.5 $[514.5 - H_2O]^+$, 294.2, 276.3, and 238.2.

Chrysogeside D (4): white, amorphous powder; $[\alpha]^{25}_D$ −4 (*c* 0.37, MeOH); IR (KBr) ν_{max} 3380, 2919, 2851, 1649, 1540, 1513, 1459, 1074, 1037 cm^{-1};, ^1H NMR and ^{13}C NMR data, Table S1; HRESIMS *m/z* 754.5852 $[M + H]^+$ (calcd for $C_{43}H_{80}NO_9$, 754.5833); ESIMS/MS *m/z* 754.7 $[M + H]^+$, 736.6 $[M + H - H_2O]^+$, 574.5 $[M + H - 180]^+$, 556.5 $[574.5 - H_2O]^+$, 538.5 $[556.5 - H_2O]^+$, 294.3 and 280.3.

Chrysogeside E (5): white, amorphous powder; $[\alpha]^{25}_D$ −3 (*c* 0.5, MeOH); IR (KBr) ν_{max} 3358, 2922, 2852, 1649, 1540, 1463, 1079, 1039 cm^{-1}; ^1H NMR and ^{13}C NMR data, Table S1; HRESIMS *m/z* 728.5646 $[M + H]^+$ (calcd for $C_{41}H_{78}NO_9$, 728.5677); ESIMS/MS *m/z* 728.6 $[M + H]^+$, 710.6 $[M + H - H_2O]^+$, 548.5 $[M + H - 180]^+$, 530.5 $[548.5 - H_2O]^+$, 296.3 and 278.3.

Chrysogedone A (6): yellow, amorphous powder; $[\alpha]^{25}_D$ −17 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 192 (3.90), 224 (4.20), 286 (4.12) nm; CD (MeOH), λ_{max} ($\Delta\varepsilon$) 206 (−3.9), 233 (+0.9), 289 (−0.6) nm; IR (KBr) ν_{max} 3482, 3392, 2961, 2934, 2874, 1715, 1641, 1557, 1459, 1381, 1296, 1188, 1104, 1071 cm^{-1}; ^1H NMR and ^{13}C NMR data, Table 2; HREIMS *m/z* 181.1099 $[M]^+$ (calcd for $C_{10}H_{15}NO_2$, 181.1103).

Chrysogedone B (7): yellow, amorphous powder; $[\alpha]^{25}_D$ −13 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 200 (3.77), 224 (4.04), 297 (3.98) nm; CD (MeOH), λ_{max} ($\Delta\varepsilon$) 208 (−8.3), 233 (+0.9), 296 (−1.2) nm; IR (KBr) ν_{max} 3481, 3392, 2961, 2924, 2865, 1716, 1645, 1558, 1447, 1177, 1101, 1026 cm^{-1}; ^1H NMR and ^{13}C NMR data, Table 2; HRESIMS *m/z* 197.1041 $[M]^+$ (calcd for $C_{10}H_{15}NO_3$, 197.1052).

Methanolysis of 1−5. By the same procedures used for alternaroside C,[3] compound **2** (20 mg) was refluxed with 5% HCl−MeOH (5 mL) for 10 h to yield methyl (2*R*,3*E*)-hydroxynonadec-3-enoate (6.1 mg) and methyl D-glucopyranoside (5.1 mg). The methyl D-glucopyranosides (7.2

mg) were refluxed with 2 N HCl in H_2O (1 mL) for 2 h to give D-glucose (3.5 mg). (2*R*,3*E*)-Hydroxynonadec-3-enoate: amorphous powder; $[\alpha]^{25}_D$ −51 (*c* 0.1, $CHCl_3$); GC-MS (30 m × 0.32 mm × 0.25 μm HP-INNoWaX MS column: He, 2 mL/min; 40 °C, 2 min, 40−250 °C, Δ 15 °C/min, 250 °C, 10 min); t_R 16.92 min; *m*/*z* (rel int) 326 (M^+, 0.3), 225 (M^+ − 101, 16), 109 (11),89 (24), 81(16), 73 (23), 57 (50), 45 (100), 43 (55); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 0.88 (3H, t, *J* = 7.1 Hz, H-19), 1.28 (26H, m, H-6-H-18), 2.05 (2H, q, *J* = 7.1 Hz, H-5), 2.87 (1H, br s, 2-OH), 3.80 (3H, 1-OCH_3), 4.61 (1H, d, *J* = 6.1 Hz, H-2), 5.49 (1H, dd, *J* = 15.4, 6.1 Hz, H-3), 5.88 (1H, dt, *J* = 15.4, 6.1 Hz, H-4); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 14.3 (CH_3, C-19), 22.8 (CH_2, C-18), 29.0−29.8 (CH_2, C-6−C-16), 32.1 (CH_2, C-17), 32.3 (CH_2, C-5), 52.9 (CH_3, −OCH_3), 71.6 (CH, C-2), 126.0 (CH, C-3), 135.3 (CH, C-4), 174.5 (C, C-1); ESIMS *m*/*z* 349 $[M + Na]^+$. Methyl D-glucopyranoside: amorphous powder; $[\alpha]^{25}_D$ +76 (*c* 0.05, MeOH); ESIMS *m*/*z* 195 $[M + H]^+$; R_f 0.50/0.56 ($CHCl_3$−MeOH−H_2O, 7:3:0.5). D-Glucose: colorless syrup: R_f 0.30/0.35 ($CHCl_3$−MeOH−H_2O, 7:3:0.5); $[\alpha]^{25}_D$ +51 (*c* 0.1, H_2O) (standard D-glucose +54/L-glucose −56). The same results were also obtained from the methanolysis of **3** and **4**. By the same procedures, compounds **1** and **5** afforded the same methyl D-glucopyranosides and D-glucose. In addition, compounds **1** (5 mg) and **5** (5 mg) also yield methyl 2*R*-hydroxyoctadec-3-enoate (2.1 mg) and 2*R*-hydroxynonadecanoate (1.9 mg), respectively. Methyl 2*R*-hydroxyoctadec-3-enoate: $[\alpha]^{25}_D$ −56 (*c* 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 0.88 (3H, t, *J* = 7.1 Hz, H-18), 1.28 (24H, m, H-6−H-17), 2.05 (2H, q, *J* = 7.1 Hz, H-5), 2.84 (1H, d, *J* = 6.0 Hz, 2-OH), 3.80 (3H, −OCH_3), 4.61 (1H, br s, H-2), 5.49 (1H, dd, *J* = 15.4, 6.1 Hz, H-3), 5.87 (1H, dt, *J* = 15.4, 6.1 Hz, H-4); ESIMS *m*/*z* 335 $[M + Na]^+$. Methyl 2*R*-hydroxynonadecanoate: $[\alpha]^{25}_D$ −50 (*c* 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 0.88 (3H, t, *J* = 7.1 Hz, H-19), 1.28 (30H, m, H-4−H-18), 1.80 (2H, m, H-3), 2.01(1H, d, *J* = 3.3 Hz, 2-OH), 3.79 (3H, −OCH_3), 4.18 (1H, td, *J* = 4.4, 3.3 Hz, H-2); ESIMS *m*/*z* 351 $[M + Na]^+$.

Preparation of the (*S*)-and (*R*)-MTPA Esters of 6 by Modified Mosher's Method. Compound **6** (each 2.0 mg) was transferred into two clean NMR tubes and was dried completely under vacuum. Deuterated pyridine (each 0.5 mL) and *R*(−)- and *S*(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (each 10 μL) were separately added into the NMR tubes quickly under a N_2 gas stream. The reaction NMR tubes were allowed to stand at room temperature for 48 h. ^{1}H NMR and ^{1}H−^{1}H COSY spectra of the corresponding (*S*)-MTPA ester (**6a**) and (*R*)-MTPA ester (**6b**) were measured directly, and the corresponding δ_H values were assigned by ^{1}H−^{1}H COSY correlations. (*S*)-MTPA ester (**6a**): ^{1}H NMR (C_5D_5N, 600 MHz) δ 7.40 (1H, d, *J* = 9.0 Hz, H-3), 8.04 (1H, d, *J* = 9.5 Hz, H-4), 5.96 (1H, t, *J* = 6.4 Hz, H-1′), 1.54 (1H, m, H-2′a), 1.41 (1H, m, H-2′b), 1.31 (1H, m, H-3′a), 1.18 (1H, m, H-3′b), 2.32 (3H, s, 6-CH_3), 0.76 (3H, t, *J* = 7.3 Hz, H-4′). (*R*)-MTPA ester (**6b**): ^{1}H NMR (C_5D_5N, 600 MHz) δ 7.39 (1H, d, *J* = 9.1 Hz, H-3), 8.03 (1H, d, *J* = 9.5 Hz, H-4), 6.05 (1H, t, *J* = 6.4 Hz, H-1′), 1.70 (1H, m, H-2′a), 1.57 (1H, m, H-2′b), 1.41 (1H, m, H-3′a), 1.28 (1H, m, H-3′b), 2.31 (3H, s, 6-CH_3), 0.82 (3H, t, *J* = 7.3 Hz, H-4′).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. NMR spectra of **1**−**7**, ESIMS/MS of **1**−**5**, GC-MS of methanolysis products of **1**−**5**, mass fragmentation pattern in compounds **1**−**5**, ^{1}H and ^{13}C NMR data of compounds **3**−**5**, and bioassay protocols used. This material is available free of charge via the Internet at http://pubs.acs.org.

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