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NOTE

Chodatiionosides A and B: two new megastigmane glycosides from *Chorisia chodatii* leaves

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Abstract Phytochemical investigation of *Chorisia chodatii* Hassl. leaves led to the isolation of an unusual rearranged megastigmane glycoside; chodatiionoside A (**1**) and another new megastigmane glycoside; chodatiionoside B (**2**), together with three known megastigmane glycosides (**3**–**5**) and one known flavonoid glycoside (**6**). Their structures were elucidated by spectroscopic methods including 1D and 2D NMR experiments (^{1}H, ^{13}C, DEPT, COSY, HSQC and HMBC) in combination with HR-ESI–MS, CD and modified Mosher's method. As a result, chodatiionoside A has been elucidated as a first example of an unusual rearranged form of megastigmane.

Keywords *Chorisia chodatii* · Bombacaceae · Chodatiionoside · Megastigmane glycoside

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Introduction

Chorisia chodatii Hassl. (family Bombacaceae) is a species of deciduous tree native to tropical and subtropical regions of the world, especially the forests of South America [1]. It is characterized by a barrel-shaped trunk generally bulging in its lower third, measuring up to 2 m in girth, and is studded with thick conical sharp spines as an adaptation to store water for dry climates [2]. *Chorisia* plants are commonly known as silk floss, drunken or bottle trees [1, 3], and are traditionally used to treat diabetes, diarrhoea, headache, peptic ulcers, fever, parasitic infections and rheumatism [4]. These trees are also cultivated worldwide for ornamental purposes due to their brilliant showy flowers as well as the economically important silky white fibers that are obtained from their fruits [1, 3]. Our previous phytochemical analysis of *C. chodatii* flowers revealed their richness in phenolics and resulted in the isolation and characterization of a number of flavonoids, coumarins and phenolic acids as well as their esters, furanoids and sterols [5]. On the other hand, none of the chemical profile of its leaves was previously studied. Consequently, in continuation of our phytochemical research on this species, we carried out the first phytochemical investigation of *C. chodatii* leaves as a source of varied secondary metabolites. The present study deals with the isolation and structural elucidation of two new megastigmane glycosides (**1**–**2**), three known megastigmane glycosides (**3**–**5**) and one known flavonoid glycoside (**6**) from the leaves of this plant (Fig. 1). Besides, the isolated compounds were tested for their 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity and cytotoxicity on the human lung cancer cell line (A549) by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

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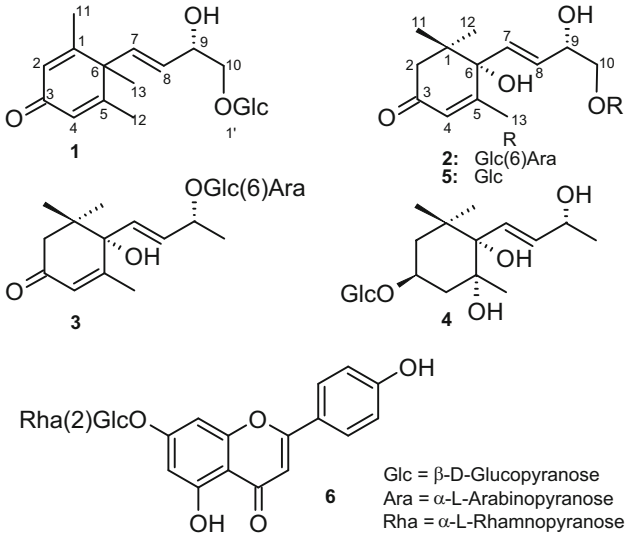

Fig. 1 Structures of two new megastigmane glycosides (**1**–**2**), three known megastigmane glycosides (**3**–**5**) and one known flavonoid glycoside (**6**) from the leaves of *Chorisia chodatii*

Results and discussion

The *n*-BuOH fraction of the 95 % EtOH extract of *C. chodatii* leaves was subjected to silica gel and octadecylsilanized (ODS) column chromatography, then HPLC to give a megastigmane glycoside with an unusual skeleton; chodatiionoside A (**1**) and another new megastigmane glycoside; chodatiionoside B (**2**), in addition to three known megastigmane glycosides; (*6S,7E,9R*)-6,9-dihydroxy-4,7-megastigmadien-3-one 9-*O*-[α-L-arabinopyranosyl-(1 → 6)-β-D-glucopyranoside] (**3**) [6], (*3S,5R,6R,7E,9S*)-megastigman-7-ene-3,5,6,9-tetrol 3-*O*-β-D-glucopyranoside (**4**) [7] and cucumegastigmane II (**5**) [8] and one known flavonoid glycoside (**6**); rhoifolin [5]. It is worth mentioning that, among various Bombacaceous plants, only one megastigmane glycoside, namely blumenol C β-D-glucopyranoside, was obtained only from *Bombax ceiba* L. flowers [9], whereas this is the first report of isolation of megastigmanes from *Chorisia* plants, and for compounds (**3–5**) from family Bombacaceae.

Compound (**1**) $[\alpha]_D^{24}$ −11.5, was obtained as an amorphous powder, and its molecular formula was determined to be $C_{19}H_{28}O_8$ by HR–ESI–MS. The IR spectrum showed absorptions indicating the presence of hydroxyl groups (3392 cm^{-1}) and a carbonyl group (1649 cm^{-1}). The ^{1}H-NMR spectrum of **1** (Table 1) displayed signals corresponding to four olefinic protons at δ_H 6.12 (2H, s), 5.74 (1H, dd, J = 15.8, 5.8 Hz) and 5.52 (1H, dd, J = 15.8, 1.5 Hz). The latter showed long range coupling (J = 1.5 Hz) with the oxymethine proton, which resonated as a one-proton multiplet at δ_H 4.36. Additionally, the ^{1}H-NMR spectrum also showed two one-proton doublet of doublets at δ_H 3.47 (J = 10.2, 7.5 Hz) and 3.92 (J = 10.2, 3.7 Hz), consistent with the diastereotopic oxymethylene protons, along with three methyl singlets at δ_H 1.42, 1.99 and 2.00. A glucosyl moiety was unambiguously deduced from the one-proton doublet at δ_H 4.28 (J = 7.7 Hz) assigned to H-1′, while the observed coupling constant was typical for β-configuration of the glucosyl unit. In the same way, the ^{13}C-NMR spectrum of **1** (Table 1) displayed six carbon signals characteristic for the β-glucopyranosyl moiety, with the anomeric carbon C-1′ resonating at δ_C 104.8, in addition to another 13 carbon resonances representing four methine, three methyl, one oxymethylene, one oxymethine, three quaternary and one ketonic carbons, in harmony with a magastigmane skeleton having a *trans* double bond between C-7 and C-8. Furthermore, the previous findings were corroborated with the aid of the ^{1}H-^{1}H

Table 1 ^{1}H- and ^{13}C-NMR spectroscopic data for chodatiionosides A and B (**1** and **2**) (600 and 150 MHz, respectively, CD_3OD)

No.	**1**		**2**	
	C	H	C	H
1	167.7	–	42.5	–
2	127.1[a]	6.12 s	50.8	2.50 d, 16.8
				2.15 d, 16.8
3	188.5	–	201.3	–
4	127.2[a]	6.12 s	127.2	5.87 q, 1.3
5	167.7	–	167.2	–
6	50.5	–	80.2	–
7	134.3	5.52 dd, 15.8, 1.5	132.9	5.91 dd, 15.8, 1.3
8	133.5	5.74 dd, 15.8, 5.8	131.7	5.78 dd, 15.8, 5.6
9	72.1	4.36 m	72.1	4.36 m
10	74.8	3.92 dd, 10.2, 3.7	75.4	3.86 m
		3.47 dd, 10.2, 7.5		3.55 m
11	20.4	1.99 s[b]	23.5	1.03 s
12	20.4	2.00 s[b]	24.6	1.01 s
13	21.5	1.42 s	19.7	1.91 d, 1.3
1′	104.8	4.28 d, 7.7	104.8	4.28 d, 7.7
2′	75.2	3.20 dd, 9.3, 7.7	75.2	3.19 dd, 9.2, 7.9
3′	78.0	3.34 m	77.8	3.34 m
4′	71.7	3.26 m	71.8	3.28 m
5′	77.9	3.27 m	76.8	3.45 m
6′	62.8	3.86 dd, 11.7, 1.8	69.8	4.12 dd, 11.1, 2.2
		3.64 dd, 11.7, 5.4		3.65 dd, 11.1, 6.4
1″			105.2	4.25 d, 6.7
2″			72.3	3.57 m
3″			74.2	3.50 m
4″			69.5	3.79 m
5″			66.8	3.84 m
				3.52 m

[a,b] Interchangeable values

COSY, HSQC and HMBC experiments (Fig. 2). The olefinic proton H-8 showed important ^{1}H-^{1}H COSY correlations with both H-7 and the oxymethine proton H-9. The latter was further correlated with the oxymethylene protons H_2-10. On the other hand, the shielded methyl protons CH_3-13 (δ_H 1.42) showed significant HMBC correlations with C-6 (δ_C 50.5), C-7 (δ_C 133.5) and C-1,5 (δ_C 167.7), confirming its attachment to C-6, whereas the other two deshielded methyl groups CH_3-11 and CH_3-12 (δ_H 1.99 and 2.00) were correlated with C-6 (δ_C 50.5), C-2 (δ_C 127.1), C-4 (δ_C 127.2) and C-1,5 (δ_C 167.7), indicating their attachment to C-1 and C-5, respectively. Other important HMBC correlations were also observed between the olefinic protons H-2,4 (δ_H 6.12) and C-11,12 (δ_C 20.4), C-6 (δ_C 50.5), C-2 (δ_C 127.1), C-4 (δ_C 127.2) and C-3 (δ_C 188.5). These findings were strongly indicative of a megastigma-1,4,7-triene-9,10-diol-3-one skeleton of the aglycone, while the glucosylation at C-10 was deduced explicitly from the observed three-bond connectivity between the anomeric proton H-1′ (δ_H 4.28) and C-10 (δ_C 74.8). The presence of the glucopyranosyl moiety was confirmed by HPLC analysis of the hydrolysate of **1**, and the absolute configuration of the sugar was simultaneously determined to be in D-series using a chiral detector. On the other hand, the modified Mosher's method [10] was applied to the aglycone (**1a**). Prior to α-methoxy-α-trifluoromethylphenyl acetic acid (MTPA) esterifications, the primary alcohol was protected as a pivaloyl ester. Figure 3 shows the results of the modified Mosher's method. Therefore, the structure of compound **1** was established to be (9*S*,1*Z*,4*Z*,7*E*)-megastigma-1,4,7-triene-9,10-diol-3-one 10-*O*-β-D-glucopyrano-side, namely chodatiionoside A as shown in Fig. 1.

Compound (**2**), $[\alpha]_D^{25}$ +21.1, was isolated as an amorphous powder, and its molecular formula was determined to be $C_{24}H_{38}O_{13}$ by HR–ESI–MS. The IR spectrum exhibited absorptions for hydroxyl groups (3395 cm^{-1} and 1073 cm^{-1}) and a carbonyl group (1649 cm^{-1}). The ^{1}H-NMR spectrum displayed signals corresponding to three olefinic protons at δ_H 5.78 (dd, J = 15.8, 5.6 Hz), 5.87 (q, J = 1.3 Hz) and 5.91 (dd, J = 15.8, 1.3 Hz). The latter exhibited long-range coupling (J = 1.3 Hz), with the oxymethine proton resonating as a one-proton multiplet at δ_H 4.36. Additionally, the ^{1}H-NMR spectrum also showed two singlet and one doublet methyl signals at δ_H 1.01, 1.03 and 1.91 (J = 1.3 Hz). Besides, two doublets proton signals at δ_H 4.25 (J = 6.7 Hz) and 4.28 (J = 7.7 Hz) were characteristic for two anomeric protons with axial configuration. Enzymatic hydrolysis of compound (**2**) yielded D-glucose and L-arabinose as the sugar components. The ^{13}C-NMR spectroscopic data showed the presence of six carbon signals assignable to a β-D-glucopyranosyl moiety, five carbon signals attributable to an α-L-arabinopyranosyl moiety and another 13 carbon resonances for four sp^2 carbons, three methyls, one methylene, one oxymethylene, one oxymethine, two quaternary carbons and one ketone, in agreement with a megastigmane skeleton with a trans double bond between C-7 and C-8. On the other hand, an arabinopyranosyl-(1 → 6)-glucopyranosyl moiety at C-10 was deduced explicitly from the two anomeric protons H-1″ and H-1′ at δ_H 4.25 (J = 6.7 Hz) and 4.28 (J = 7.7 Hz), respectively, and their corresponding oxymethine carbon signals at δ_C 104.8 for C-1′ and 105.2 for C-1″ in the ^{13}C-NMR spectrum. Likewise, the significant HMBC correlation between the anomeric proton (H-1′) of the glucopyranosyl moiety and C-10 (δ_C 75.4) of the aglycone evidently indicated the glycosylation at the hydroxyl group of C-10 as shown in Fig. 4. In addition, the (1 → 6) interglycosidic linkage was clearly determined from the characteristic downfield shift of C-6′ at δ_C 69.8 compared with that of an unsubstituted β-D-glucopyranoside [11], and was also confirmed by the observed HMBC correlation between the anomeric proton (H-1″) of

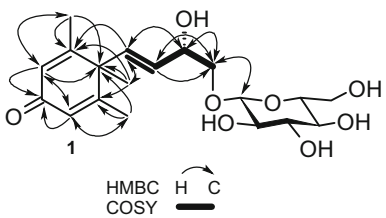

Fig. 2 Results of ^{1}H-^{1}H COSY, HSQC and HMBC experiments

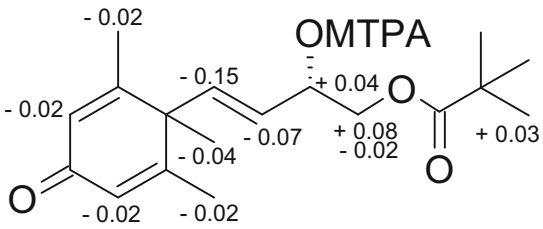

Fig. 3 Structure of compound **1**: (9*S*,1*Z*,4*Z*,7*E*)-megastigma-1,4,7-triene-9,10-diol-3-one 10-*O*-β-D-glucopyranoside (chodatiionoside A)

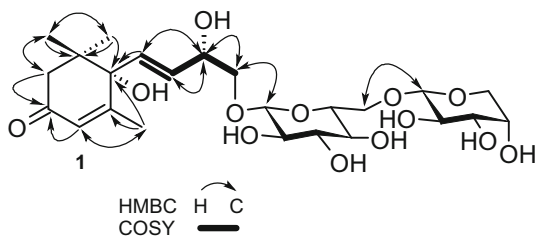

Fig. 4 Significant HMBC correlation between the anomeric proton (H-1') of the glucopyranosyl moiety and C-10 (δ C 75.4) of the aglycone indicates glycosylation at the hydroxyl group of C-10

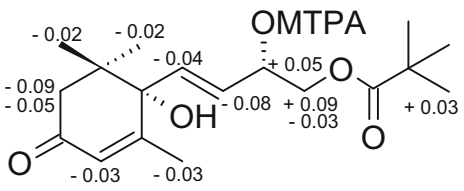

Fig. 5 Structure of compound **2**: (6*S*,9*S*,4*Z*,7*E*)-megastigma-4,7-diene-6,9,10-triol-3-one 10-*O*-α-L-arabinopyranosyl-(1''→6')-β-D-glucopyranoside (chodatiionoside B)

the arabinopyranosyl moiety and C-6′ of the glucopyranosyl moiety. Furthermore, the previous findings were substantiated with the aid of the ^{1}H-^{1}H COSY, HSQC and HMBC experiments (Fig. 4). The olefinic proton H-8 showed important ^{1}H-^{1}H COSY correlations with both H-7 and the oxymethine proton H-9. The latter was further correlated with the oxymethylene protons H_2-10. On the other hand, the absolute configuration at C-6 was determined to be *S*, judging from the positive and negative Cotton effects at 238 nm and 303 nm, respectively, in the CD spectrum [9]. Further, the modified Mosher's method was applied to the aglycone (**2a**). Prior to MTPA esterifications, the primary alcohol was protected as a pivaloyl ester. Figure 5 shows the results of the modified Mosher's analysis. Consequently, the structure of compound **2** was elucidated to be (6*S*,9*S*,4*Z*,7*E*)-megastigma-4,7-diene-6,9,10-triol-3-one 10-*O*-α-L-arabinopyranosyl-(1″ → 6′)-β-D-glucopyranoside, namely chodatiionoside B as shown in Fig. 1.

Compounds **1**–**6** were examined for their DPPH radical-scavenging activity and tumor cell growth inhibitory activity toward A549 by means of the MTT assay. The results were compared with those of trolox ($IC_{50} = 16.6 \pm 2.2$ μM) and doxorubicin ($IC_{50} = 0.90 \pm 0.02$ μM), as positive controls, respectively. However, the results revealed that none of the isolated compounds have significant cytotoxicity or DPPH radical scavenging properties ($IC_{50} > 100$ μM).

Experimental

General experimental procedures

Optical rotations and CD data were measured on a JASCO P-1030 and Jasco J-720 polarimeters (Jasco, Easton, MD), respectively. IR and UV spectra were obtained on Horiba FT-710 Fourier transform infrared (Horiba, Kyoto, Japan) and Jasco V-520 UV/Vis spectrophotometers, respectively. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AVANCE 600 MHz spectrometer (Bruker, Billerica, MA). HR-ESI–MS spectra were obtained using a LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific, Waltham, MA). Silica gel 60 (Merck, Darmstardt, Germany; 60–120 mesh) was used for column chromatography (CC), whereas reversed-phase [octadecylsilanised silica gel (ODS)] open CC (RPCC) was performed on Cosmosil 75C_{18}-OPN (Nacalai Tesque, Kyoto, Japan) ($\Phi = 2$ cm, $L = 40$ cm, 10 g fractions were collected). High-performance liquid chromatography (HPLC) was performed on an ODS column (Inertsil ODS-3; GL Science, Tokyo, Japan; $\Phi = 10$ mm, $L = 25$ cm, flow rate: 2.0 ml/min), using a refractive index and/or a UV detector. HPLC analysis of sugars obtained after hydrolysis was carried on an amino column [Shodex Asahipak NH_2P-50 4E (4.6 mm × 250 mm), CH_3CN-H_2O (3:1), 1 ml/min], using a chiral detector (JASCO OR-2090*plus*). Pre-coated silica gel 60 GF254 plates (Merck; 20 × 20 cm, 0.25 mm in thickness) were used for TLC analyses. Spots were visualized by spraying with sulfuric acid (10 % in ethanol) and heating at 150 °C on a hot plate. The human lung cancer cell line (A549) was obtained from the Japanese Collection of Research Bioresources (JCRB) Cell Bank, National Institute of Biomedical Innovation. (*R*)- and (*S*)-MTPA were obtained from Wako Pure Chemical Industries (Tokyo, Japan).

Plant material

Leaves of *C. chodatii* were collected before the flowering stage in July 2010 from plants cultivated in the campus of Minia University, Minia, Egypt. Authentication of the plant species was established by Prof. Ahmed Abdel-Monem, Department of Horticulture, Faculty of Agriculture, Minia University, Minia, Egypt. A voucher specimen (Mn-Ph-Cog-001) has been deposited in the herbarium of Pharmacognosy Department, Faculty of Pharmacy, Minia University, Minia, Egypt.

Extraction and isolation

The air-dried powdered leaves (6.58 kg) were exhaustively extracted with 95 % ethanol (10 L × 10) and concentrated under reduced pressure to a syrupy consistency. The solvent-free residue (524 g) was suspended in distilled water (800 mL) and successively extracted with light petroleum ether, $CHCl_3$, EtOAc and *n*-BuOH. The petroleum ether, $CHCl_3$, EtOAc and *n*-BuOH fractions were concentrated under reduced pressure to give residues of 204.5, 13.9, 24.4 and 26.5 g, respectively. The remaining aqueous layer was concentrated to furnish a water-soluble fraction (245.8 g).

The *n*-BuOH fraction (26.5 g) was fractionated by CC over silica gel (600 g), ($\Phi = 60$ mm, $L = 60$ cm), using $CHCl_3$-MeOH gradient system, 300 mL fractions were collected, and similar fractions were combined together to yield nine fractions (B-1 to B-9). Fraction B-6 (2.90 g) was

purified on RPCC, giving 13 subfractions. Subfraction B-6-3 (181.2 mg) was further purified by HPLC (35 % MeOH) to provide compounds **3** (8.1 mg) and **1** (20.3 mg). Fraction B-7 (4.12 g) was purified on RPCC, yielding ten subfractions. Subfraction B-7-2 (219.6 mg) was further purified by HPLC (30 % MeOH) to furnish compounds **2** (12.8 mg) and **4** (8.1 mg). Subfraction B-7-5 afforded compound **6** (35.4 mg) as a yellow powder. Fraction B-8 (1.35 g) was purified on RPCC by the procedure described above with 10–100 % gradient elution, producing 11 subfractions. The subfraction B-8-2 (91.3 mg) was purified by HPLC (30 % MeOH) to give compound **5** (11.8 mg).

*Chodatiionoside A (**1**)*

Amorphous powder; $[\alpha]_D^{25}$ −11.5 (*c* 1.35, MeOH); IR ν_{max} (film) cm^{-1}: 3392, 2930, 2879, 1649, 1454, 1375, 1074; UV λ_{max} (MeOH) nm (log ε): 237 (3.84); ^{1}H-NMR (600 MHz, CD_3OD) and ^{13}C-NMR (150 MHz, CD_3OD): Table 1; HR-ESI–MS (positive-ion mode) *m/z*: 407.1680 $[M+Na]^+$ (Calcd for $C_{19}H_{28}O_8Na$: 407.1676).

*Chodatiionoside B (**2**)*

Amorphous powder; $[\alpha]_D^{25}$ +21.1 (*c* = 0.73, MeOH); IR ν_{max} (film) cm^{-1}: 3395, 2936, 2880, 1649, 1455, 1392, 1073 cm^{-1}; UV λ_{max} (MeOH) nm (log ε): 232 (4.17); CD (*c* 1.37 × 10^{-5} M, MeOH) nm ($\Delta\varepsilon$): 238 (+6.77), 303 (−0.55); ^{1}H-NMR (600 MHz, CD_3OD) and ^{13}C-NMR (150 MHz, CD_3OD): Table 1; HR-ESI–MS (positive-ion mode) *m/z*: 557.2205 $[M+Na]^+$ (Calcd for $C_{24}H_{38}O_{13}Na$: 557.2205).

Enzymatic hydrolysis of chodatiionoside A (1) to chodatiionol A (1a)

Chodatiionoside A (**1**) (10.5 mg) was hydrolyzed with crude β-glucosidase (10 mg) in 1 mL 100 mM acetate buffer (pH 5.0) at 37 °C for 18 h. The reaction solvent was evaporated off, and the residue was purified by preparative TLC [$CHCl_3$-MeOH, 4:1] to give the aglycone (**1a**) (4.6 mg, yield 76 %) and D-glucose.

*Chodatiionol A (**1a**)*

Amorphous powder; $[\alpha]_D^{24}$ −20.2 (*c* 0.46, MeOH); IR ν_{max} (film) cm^{-1}: 3324, 2925, 2873, 1661, 1473, 1383, 1074; UV λ_{max} (MeOH) nm (log ε): 237 (4.20); ^{1}H-NMR (CD_3OD, 600 MHz) δ: 6.12 (2H, s, H-2,4), 5.74 (1H, dd, *J* = 15.8, 5.9 Hz, H-8), 5.47 (1H, dd, *J* = 15.8, 1.3 Hz, H-7), 4.15 (1H, m, H-9), 3.49 (2H, m, H_2-10), 2.00 (3H, s, H_3-11 or 12), 1.99 (3H, s, H_3-12 or 11), 1.42 (3H, s, H_3-13); ^{13}C-NMR (CD_3OD, 150 MHz) δ: 188.6 (C-3), 167.9 (C-1 or 5), 167.8 (C-5 or 1), 134.6 (C-7), 133.9 (C-8), 127.2 (C-2 or 4), 127.1 (C-4 or 2), 73.7 (C-9), 67.2 (C-10), 50.5 (C-6), 21.6 (C-13), 20.4 (C-11, 12); HR-ESI–MS (positive-ion mode) *m/z*: 245.1147 $[M+Na]^+$ (Calcd for $C_{13}H_{18}O_3Na$: 245.1148).

Preparation of 10-*O*-pivalate (1b) from chodatiionol A (1a)

The aglycone (**1a**) (3.0 mg) was dissolved in 100 μL dry pyridine and 20 μL pivaloyl chloride was added. The reaction was performed for 6 h at 25 °C; 1 mL H_2O was added, followed by extraction with 2 mL EtOAc twice. The organic layer was dried over Na_2SO_4 and evaporated. The residue was then purified by preparative TLC [$CHCl_3$–$(CH_3)_2CO$, 10:1] to give 1.6 mg (39 %) of pivalate (**1b**).

*10-O-pivaloyl chodatiionol A (**1b**)*

Amorphous powder; ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 6.14 (2H, br s, H-2,4), 5.65 (1H, dd, *J* = 16.0, 5.6 Hz, H-8), 5.55 (1H, dd, *J* = 16.0, 1.3 Hz, H-7), 4.44 (1H, m, H-9), 4.18 (1H, dd, *J* = 11.4, 3.5 Hz, H-10a), 4.05 (1H, dd, *J* = 11.4, 6.8 Hz, H-10b), 1.94 (3H, s, H_3-11 or 12), 1.93 (3H, s, H_3-12 or 11), 1.39 (3H, s, H_3-13), 1.23 (9H, s, H_3-×3); ^{13}C-NMR (CD_3OD, 150 MHz) δ: 185.9 (C-3), 178.7 (C = O), 162.5 (C-1 or 5), 162.4 (C-5 or 1), 134.5 (C-7), 130.8 (C-8), 127.32 (C-2 or 4), 127.26 (C-4 or 2), 70.5 (C-9), 68.0 (C-10), 48.5 (C-6), 38.9 (C), 27.2 (CH_3-×3), 20.8 (C-13), 20.01 (C-11 or 12), 19.97 (C-11 or 12), HR-ESI–MS (positive-ion mode) *m/z*: 329.1726 $[M+Na]^+$ (Calcd for $C_{18}H_{26}O_4Na$: 329.1723).

Preparation of (*R*)- and (*S*)-9-*O*-MTPA esters (1c and 1d) from 10-*O*-pivaloyl chodatiionol A (1b)

A solution of **1b** (0.4 mg) in 1 mL dehydrated CH_2Cl_2 was reacted with (*R*)-MTPA (32 mg) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) (42 mg) and *N*,*N*-dimethyl-4-aminopyridine (DMAP) (12 mg), with the mixture being occasionally stirred at 37 °C for 12 h. After addition of 1 mL CH_2Cl_2, the solution was washed with H_2O (1 mL), 5 % HCl (1 mL), $NaHCO_3$-saturated H_2O and then with brine (1 mL), successively. The organic layer was dried over Na_2SO_4 and then evaporated under reduced pressure. The residue was purified by preparative TLC [silica gel (0.25 mm thickness), developed with $CHCl_3$-$(CH_3)_2CO$ (20:1) to furnish an ester, **1c** (0.3 mg, 44 %). Through a similar procedure, **1d** (0.2 mg, 29 %) was prepared from **1b** (0.4 mg) using (*S*)-MTPA (30 mg), EDC (45 mg) and 4-DMAP (16 mg).

*10-O-pivaloyl chodatiionol A 9-O-(R)-MTPA ester (**1c**)*

Amorphous powder; ^{1}H-NMR ($CDCl_3$, 600 MHz) *δ:* 7.59–7.50 (2H, aromatic protons), 7.43–7.37 (3H, m, aromatic protons), 6.15 (1H, q, $J = 1.4$ Hz, H-2 or 4), 6.14 (1H, q, $J = 1.4$ Hz, H-4 or 2), 5.68 (1H, ddd, $J = 7.1$, 6.2, 3.6 Hz, H-9), 5.64 (1H, d, $J = 15.5$ Hz, H-7), 5.60 (1H, dd, $J = 15.5$, 6.2 Hz, H-8), 4.29 (1H, dd, $J = 12.1$, 3.6 Hz, H-10a), 4.13 (1H, dd, $J = 12.1$, 7.1 Hz, H-10b), 3.59 (3H, q, $J = 1.0$ Hz, –OCH_3), 1.87 (3H, s, H_3-11 or 12), 1.86 (3H, s, H_3-12 or 11), 1.36 (3H, s, H_3-13), 1.15 (9H, s, CH_3-×3); HR-ESI–MS (positive-ion mode) *m/z*: 545.2115 $[M+Na]^+$ (Calcd for $C_{28}H_{33}O_6F_3Na$: 545.2121).

*10-O-pivaloyl chodatiionol A 9-O-(S)-MTPA ester (**1d**)*

Amorphous powder; ^{1}H-NMR ($CDCl_3$, 400 MHz) *δ:* 7.60–7.50 (2H, aromatic protons), 7.43–7.37 (3H, m, aromatic protons), 6.13 (1H, q, $J = 1.3$ Hz, H-2 or 4), 6.12 (1H, q, $J = 1.3$ Hz, H-4 or 2), 5.72 (1H, ddd, $J = 7.4$, 5.5, 3.3 Hz, H-9), 5.53 (1H, dd, $J = 15.8$, 5.5 Hz, H-8), 5.49 (1H, d, $J = 15.8$ Hz, H-7), 4.37 (1H, dd, $J = 12.0$, 3.3 Hz, H-10a), 4.11 (1H, dd, $J = 12.0$, 7.4 Hz, H-10b), 3.59 (3H, q, $J = 1.0$ Hz, –OCH_3), 1.85 (3H, s, H_3-11 or 12), 1.84 (3H, s, H_3-12 or 11), 1.32 (3H, s, H_3-13), 1.18 (9H, s, CH_3- ×3); HR-ESI–MS (positive-ion mode) *m/z*: 545.2116 $[M+Na]^+$ (Calcd for $C_{28}H_{33}O_6F_3Na$: 545.2121).

Enzymatic hydrolysis of chodatiionoside B (2) to chodatiionol B (2a)

Compound **2** (4 mg) was hydrolyzed in a similar manner as for **1**. The usual workup gave 0.6 mg (33 %) of chodatiioionol B (**2a**) and D-glucose and L-arabinose.

*Chodatiionol B (**2a**)*

Amorphous powder; $[\alpha]_D^{24}$ +143.3 (*c* 0.06, MeOH); IR ν_{max} (film) cm^{-1}: 3419, 2980, 2944, 1651, 1563, 1414, 1120, 1075, 1043, 1011; UV λ_{max} (MeOH) nm (log ε): 238 (3.83); CD (*c* 2.50×10^{-5} M, MeOH) nm (Δε): 240 (+14.7), 330 (−0.82); ^{1}H-NMR (CD_3OD, 600 MHz) *δ:* 5.89 (1H, dd, $J = 15.5$, 1.2 Hz, H-7), 5.88 (1H, s, H-4), 5.79 (1H, dd, $J = 15.5$, 5.5 Hz, H-8), 4.20 (1H, dddd, $J = 7.0$, 5.5, 4.9, 1.2 Hz, H-9), 3.50 (1H, dd, $J = 11.2$, 4.9 Hz, H-10a), 3.46 (1H, dd, $J = 11.2$, 7.0 Hz, H-10b), 2.51 (1H, d, $J = 16.8$ Hz, H-2a), 2.16 (1H, d, $J = 16.8$ Hz, H-2b), 1.92 (3H, d, $J = 1.3$ Hz, H_3-13), 1.04 (3H, s, H_3-11), 1.02 (3H, s, H_3-12); ^{13}C-NMR (CD_3OD, 150 MHz) *δ:* 201.4 (C-3), 167.5 (C-5), 132.7 (C-7), 132.6 (C-8), 127.3 (C-4), 80.3 (C-6), 73.8 (C-9), 67.4 (C-10), 50.9 (C-2), 42.5 (C-1), 24.7 (C-12), 23.6 (C-11), 19.7 (C-13); HR-ESI–MS (positive-ion mode) *m/z*: 263.1255 $[M+Na]^+$ (Calcd for $C_{13}H_{20}O_4Na$: 263.1254).

Preparation of 10-*O*-pivalate (2b) from chodatiionol B (2a)

In a similar manner as for the preparation of **1b** from **1a**, **2b** was prepared from **2a** (0.6 mg) with 20 μL pivaloyl chloride. The usual workup gave 0.8 mg (99 %) of pivaloyl ester (**4b**).

*10-O-pivaloyl chodatiionol B (**2b**)*

Amorphous powder; ^{1}H-NMR ($CDCl_3$, 400 MHz) *δ:* 5.97 (1H, dd, $J = 15.6$, 1.4 Hz, H-7), 5.91 (1H, br s, H-4), 5.84 (1H, dd, $J = 15.6$, 5.3 Hz, H-8), 4.48 (1H, dddd, $J = 6.9$, 5.3, 3.7, 1.4 Hz, H-9), 4.19 (1H, dd, $J = 11.5$, 3.7 Hz, H-10a), 4.05 (1H, dd, $J = 11.5$, 6.9 Hz, H-10b), 2.46 (1H, d, $J = 17.0$ Hz, H-2a), 2.27 (1H, d, $J = 17.0$ Hz, H-2b), 1.90 (3H, d, $J = 1.3$ Hz, H_3-13), 1.22 (9H, s, CH_3– × 3), 1.09 (3H, s, H_3-11), 1.02 (3H, s, H_3-12); HR–ESI–MS (positive-ion mode) *m/z*: 347.1827 $[M+Na]^+$ (Calcd for $C_{18}H_{28}O_5Na$: 347.1829).

Preparation of (*R*)- and (*S*)-9-*O*-MTPA esters (2c and 2d) from 10-*O*-pivaloyl chodatiionol B (2b)

In a similar manner as for the preparation of **1c** and **1d** from **1b**, **2c** and **2d** were prepared from **2b** (0.4 mg each) with the respective amounts of the reagents, (*R*)- and (*S*)-MPTA (42 mg and 36 mg), EDC (40 mg and 42 mg) and DMAP (16 mg and 20 mg). The usual workup gave 0.4 mg (**2c**, 60 %) and 0.3 mg (**2d**, 45 %) of esters, respectively.

*10-O-pivaloyl chodatiionol B 9-O-(R)-MTPA ester (**2c**)*

Amorphous powder; ^{1}H-NMR ($CDCl_3$, 400 MHz) *δ*: 7.53–7.50 (2H, aromatic protons), 7.42–7.36 (3H, m, aromatic protons), 6.00 (1H, dd, $J = 15.4$, 0.9 Hz, H-7), 5.91 (1H, br s, H-4), 5.88 (1H, dd, $J = 15.4$, 7.1 Hz, H-8), 5.74 (1H, dddd,, $J = 7.3$, 7.1, 3.5, 0.9 Hz, H-9), 4.30 (1H, dd, $J = 12.1$, 3.5 Hz, H-10a), 4.15 (1H, dd, $J = 12.1$, 7.3 Hz, H-10b), 3.50 (3H, s, –OCH_3), 2.37 (1H, d, $J = 17.3$ Hz, H-2a), 2.27 (1H, d, $J = 17.3$ Hz, H-2b), 1.85 (3H, d, $J = 1.3$ Hz, H_3-13), 1.15 (9H, s, CH_3– × 3), 1.07 (3H, s, H_3-11), 0.94 (3H, s, H_3-12); HR-ESI–MS (positive-ion mode) *m/z*: 563.2224 $[M+Na]^+$ (Calcd for $C_{28}H_{35}O_7F_3Na$: 563.2227).

*10-O-pivaloyl chodatiionol B 9-O-(S)-MTPA ester (**2d**)*

Amorphous powder; ^{1}H-NMR ($CDCl_3$, 400 MHz) *δ*: 7.54–7.50 (2H, m, aromatic protons), 7.41–7.35 (3H, m,

aromatic protons), 5.88 (1H, br s, H-4), 5.86 (1H, d, $J = 15.0$ Hz, H-7), 5.80 (1H, dd, $J = 15.0$, 6.2 Hz, H-8), 5.79 (1H, m, H-9), 4.39 (1H, dd, $J = 12.4$, 2.8 Hz, H-10a), 4.12 (1H, dd, $J = 12.4$, 7.2 Hz, H-10b), 3.58 (3H, q, $J = 1.0$ Hz, $-OCH_3$), 2.28 (1H, d, $J = 17.4$ Hz, H-2a), 2.22 (1H, d, $J = 17.4$ Hz, H-2b), 1.82 (3H, d, $J = 1.3$ Hz, H_3-13), 1.18 (9H, s, CH_3-×3), 1.05 (3H, s, H_3-11), 0.92 (3H, s, H_3-12); HR-ESI–MS (positive-ion mode) *m/z*: 563.2220 $[M+Na]^+$ (Calcd for $C_{28}H_{35}O_7F_3Na$: 563.2227).

Sugar analysis

Sugars obtained from compounds **1** and **2** by enzymatic hydrolysis were analyzed with a chiral detector (JASCO OR-2090plus) on an amino column [Asahipak NH_2P-50 4E, CH_3CN-H_2O (3:1), 1 mL/min] in comparison with authentic sugars (D-glucose and L-arabinose). Compound **1** gave a peak for D-(+)-glucose at retention time of 8.32 min, whereas compound **2** gave peaks for L-(+)-arabinose and D-(+)-glucose at retention times of 6.10 min and 8.32 min, respectively.

DPPH radical scavenging activity

Determination of the free radical scavenging activity was carried out using a quantitative DPPH assay. The absorbance with various concentrations of the test compounds dissolved in methanol (100 μL) in 96-well microtiter plates was measured at 515 nm as A_{blank}. Then, a 200 μM DPPH solution (100 μL) was added to each well, followed by incubation in a dark chamber at room temperature for 30 min. The absorbance was then measured again as A_{sample}. The % inhibition of free radicals was calculated according to the following equation:

$$\% \text{ Inhibition} = [1 - (A_{\text{sample}} - A_{\text{blank}})/(A_{\text{control}} - A_{\text{blank}})] \times 100$$

where A_{control} is the absorbance of the control reaction mixture containing DMSO and all reagents, except for the test compound. IC_{50} was determined as the sample concentration required to inhibit the formation of the DPPH radical by 50 % [12]. Measurements were carried out in triplicate.

Human cancer cell growth inhibition assay

This assay was performed using the human lung cancer cell line (A549) and the viability was estimated by means of the colorimetric MTT assay. Dulbecco's modified Eagle's medium supplemented with 10 % heat-inactivated FBS, 5.6 μg/mL amphotericin B and 100 μg/mL kanamycin was used as the cell culture medium. The test compounds were dissolved in DMSO and then added to the wells of 96-well microtiter plates to the final concentration of 1 %. A549 cells (5×10^3 cells/well) were cultured in a 5 % CO_2 incubator at 37 °C for 72 h, then a MTT solution was added to each well and the plates were incubated for further 1.5 h. The formed formazan precipitates were then dissolved in DMSO and the optical density value for each well was measured at 540 nm using a microplate reader. Doxorubicin was used as a positive control. The cell growth inhibition was calculated using the following equation:

$$\% \text{ Inhibition} = [1 - (A_{\text{sample}} - A_{\text{blank}})/(A_{\text{control}} - A_{\text{blank}})] \times 100$$

where A_{control} is the absorbance of the control reaction mixture containing DMSO and all reagents except for the test compound. IC_{50} was determined as the sample concentration required to inhibit the formation of MTT formazan by 50 % [13].

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