

ORIGINAL ARTICLE

New dinapinone derivatives, potent inhibitors of triacylglycerol synthesis in mammalian cells, produced by *Talaromyces pinophilus* FKI-3864

Mio Kawaguchi¹, Ryuji Uchida¹, Satoshi Ohte¹, Natsuki Miyachi¹, Keisuke Kobayashi¹, Noriko Sato¹, Kenichi Nonaka², Rokuro Masuma², Takashi Fukuda¹, Tadashi Yasuhara¹ and Hiroshi Tomoda¹

Eight new dinapinones, AB1, AB2, AC1, AC2, AD1, AD2, AE1 and AE2, were isolated from the culture broth of *Talaromyces pinophilus* FKI-3864. The structures of these dinapinones were elucidated by various NMR experiments. All these dinapinones possessed the same biaryl dihydronaphthopyranone skeleton consisting of a heterodimer with one monapinone A and one different monapinone. Dinapinones AB1 and AB2, consisting of monapinones A and B, were atropisomers. Similarly, dinapinones AC1 and AC2, consisting of monapinones A and C, dinapinones AD1 and AD2, consisting of monapinones A and D, and dinapinones AE1 and AE2, consisting of monapinones A and E, were atropisomers. Dinapinone AB2 showed potent inhibition of triacylglycerol (TG) synthesis in intact mammalian cells with an IC₅₀ value of 1.17 μM, whereas the other dinapinones showed weak inhibition of TG synthesis.

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Keywords: atropisomer; dinapinone; structure elucidation; *Talaromyces pinophilus*; triacylglycerol synthesis inhibitor

INTRODUCTION

Triacylglycerol (TG) is an energy-storage molecule and thus a common form of fat found in both food and the body. TG synthesis is important in many metabolic processes in mammals, including lactation, energy storage in fat and muscle, fat absorption in the intestine and the assembly of lipoprotein particles in the liver and small intestine; however, excess accumulation of TG in certain organs and tissue causes fatty liver, obesity and hypertriglyceridemia.^{1–4} In the course of screening microbial metabolites for inhibitors of TG synthesis using intact Chinese hamster ovary-K1 (CHO-K1) cells,⁵ we discovered new dihydronaphthopyranone-containing compounds named dinapinones A1 (DPA1; **1**) and A2 (DPA2; **2**) and monapinones A (**11**) to E (**15**) (Figure 1) in the culture broth of *Talaromyces pinophilus* (originally named *Penicillium pinophilum*) FKI-3864.^{5–7} We reported that **1** and **2**, homodimers of **11** were atropisomers. Furthermore, the absolute stereochemistries of **1** and **2** were elucidated by *in vitro* enzymatic conversion of structure-defined **11** to **1** and **2**.⁷

Continuous isolation study from the culture broth of *T. pinophilus* FKI-3864 enabled us to obtain eight new dinapinones that were heterodimers of monapinones (Figure 1). In this study, the fermentation, isolation, structure elucidation and inhibitory effects on TG synthesis in CHO-K1 cells of dinapinones are described.

RESULTS

Isolation of dinapinones

Isolation procedure of dinapinones from the culture broth obtained by the fermentation of *T. pinophilus* FKI-3864 is shown in Figure 2. LC/UV analysis-guided isolation of dinapinones was carried out. The 14-day-old culture broth (10 l) was extracted with acetone (10 l). After concentrating the mixture to remove acetone, the aqueous solution was adjusted to pH 3 with 0.5 M HCl and extracted with EtOAc (10 l). The organic layer was dried over Na₂SO₄ and concentrated *in vacuo* to dryness to yield a brown material (4.8 g). The material was dissolved in a small amount of MeOH and applied to an ODS column (intradermal (i.d.) 40 × 270 mm; Senshu Scientific, Tokyo, Japan) and stepwise eluted with 60, 70, 80 and 90% MeOH-0.05% H₃PO₄ solvents (900 ml each).

The fractions of 90% MeOH-0.05% H₃PO₄ were concentrated *in vacuo* and extracted with EtOAc to give a brown material (319.3 mg). This material was dissolved in MeOH and purified by preparative HPLC (column, CAPCELL PAK C₁₈ UG 120 (i.d. 20 × 250 mm; Shiseido, Tokyo, Japan); mobile phase, 80% CH₃CN-0.05% H₃PO₄; flow rate, 8 ml min⁻¹; detection, UV at 210 nm). Under these conditions, dinapinones A (DPA) and AB (DPAB) were eluted as peaks with retention times of 14 and 17 min, respectively. The fractions were concentrated *in vacuo* to dryness to give DPA

¹Graduate School of Pharmaceutical Sciences, Kitasato University, Tokyo, Japan and ²Kitasato Institute for Life Sciences, Kitasato University, Tokyo, Japan
Correspondence: Professor H Tomoda, Graduate School of Pharmaceutical Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan.
E-mail: tomodah@pharm.kitasato-u.ac.jp

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Dinapinone	Structure	R
A1 (<i>M</i> -DPA1; 1)		
A2 (<i>P</i> -DPA2; 2)		
AB1 (<i>M</i> -DPAB1; 3)		
AB2 (<i>P</i> -DPAB2; 4)		
AC1 (<i>M</i> -DPAC1; 5)		
AC2 (<i>P</i> -DPAC2; 6)		
AD1 (<i>M</i> -DPAD1; 7)		
AD2 (<i>P</i> -DPAD2; 8)		
AE1 (<i>M</i> -DPAE1; 9)		
AE2 (<i>P</i> -DPAE2; 10)		
Monapinone		R
A (11)		
A' (11')		
B (12)		
B' (12')		
C (13)		
D (14)		
E (15)		

Figure 1 Structures of 1–15.

(212.8 mg) and DPAB (6.2 mg), both of which were a mixture of stereoisomers. As well as DPA,⁵ the stereoisomers of DPAB were separated by HPLC using a reverse-phase C30 column under the following conditions: column, Develosil C30 (i.d. 20 × 250 mm; Nomura Chemical, Aichi, Japan); mobile phase, 90% CH₃CN-0.05% H₃PO₄; flow rate, 8 ml min⁻¹; detection, UV at 210 nm. Dinapinones AB1 (3) and AB2 (4) were eluted as peaks with retention times of 20 and 24 min, respectively. These fractions were pooled and concentrated to remove CH₃CN. The aqueous solutions were extracted with EtOAc, and the organic layers were concentrated to dryness to give 3 (1.6 mg) and 4 (2.5 mg) as yellow amorphous solids.

The fractions of 80% MeOH-0.05% H₃PO₄ were concentrated *in vacuo* and extracted with EtOAc to give a brown material (217.8 mg). This material was dissolved in MeOH and purified by

preparative HPLC (column, CAPCELL PAK C₁₈ UG 120 (i.d. 20 × 250 mm); mobile phase, 50% CH₃CN-0.05% H₃PO₄; flow rate, 8 ml min⁻¹; detection, UV at 210 nm). Under these conditions, dinapinones AC (DPAC), AD (DPAD) and AE (DPAE) were eluted as peaks with retention times of 26, 36 and 41 min, respectively. The fractions were concentrated *in vacuo* to dryness to give DPAC (30.0 mg), DPAD (90.3 mg) and DPAE (7.4 mg). Similarly, to separate stereoisomers dinapinones AC1 (5) and AC2 (6), DPAC was subjected to HPLC under the following conditions: column, Develosil C30 (i.d. 20 × 250 mm); mobile phase, 50% CH₃CN-0.05% H₃PO₄; flow rate, 8 ml min⁻¹; detection, UV at 210 nm. Stereoisomers 5 and 6 were eluted as peaks with retention times of 55 and 60 min, respectively. By the same treatment, pure 5 (8.6 mg) and 6 (9.1 mg) were obtained as yellow amorphous solids. DPAD was subjected to HPLC under the following conditions: column, Develosil C30 (i.d. 20 × 250 mm);

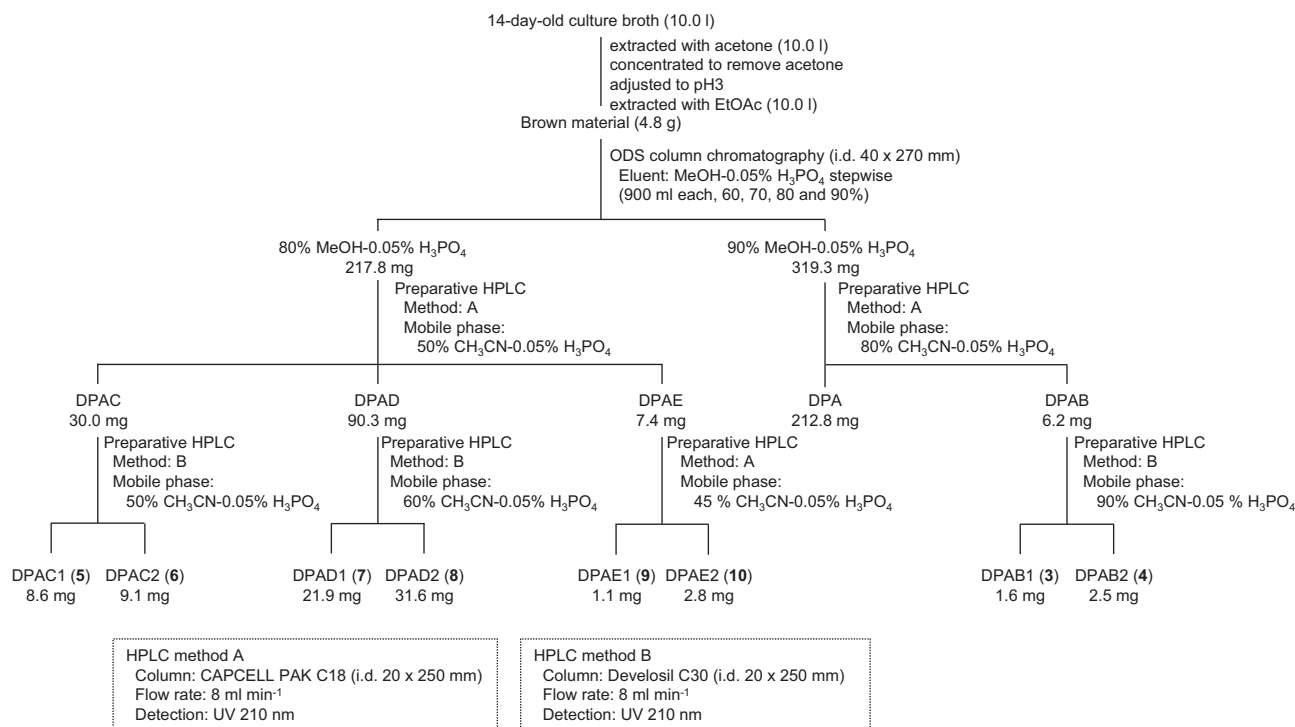


Figure 2 Isolation procedure of 3–10.

mobile phase, 60% CH₃CN-0.05% H₃PO₄; flow rate, 8 ml min⁻¹; detection, UV at 210 nm. Dinapinones AD1 (7) and AD2 (8) were eluted as peaks with retention times of 28 and 30 min, respectively. By the same treatment, pure 7 (21.9 mg) and 8 (31.6 mg) were obtained as yellow amorphous solids. DPAE was subjected to HPLC under the following conditions: column, CAPCELL PAK C₁₈ UG 120 (i.d. 20 × 250 mm); mobile phase, 45% CH₃CN-0.05% H₃PO₄; flow rate, 8 ml min⁻¹; detection, UV at 210 nm. Dinapinone AE1 (9) and AE2 (10) were eluted as peaks with retention times of 77 and 73 min, respectively. By the same treatment, pure 9 (1.1 mg) and 10 (2.8 mg) were obtained as yellow amorphous solids.

Physicochemical properties of dinapinones

The physicochemical properties of dinapinones (3–10) are summarized in Table 1. The compounds had similar UV spectra with absorption maxima at 221–226, 268–269 and 382–387 nm. The IR absorption at 1637–1638, 2923–2931 and 3394–3400 cm⁻¹ suggested the presence of a carbonyl group, alkyl chain and hydroxyl group in their structures. These data were similar to those of 1 and 2,⁷ indicating that they share the same skeleton.

Structure elucidation of dinapinones

Dinapinones AB1 (3) and AB2 (4): Dinapinone AB1 (3) showed a molecular ion peak *m/z* 813 [M + Na]⁺ in ESI-TOF-MS, and the molecular formula C₄₄H₅₄O₁₃ was assigned on the basis of its HRESI-TOF-MS (*m/z* 813.3448 (M + Na)⁺, Δ -1.4 mmu), indicating 18 degrees of unsaturation. The ¹H and ¹³C NMR spectra of 3 (Tables 2 and 3) showed 51 protons and 44 carbons signals, which were confirmed by 2D NMR correlation. The multiplicity of the carbon signals was classified into 2 methyl carbons, 2 oxygenated methyl carbons, 13 *sp*³ methylene carbons, 4 *sp*² methine carbons, 5 *sp*³ oxygenated methine carbons, 10 *sp*² quaternary carbons, 6 *sp*²

oxygenated quaternary carbons and 2 ester carbonyl carbons by analysis of HSQC data (Table 3).

As shown by the bold lines in Figure 3, the partial structure I of 3 was elucidated by ¹H-¹H COSY and TOCSY spectra. ¹H-¹³C long-range couplings of ²*J* and ³*J* in the HMBC are also shown in Figure 3a, leading to the following results: The cross peaks from the *sp*³ oxymethine proton H-3 (δ 4.89) to the ester carbonyl carbon C-1 (δ 171.28) and the *sp*² quaternary carbon C-4a (δ 132.69), from the *sp*³ methylene proton H₂-4 (δ 3.08, 3.14) to C-4a, the *sp*² methine carbon C-5 (δ 116.23) and the *sp*² quaternary carbon C-10a (δ 99.3), from the *sp*² methine proton H-5 (δ 6.97) to the *sp*³ methylene carbon C-4 (δ 32.99), C-4a, C-5a (δ 140.09), the *sp*² quaternary carbon C-9a (δ 108.4) and C-10a and from OH-10 (δ 13.71) to C-9a, the *sp*² oxygenated quaternary carbon C-10 (δ 162.85) and C-10a indicated the presence of a 10-oxy-3,5a,9a-trisubstituted isochroman-1-one moiety containing the partial structure I. The cross peaks from the *sp*² methine proton H-6 (δ 6.71) to the *sp*² oxygenated quaternary carbon C-7 (δ 161.5), the *sp*² quaternary carbon C-8 (δ 108.2) and C-9a, from OH-9 (δ 9.68) to C-8 and the *sp*² oxygenated quaternary carbon C-9 (δ 155.4) and from the *sp*³ oxygenated methyl proton H₃-11 (δ 3.85) to C-7 indicated the presence of a 7-methoxy-9-oxy-5a,8,9a-trisubstituted phenol moiety. This moiety was connected to the 10-oxy-3,5a,9a-trisubstituted isochroman-1-one moiety by the cross peaks from H-5 to C-6 (δ 98.1) and from H-6 to C-5 and the ⁴*J* cross peaks from H-5 to C-9, from H-6 to C-10 and from OH-9 to C-7 in HMBC experiments, indicating the presence of the partial structure II (Figure 3), which shares the same planar structure of monapinone A (11) (named monapinone A'; 11').

Similarly, as shown by the bold lines in Figure 3, the partial structure I' was elucidated by ¹H-¹H COSY and TOCSY spectra. ¹H-¹³C long-range couplings of ²*J* and ³*J* in the HMBC are also shown in Figure 3a, leading to the following results. Cross peaks from

Table 1 Physicochemical properties of 3–10

	DPABI (3)	DPAB2 (4)	DPAC1 (5)	DPAC2 (6)	DPAD1 (7)	DPAD2 (8)	DPAE1 (9)	DPAE2 (10)
Appearance	Pale brown powder	Pale brown powder	Pale brown powder	Pale brown powder	Pale brown powder	Pale brown powder	Pale brown powder	Pale brown powder
M_w	790	790	866	866	822	822	850	850
Molecular formula	$C_{44}H_{54}O_{13}$	$C_{44}H_{54}O_{13}$	$C_{46}H_{58}O_{16}$	$C_{46}H_{58}O_{16}$	$C_{44}H_{54}O_{15}$	$C_{44}H_{54}O_{15}$	$C_{46}H_{58}O_{15}$	$C_{46}H_{58}O_{15}$
HRESI-TOF-MS (m/z)								
Calcd	813.3462 [M + Na] ⁺	813.3462 [M + Na] ⁺	889.3623 [M + Na] ⁺	889.3623 [M + Na] ⁺	845.3360 [M + Na] ⁺	845.3360 [M + Na] ⁺	873.3673 [M + Na] ⁺	873.3673 [M + Na] ⁺
Found	813.3448 [M + Na] ⁺	813.3427 [M + Na] ⁺	889.3610 [M + Na] ⁺	889.3586 [M + Na] ⁺	845.3329 [M + Na] ⁺	845.3358 [M + Na] ⁺	873.3668 [M + Na] ⁺	873.3668 [M + Na] ⁺
UV (MeOH) λ_{max} nm (ϵ)	222 (24.200)	222 (25.700)	223 (24.000)	224 (22.500)	226 (12.800)	225 (19.600)	221 (28.600)	221 (26.500)
	269 (78.800)	269 (86.000)	269 (70.100)	269 (73.300)	268 (51.000)	269 (55.000)	269 (80.400)	269 (75.500)
	382 (22.000)	382 (23.400)	383 (20.600)	383 (22.100)	387 (16.400)	387 (18.200)	380 (19.000)	382 (20.700)
$[\alpha]_D^{25}$ (c 0.1, MeOH)	-200.1 ^a	+265.4 ^b	-124.1 ^c	+237.4 ^d	-141.9 ^e	+160.3 ^f	-155.4 ^g	+139.8 ^h
IR (KBr) ν_{max} (cm ⁻¹)	3399, 2926, 1637	3400, 2928, 1638	3399, 2925, 1638	3394, 2923, 1637	3399, 2931, 1637	3400, 2928, 1637	3399, 2928, 1637	3399, 2925, 1637
CD (MeOH)	287 (-30.9)	284 (-45.8)	286 (-22.5)	285 (+34.8)	284 (-27.0)	275 (+52.3)	286 (-29.8)	281 (+38.5)
$\lambda_{extremum}$ ($\Delta\epsilon$)	248 (+63.2)	246 (-50.9)	247 (+42.2)	245 (-38.0)	248 (+45.0)	257 (-63.0)	250 (+58.5)	249 (-48.6)

Abbreviation: DP, dinapinone.

the sp^3 oxymethine proton H-3' (δ 4.86) to the ester carbonyl carbon C-1' (δ 171.25) and the sp^2 quaternary carbon C-4a' (δ 132.72), from the sp^3 methylene proton H₂-4' (δ 3.06, 3.12) to C-4a', the sp^2 methine carbon C-5' (δ 116.26) and the sp^2 quaternary carbon C-10a' (δ 99.3), from the sp^2 methine proton H-5' (δ 6.97) to the sp^3 methylene carbon C-4' (δ 33.08), C-4a', the sp^2 quaternary carbon C-5a' (δ 140.08), the sp^2 quaternary carbon C-9a' (δ 108.4) and C-10a' and from OH-10' (δ 13.70) to C-9a', the sp^2 oxygenated quaternary carbon C-10' (δ 162.87) and C-10a' indicated the presence of 10'-oxy-3',5a',9a'-trisubstituted isochroman-1-one moiety containing the partial structure I'. The cross peaks from the sp^2 methine proton H-6' (δ 6.71) to the sp^2 oxygenated quaternary carbon C-7' (δ 161.5), the sp^2 quaternary carbon C-8' (δ 108.2) and C-9a', from OH-9' (δ 9.68) to C-8' and the sp^2 oxygenated quaternary carbon C-9' (δ 155.4) and from the sp^3 oxygenated methyl proton H₃-11' (δ 3.85) to C-7' indicated the presence of a 7'-methoxy-9'-oxy-5a',8',9a'-trisubstituted phenol moiety. The moiety was connected to the 10'-oxy-3',5a',9a'-trisubstituted isochroman-1-one moiety by the cross peaks from H-5' to C-6' (δ 98.1) and from H-6' to C-5' and the ⁴J cross peaks from H-5' to C-9', from H-6' to C-10' and from 9'-OH to C-7' in HMBC experiments, indicating the presence of the partial structure II' (Figure 3), which shares the same planar structure of monapinone B (12) (named monapinone B'; 12').

The chemical shifts and the molecular formula of 3 indicated that three hydroxyl groups were attached to C-13 (δ 69.6), C-15 (δ 73.3) and C-13' (δ 69.1). Furthermore, ¹³C NMR chemical shift data of the partial structures II and II' were almost identical to those of II' and 12', except that the methine C-8 signals (δ 101.2 and δ 101.6) in II' and 12'⁷ was replaced by quaternary carbon signal (C-8; δ 108.2 and C-8'; δ 108.2) in 3. According to the carbon signals of C-8 and C-8', 3 should have an 8,8'-linkage between the partial structures II and II'. This planar structure of 3 fulfilled the molecular formula and the degree of unsaturation. Thus, 3 was a heterodimer of II' and 12' via an 8,8'-linkage.

The ¹H and ¹³C NMR spectra (Tables 2 and 3) and 2D NMR (¹H-¹H COSY, HSQC and HMBC) data of 4 resembled those of 3. Accordingly, the planar structure of 4 was also elucidated to be the same as 3.

Dinapinones AC1 (5) and AC2 (6): The molecular formula of 5 is $C_{46}H_{58}O_{16}$ on the basis of HRESI-TOF-MS (m/z 889.3610 [M + Na]⁺, Δ -1.3 mmu) indicating that 5 contains two more oxygen atoms than 1. Comparison of the ¹H and ¹³C NMR spectra (Tables 2 and 3) between 1⁷ and 5 indicated that the methylenes at C-17' (δ_C 24.9, δ_{H2} 1.30) and C-19' (δ_C 22.6, δ_{H2} 1.30) in 1 are replaced by the oxygenated methines at C-17' (δ_C 70.1, δ_H 3.96) and C-19' (δ_C 67.3, δ_H 3.96) in 5, supporting the presence of monapinone C (13) substructure. Accordingly, 5 was a heterodimer of 1' and 13 via an 8,8'-linkage. (Figure 1), which satisfied the degrees of unsaturation and the molecular formula.

The ¹H and ¹³C NMR spectra (Tables 2 and 3) and 2D NMR (¹H-¹H COSY, HSQC and HMBC) data of 6 resembled those of 5. Accordingly, the planar structure of 6 was also elucidated to be the same as 5.

Dinapinones AD1 (7) and AD2 (8): The molecular formula of 7 is $C_{44}H_{54}O_{15}$ on the basis of HRESI-TOF-MS (m/z 845.3329 [M + Na]⁺, Δ -3.1 mmu), indicating 7 contains two more oxygen atoms than 3. Comparison of the ¹H and ¹³C NMR spectra (Table 2) between 3 and 7 indicated that the methylenes at C-15' (δ_C 25.13 δ_{H2} 1.34) and C-17' (δ_C 22.58, δ_{H2} 1.33) in 3 are replaced by oxygenated methines at C-15' (δ_C 70.1, δ_H 3.98) and C-17' (δ_C 67.3, δ_H 3.97) in 7, supporting the presence of a monapinone D (14) substructure.

Table 2 ¹H NMR chemical shifts of 1–10

No.	DPABI (3) ^a			DPAB2 (4) ^a			DPACI (5) ^b			DPAC2 (6) ^b				
	δ _H	J (Hz)	δ _H	J (Hz)	δ _H	J (Hz)	δ _H	J (Hz)	δ _H	J (Hz)	δ _H	J (Hz)		
3	4.89	ddd	3.6, 5.6, 7.0, 10.0	4.89	1H	ddd	3.6, 6.0, 6.4, 10.0	4.88	1H	ddd	4.82	1H	ddd	3.0, 5.4, 6.2, 9.2
4a	3.08	dd	10.0, 16.4	3.08	1H	dd	9.0, 16.8	3.04	1H	dd	2.96	1H	dd	9.2, 15.0
b	3.14	dd	3.6, 16.4	3.14	1H	dd	3.6, 16.8	3.20	1H	dd	3.12	1H	dd	3.0, 15.0
5	6.97	1H	s	6.97	1H	s	s	7.08	1H	s	6.97	1H	s	s
6	6.71	1H	s	6.71	1H	s	s	6.81	1H	s	6.73	1H	s	s
11	3.85	3H	s	3.85	3H	s	s	3.80	3H	s	3.76	3H	s	s
12a	1.89	1H	ddd	1.88	1H	ddd	4.0, 6.0, 14.6	1.97	1H	ddd	1.94	1H	ddd	4.0, 5.4, 14.0
b	2.14	1H	ddd	2.14	1H	ddd	6.4, 8.0, 14.6	2.09	1H	ddd	2.05	1H	ddd	6.2, 7.4, 14.0
13	4.22	1H	ddd	4.20	1H	ddd	2.6, 4.0, 8.0, 9.4	4.08	1H	ddd	4.08	1H	ddd	3.4, 4.0, 7.4, 7.4
14	1.62	1H	ddd	1.61	1H	ddd	9.4, 9.4, 14.2	1.65	1H	ddd	1.64	1H	ddd	7.4, 7.4, 14.6
15	1.67	1H	ddd	1.67	1H	ddd	2.6, 2.6, 14.2	1.70	1H	ddd	1.72	1H	ddd	3.4, 3.4, 14.6
16a	3.93	1H	m	3.93	1H	m	m	3.79	1H	m	3.79	1H	m	m
b	1.49	2H	m	1.50	2H	m	m	1.45	1H	m	1.44	2H	m	m
17a	1.32	2H	m	1.32	2H	m	m	1.48	1H	m	1.34	1H	m	m
b	1.33	2H	m	1.31	2H	m	m	1.37	2H	m	1.46	1H	m	m
18a	1.33	2H	m	1.31	2H	m	m	1.32	2H	m	1.32	2H	m	m
b	1.33	2H	m	1.33	2H	m	m	1.32	2H	m	1.32	2H	m	m
19	1.33	2H	m	1.33	2H	m	m	1.35	2H	m	1.32	2H	m	m
20	0.90	3H	t	0.90	3H	t	6.6	0.92	3H	t	0.91	3H	t	6.6
9-OH	9.68	1H	s	9.68	1H	s	s	9.68	1H	s	9.68	1H	s	s
10-OH	13.71	1H	s	13.72	1H	s	s	13.72	1H	s	13.71	1H	s	s
3'	4.86	1H	ddd	4.86	1H	ddd	3.6, 6.0, 6.8, 10.0	4.88	1H	ddd	4.82	1H	ddd	3.0, 5.4, 6.2, 9.2
4'a	3.06	1H	dd	3.07	1H	dd	10.0, 16.8	3.04	1H	dd	2.96	1H	dd	9.2, 15.0
b	3.12	1H	dd	3.12	1H	dd	3.6, 16.8	3.20	1H	dd	3.12	1H	dd	3.0, 15.0
5'	6.97	1H	s	6.97	1H	s	s	7.08	1H	s	6.97	1H	s	s
6'	6.71	1H	s	6.71	1H	s	s	6.81	1H	s	6.73	1H	s	s
11'	3.85	3H	s	3.85	3H	s	s	3.80	3H	s	3.76	3H	s	s
12'a	1.91	1H	ddd	1.92	1H	ddd	3.4, 6.0, 14.4	1.97	1H	ddd	1.94	1H	ddd	4.0, 5.4, 14.0
b	2.08	1H	ddd	2.08	1H	ddd	6.8, 8.6, 14.4	2.09	1H	ddd	2.05	1H	ddd	6.2, 7.4, 14.0
13'	3.95	1H	m	3.93	1H	m	m	4.10	1H	ddd	4.08	1H	ddd	3.4, 4.0, 7.4, 7.4
14'a	1.53	2H	m	1.54	2H	m	m	1.75	1H	ddd	1.64	1H	ddd	7.4, 7.4, 14.6
b	1.34	2H	m	1.35	2H	m	m	1.68	1H	ddd	1.72	1H	ddd	3.4, 3.4, 14.6
15'	1.30	2H	m	1.31	2H	m	m	4.02	1H	ddd	4.02	1H	ddd	3.4, 4.0, 7.4, 8.0
16a	1.30	2H	m	1.31	2H	m	m	1.63	1H	ddd	1.58	1H	ddd	8.0, 8.0, 15.0
b	1.33	2H	m	1.33	2H	m	m	1.67	1H	ddd	1.62	1H	ddd	4.0, 4.0, 15.0
17'	0.91	3H	t	0.91	3H	t	6.6	3.96	1H	ddd	3.95	1H	ddd	4.0, 4.0, 8.0, 8.0
18'a	9.68	1H	s	9.68	1H	s	s	1.57	1H	ddd	1.55	1H	ddd	4.0, 4.0, 15.0
b	13.70	1H	s	13.71	1H	s	s	1.64	1H	ddd	1.60	1H	ddd	8.0, 8.0, 15.0
19'	—	—	—	—	—	—	—	3.96	1H	m	3.95	1H	m	m
20'	—	—	—	—	—	—	—	1.19	3H	d	1.18	3H	d	6.8
9'-OH	9.68	1H	s	9.68	1H	s	s	9.68	1H	s	9.68	1H	s	s
10'-OH	13.70	1H	s	13.71	1H	s	s	13.71	1H	s	13.71	1H	s	s

Abbreviation: DP, dinapinone.
^aChemical shifts are shown as δ values (p.p.m.) relative to CDCl₃ at 7.26 p.p.m. for ¹H NMR.
^bChemical shifts are shown as δ values (p.p.m.) relative to CD₃OD at 3.31 p.p.m. for ¹H NMR.
^cChemical shifts are quoted from Uchida *et al.*⁷

Table 2 (Continued)

No.	DPAD1 (7) ^b			DPAD2 (8) ^b			DPAE1 (9) ^b			DPAE2 (10) ^b		
	δ_H	J (Hz)	δ_H	J (Hz)	δ_H	J (Hz)	δ_H	J (Hz)	δ_H	J (Hz)	δ_H	J (Hz)
3	4.88	3.0, 5.4, 7.0, 10.8	4.84	5.4, 7.0, 11.0	4.88	5.4, 7.0, 11.0	4.87	2.4, 6.2, 6.4, 10.6	4.87	2.4, 6.2, 6.4, 10.6	4.87	3.0, 6.0, 6.0, 11.0
4a	3.02	10.8, 16.2	2.96	11.0, 16.6	3.04	11.0, 16.6	3.01	10.6, 16.4	3.01	10.6, 16.4	3.01	11.0, 15.0
b	3.19	3.0, 16.2	3.12	16.6	3.21	16.6	3.18	2.4, 16.4	3.18	2.4, 16.4	3.18	3.0, 15.0
5	7.07		6.98		7.12		7.06		7.06		7.06	
6	6.79		6.74		6.82		6.80		6.80		6.80	
11	3.81		3.76		3.81		3.79		3.79		3.79	
12a	1.96	4.4, 5.4, 14.4	1.96	3.6, 5.4, 14.0	1.97	3.6, 5.4, 14.0	1.94	4.0, 6.4, 13.0	1.94	4.0, 6.4, 13.0	1.94	4.2, 6.0, 13.0
b	2.07	7.0, 8.0, 14.4	2.06	7.0, 8.0, 14.0	2.07	7.0, 8.0, 14.0	2.07	6.2, 7.8, 13.0	2.06	6.2, 7.8, 13.0	2.06	6.0, 7.4, 13.0
13	4.09	4.4, 4.6, 8.0, 8.0	4.07	3.6, 4.0, 8.0, 8.0	4.08	3.6, 4.0, 8.0, 8.0	4.08	3.0, 4.0, 7.8, 8.0	4.08	3.0, 4.0, 7.8, 8.0	4.08	3.0, 4.2, 7.4, 8.0
14a	1.65	8.0, 8.0, 13.4	1.65	8.0, 8.0, 14.0	1.66	8.0, 8.0, 14.0	1.66	8.0, 8.0, 13.4	1.65	8.0, 8.0, 13.4	1.65	8.0, 8.0, 13.0
b	1.70	4.6, 4.6, 13.4	1.72	4.4, 4.4, 14.0	1.71	4.4, 4.4, 14.0	1.71	3.0, 3.0, 13.4	1.70	3.0, 3.0, 13.4	1.70	3.0, 3.0, 13.0
15	3.78		3.78		3.79		3.79		3.79		3.79	
16a	1.40		1.44		1.48		1.48		1.48		1.48	
b	1.35		1.34		1.52		1.52		1.52		1.52	
17a	1.45		1.45		1.38		1.38		1.36		1.36	
b	1.29		1.31		1.30		1.30		1.29		1.29	
18a	1.33		1.33		1.32		1.32		1.32		1.32	
b	1.35		1.33		1.34		1.34		1.34		1.34	
19	0.92	7.0	0.90	6.8	0.92	6.8	0.92	7.0	0.92	7.0	0.92	6.4
20												
9-OH												
10-OH												
3'	4.88	3.0, 6.2, 6.4, 10.8	4.84	6.0, 6.2, 11.0	4.88	6.0, 6.2, 11.0	4.88	2.4, 6.2, 6.4, 10.6	4.87	2.4, 6.2, 6.4, 10.6	4.87	3.0, 6.0, 6.0, 11.0
4'a	3.02	10.8, 16.8	2.96	11.0, 16.6	3.04	11.0, 16.6	3.04	10.6, 16.4	3.01	10.6, 16.4	3.01	11.0, 15.0
b	3.19	3.0, 16.8	3.12	16.6	3.21	16.6	3.21	2.4, 16.4	3.18	2.4, 16.4	3.18	3.0, 15.0
5'	7.07		6.98		7.10		7.10		7.06		7.06	
6'	6.79		6.74		6.82		6.82		6.80		6.80	
11'	3.80		3.76		3.81		3.81		3.79		3.79	
12'a	1.94	5.0, 6.4, 14.4	1.92	4.4, 6.0, 14.0	1.97	4.4, 6.0, 14.0	1.97	4.0, 6.4, 13.0	1.94	4.0, 6.4, 13.0	1.94	4.2, 6.0, 13.0
b	2.08	6.2, 7.4, 14.4	2.08	6.2, 7.0, 14.0	2.07	6.2, 7.0, 14.0	2.07	6.2, 7.8, 13.0	2.06	6.2, 7.8, 13.0	2.06	6.0, 7.4, 13.0
13'	4.08	4.2, 5.0, 7.0, 7.4	4.06	4.4, 4.4, 7.0, 7.2	4.08	4.4, 4.4, 7.0, 7.2	4.08	3.0, 4.0, 7.8, 8.0	4.08	3.0, 4.0, 7.8, 8.0	4.08	3.0, 4.2, 7.4, 8.0
14'a	1.69	7.0, 7.0, 13.4	1.68	7.2, 7.2, 14.0	1.66	7.2, 7.2, 14.0	1.66	8.0, 8.0, 13.4	1.65	8.0, 8.0, 13.4	1.65	8.0, 8.0, 13.0
b	1.74	4.2, 4.2, 13.4	1.74	4.4, 4.4, 14.0	1.71	4.4, 4.4, 14.0	1.71	3.0, 3.0, 13.4	1.70	3.0, 3.0, 13.4	1.70	3.0, 3.0, 13.0
15'	3.98	4.2, 4.4, 7.0, 7.4	3.98	4.4, 4.6, 7.2, 7.2	3.79	4.4, 4.6, 7.2, 7.2	3.79		3.79		3.79	
16'a	1.59	4.4, 4.4, 14.4	1.58	4.6, 4.6, 13.6	1.56	4.6, 4.6, 13.6	1.56		1.48		1.48	
b	1.64	7.4, 7.4, 14.4	1.62	7.2, 7.2, 13.6	1.62	7.2, 7.2, 13.6	1.62		1.52		1.52	
17'	3.97		3.96	6.3	3.96	6.3	3.96		1.36		1.36	
18'a	1.19		1.18		1.36		1.36		1.34		1.34	
b					1.40		1.40		1.40		1.40	
19'					1.56		1.56		1.56		1.56	
20'					3.56		3.56	6.8	3.57	6.8	3.57	6.8

Abbreviation: DP, dinapinone.

^aChemical shifts are shown as δ values (p.p.m.) relative to CDCl₃ at 7.26 p.p.m. for ¹H NMR.^bChemical shifts are shown as δ values (p.p.m.) relative to CD₃OD at 3.31 p.p.m. for ¹H NMR.^cChemical shifts are quoted from Uchida *et al.*

Table 2 (Continued)

No.	DPA1 (1) ^{a,c}		DPA2 (2) ^{a,c}	
	δ_H	J (Hz)	δ_H	J (Hz)
3	4.88	ddd	4.88	ddd
4a	3.07	dd	3.07	dd
b	3.13	dd	3.13	dd
5	6.97	s	6.97	s
6	6.71	s	6.71	s
11	3.84	s	3.84	s
12a	1.89	ddd	1.88	ddd
b	2.14	ddd	2.14	ddd
13	4.22	dddd	4.20	dddd
14a	1.62	ddd	1.62	ddd
b	1.68	ddd	1.67	ddd
15	3.93	m	3.92	m
16a	1.49	m	1.49	m
b	1.30	m	1.30	m
17a	1.30	m	1.30	m
b	1.30	m	1.30	m
18a	1.30	m	1.30	m
b	1.30	m	1.30	m
19	0.90	t	0.90	t
20	9.68	s	9.67	s
9-OH	13.70	s	13.70	s
10-OH	4.88	dddd	4.88	dddd
3'	3.07	dd	3.07	dd
4'a	3.13	dd	3.13	dd
b	6.97	s	6.97	s
5'	6.71	s	6.71	s
6'	3.84	s	3.84	s
11'	1.89	ddd	1.88	ddd
12'a	2.14	ddd	2.14	ddd
b	4.22	dddd	4.20	dddd
13'	1.62	ddd	1.62	ddd
14'a	1.68	ddd	1.67	ddd
b	3.93	m	3.92	m
15'	1.49	m	1.49	m
16'a	1.30	m	1.30	m
b	1.30	m	1.30	m
17'	1.30	m	1.30	m
18'a	1.30	m	1.30	m
b	0.90	t	0.90	t
19'	9.68	s	9.67	s
20'	13.70	s	13.70	s
9'-OH	4.88	dddd	4.88	dddd
10'-OH	3.07	dd	3.07	dd
3''	3.13	dd	3.13	dd
4''a	6.97	s	6.97	s
5''	6.71	s	6.71	s
6''	3.84	s	3.84	s
11''	1.89	ddd	1.88	ddd
12''a	2.14	ddd	2.14	ddd
b	4.22	dddd	4.20	dddd
13''	1.62	ddd	1.62	ddd
14''a	1.68	ddd	1.67	ddd
b	3.93	m	3.92	m
15''	1.49	m	1.49	m
16''a	1.30	m	1.30	m
b	1.30	m	1.30	m
17''	1.30	m	1.30	m
18''a	1.30	m	1.30	m
b	0.90	t	0.90	t
19''	9.68	s	9.67	s
20''	13.70	s	13.70	s
9''-OH	4.88	dddd	4.88	dddd
10''-OH	3.07	dd	3.07	dd
3'''	3.13	dd	3.13	dd
4'''a	6.97	s	6.97	s
5'''	6.71	s	6.71	s
6'''	3.84	s	3.84	s
11'''	1.89	ddd	1.88	ddd
12'''a	2.14	ddd	2.14	ddd
b	4.22	dddd	4.20	dddd
13'''	1.62	ddd	1.62	ddd
14'''a	1.68	ddd	1.67	ddd
b	3.93	m	3.92	m
15'''	1.49	m	1.49	m
16'''a	1.30	m	1.30	m
b	1.30	m	1.30	m
17'''	1.30	m	1.30	m
18'''a	1.30	m	1.30	m
b	0.90	t	0.90	t
19'''	9.68	s	9.67	s
20'''	13.70	s	13.70	s
9'''-OH	4.88	dddd	4.88	dddd
10'''-OH	3.07	dd	3.07	dd
3''''	3.13	dd	3.13	dd
4''''a	6.97	s	6.97	s
5''''	6.71	s	6.71	s
6''''	3.84	s	3.84	s
11''''	1.89	ddd	1.88	ddd
12''''a	2.14	ddd	2.14	ddd
b	4.22	dddd	4.20	dddd
13''''	1.62	ddd	1.62	ddd
14''''a	1.68	ddd	1.67	ddd
b	3.93	m	3.92	m
15''''	1.49	m	1.49	m
16''''a	1.30	m	1.30	m
b	1.30	m	1.30	m
17''''	1.30	m	1.30	m
18''''a	1.30	m	1.30	m
b	0.90	t	0.90	t
19''''	9.68	s	9.67	s
20''''	13.70	s	13.70	s
9''''-OH	4.88	dddd	4.88	dddd
10''''-OH	3.07	dd	3.07	dd
3'''''	3.13	dd	3.13	dd
4'''''a	6.97	s	6.97	s
5'''''	6.71	s	6.71	s
6'''''	3.84	s	3.84	s
11'''''	1.89	ddd	1.88	ddd
12'''''a	2.14	ddd	2.14	ddd
b	4.22	dddd	4.20	dddd
13'''''	1.62	ddd	1.62	ddd
14'''''a	1.68	ddd	1.67	ddd
b	3.93	m	3.92	m
15'''''	1.49	m	1.49	m
16'''''a	1.30	m	1.30	m
b	1.30	m	1.30	m
17'''''	1.30	m	1.30	m
18'''''a	1.30	m	1.30	m
b	0.90	t	0.90	t
19'''''	9.68	s	9.67	s
20'''''	13.70	s	13.70	s
9'''''-OH	4.88	dddd	4.88	dddd
10'''''-OH	3.07	dd	3.07	dd
3''''''	3.13	dd	3.13	dd
4''''''a	6.97	s	6.97	s
5''''''	6.71	s	6.71	s
6''''''	3.84	s	3.84	s
11''''''	1.89	ddd	1.88	ddd
12''''''a	2.14	ddd	2.14	ddd
b	4.22	dddd	4.20	dddd
13''''''	1.62	ddd	1.62	ddd
14''''''a	1.68	ddd	1.67	ddd
b	3.93	m	3.92	m
15''''''	1.49	m	1.49	m
16''''''a	1.30	m	1.30	m
b	1.30	m	1.30	m
17''''''	1.30	m	1.30	m
18''''''a	1.30	m	1.30	m
b	0.90	t	0.90	t
19''''''	9.68	s	9.67	s
20''''''	13.70	s	13.70	s
9''''''-OH	4.88	dddd	4.88	dddd
10''''''-OH	3.07	dd	3.07	dd
3'''''''	3.13	dd	3.13	dd
4'''''''a	6.97	s	6.97	s
5'''''''	6.71	s	6.71	s
6'''''''	3.84	s	3.84	s
11'''''''	1.89	ddd	1.88	ddd
12'''''''a	2.14	ddd	2.14	ddd
b	4.22	dddd	4.20	dddd
13'''''''	1.62	ddd	1.62	ddd
14'''''''a	1.68	ddd	1.67	ddd
b	3.93	m	3.92	m
15'''''''	1.49	m	1.49	m
16'''''''a	1.30	m	1.30	m
b	1.30	m	1.30	m
17'''''''	1.30	m	1.30	m
18'''''''a	1.30	m	1.30	m
b	0.90	t	0.90	t
19'''''''	9.68	s	9.67	s
20'''''''	13.70	s	13.70	s
9'''''''-OH	4.88	dddd	4.88	dddd
10'''''''-OH	3.07	dd	3.07	dd
3''''''''	3.13	dd	3.13	dd
4''''''''a	6.97	s	6.97	s
5''''''''	6.71	s	6.71	s
6''''''''	3.84	s	3.84	s
11''''''''	1.89	ddd	1.88	ddd
12''''''''a	2.14	ddd	2.14	ddd
b	4.22	dddd	4.20	dddd
13''''''''	1.62	ddd	1.62	ddd
14''''''''a	1.68	ddd	1.67	ddd
b	3.93	m	3.92	m
15''''''''	1.49	m	1.49	m
16''''''''a	1.30	m	1.30	m
b	1.30	m	1.30	m
17''''''''	1.30	m	1.30	m
18''''''''a	1.30	m	1.30	m
b	0.90	t	0.90	t
19''''''''	9.68	s	9.67	s
20''''''''	13.70	s	13.70	s
9''''''''-OH	4.88	dddd	4.88	dddd
10''''''''-OH	3.07	dd	3.07	dd
3'''''''''	3.13	dd	3.13	dd
4'''''''''a	6.97	s	6.97	s
5'''''''''	6.71	s	6.71	s
6'''''''''	3.84	s	3.84	s
11'''''''''	1.89	ddd	1.88	ddd
12'''''''''a	2.14	ddd	2.14	ddd
b	4.22	dddd	4.20	dddd
13'''''''''	1.62	ddd	1.62	ddd
14'''''''''a	1.68	ddd	1.67	ddd
b	3.93	m	3.92	m
15'''''''''	1.49	m	1.49	m
16'''''''''a	1.30	m	1.30	m
b	1.30	m	1.30	m
17'''''''''	1.30	m	1.30	m
18'''''''''a	1.30	m	1.30	m
b	0.90	t	0.90	t
19'''''''''	9.68	s	9.67	s
20'''''''''	13.70	s	13.70	s
9'''''''''-OH	4.88	dddd	4.88	dddd
10'''''''''-OH	3.07	dd	3.07	dd
3''''''''''	3.13	dd	3.13	dd
4''''''''''a	6.97	s	6.97	s
5''''''''''	6.71	s	6.71	s
6''''''''''	3.84	s	3.84	s
11''''''''''	1.89	ddd	1.88	ddd
12''''''''''a	2.14	ddd	2.14	ddd
b	4.22	dddd	4.20	dddd
13''''''''''	1.62	ddd	1.62	ddd
14''''''''''a	1.68	ddd	1.67	ddd
b	3.93	m	3.92	m
15''''''''''	1.49	m	1.49	m
16''''''''''a	1.30	m	1.30	m
b	1.30	m	1.30	m
17''''''''''	1.30	m	1.30	m
18''''''''''a	1.30	m	1.30	m
b	0.90	t	0.90	t
19''''''''''	9.68	s	9.67	s
20''''''''''	13.70	s	13.70	s
9''''''''''-OH	4.88	dddd	4.88	dddd
10''''''''''-OH	3.07	dd	3.07	dd
3'''''''''''	3.13	dd	3.13	dd
4'''''''''''a	6.97	s	6.97	s
5'''''''''''	6.71	s	6.71	s
6'''''''''''	3.84	s	3.84	s
11'''''''''''	1.89	ddd	1.88	ddd
12'''''''''''a	2.14	ddd	2.14	ddd
b	4.22	dddd	4.20	dddd
13'''''''''''	1.62	ddd	1.62	ddd
14'''''''''''a	1.68	ddd	1.67	ddd
b	3.93	m	3.92	m
15'''''''''''	1.49	m	1.49	m
16'''''''''''a	1.30	m	1.30	m
b	1.30	m	1.30	m
17'''''''''''	1.30	m	1.30	m
18'''''''''''a	1.30	m	1.30	m
b	0.90	t	0.90	t
19'''''''''''	9.68	s	9.67	s
20'''''''''''	13.70	s	13.70	s
9'''''''''''-OH	4.88	dddd	4.88	dddd
10'''''''''''-OH	3.07			

Table 3 ^{13}C NMR chemical shifts of 1–10

No.	DPAB1 (3) ^a		DPB2 (4) ^a		DPAC1 (5) ^b		DPAC2 (6) ^b		DPAD1 (7) ^b		DPD2 (8) ^b		DPAE1 (9) ^b		DPAE2 (10) ^b		DPA1 (1) ^{a,c}		DPA2 (2) ^{a,c}	
	δ_c		δ_c		δ_c		δ_c		δ_c		δ_c		δ_c		δ_c		δ_c		δ_c	
1	171.28	s	171.28	s	172.8	s	170.8	s	172.9	s	172.8	s	172.9	s	172.8	s	171.3	s	171.3	s
3	78.19	d	78.14	d	79.3	d	78.7	d	79.3	d	79.2	d	79.3	d	79.3	d	78.18	d	78.13	d
4	32.99	t	32.85	t	33.7	t	33.9	t	33.7	t	33.7	t	33.7	t	33.7	t	32.98	t	32.84	t
4a	132.69	s	132.67	s	134.7	s	133.1	s	134.6	s	134.5	s	134.7	s	134.6	s	132.73	s	132.7	s
5	116.23	d	116.24	d	117.5	d	116.5	d	117.6	d	117.6	d	117.6	d	117.6	d	116.26	d	116.28	d
5a	140.09	s	140.1	s	141.6	s	140.0	s	141.6	s	141.5	s	141.6	s	141.6	s	140.1	s	140.1	s
6	98.1	d	98.1	d	99.1	d	98.5	d	99.2	d	99.2	d	99.1	d	99.2	d	98.1	d	98.1	d
7	161.5	s	161.5	s	162.9	s	161.3	s	162.9	s	162.9	s	163.0	s	162.9	s	161.5	s	161.5	s
8	108.2	s	108.2	s	109.7	s	108.8	s	109.8	s	109.8	s	109.8	s	109.8	s	108.4	s	108.4	s
9	155.4	s	155.4	s	156.5	s	154.6	s	156.3	s	154.3	s	156.4	s	156.3	s	155.4	s	155.4	s
9a	108.4	s	108.5	s	109.4	s	108.2	s	109.3	s	109.2	s	109.3	s	109.3	s	108.2	s	108.2	s
10	162.85	s	162.84	s	162.9	s	161.0	s	163.3	s	162.9	s	163.4	s	163.3	s	162.9	s	162.82	s
10a	99.3	s	99.4	s	100.7	s	99.6	s	100.6	s	100.5	s	100.7	s	100.6	s	99.3	s	99.3	s
11	56.0	q	56.0	q	56.2	q	56.2	q	56.2	q	56.3	q	56.2	q	56.2	q	55.9	q	55.9	q
12	42.2	t	42.1	t	43.1	t	43.2	t	43.1	t	43.1	t	43.0	t	43.1	t	42.25	t	42.14	t
13	69.6	d	69.6	d	67.7	d	67.4	d	67.6	d	67.7	d	68.0	d	68.0	d	69.6	d	69.54	d
14	42.9	t	42.9	t	45.6	t	45.6	t	44.9	t	44.9	t	44.92 ^{d1}	t	44.9	t	42.91	t	42.91	t
15	73.3	d	73.4	d	71.3	d	71.0	d	71.3	d	71.4	d	71.29 ^{d2}	d	71.21 ^{d5}	d	73.36	d	73.36	d
16	38.4	t	38.4	t	38.8	t	39.0	t	38.8	t	38.8	t	38.76 ^{d3}	t	38.74 ^{d6}	t	38.38	t	38.4	t
17	24.94	t	24.9	t	26.2	t	26.6	t	26.3	t	26.3	t	26.34 ^{d4}	t	26.22 ^{d7}	t	24.9	t	24.9	t
18	31.73	t	31.7	t	33.1	t	33.4	t	33.1	t	33.1	t	33.1	t	33.1	t	31.7	t	31.7	t
19	22.61	t	22.60	t	23.7	t	24.2	t	23.8	t	23.8	t	23.7	t	23.7	t	22.6	t	22.6	t
20	14.00	q	14.00	q	14.4	q	15.0	q	14.4	q	14.4	q	14.4	q	14.4	q	14.0	q	14.0	q
1'	171.25	s	171.24	s	172.8	s	170.8	s	172.9	s	172.8	s	172.9	s	172.8	s	171.3	s	171.3	s
3'	78.81	d	78.77	d	79.3	d	78.7	d	79.3	d	79.2	d	79.3	d	79.3	d	78.18	d	78.13	d
4'	33.08	t	33.0	t	33.7	t	33.9	t	33.7	t	33.7	t	33.7	t	33.7	t	32.98	t	32.84	t
4a'	132.72	s	132.71	s	134.7	s	133.1	s	134.6	s	134.5	s	134.7	s	134.6	s	132.73	s	132.7	s
5'	116.26	d	116.28	d	117.5	d	116.5	d	117.6	d	117.6	d	117.6	d	117.6	d	116.26	d	116.28	d
5a'	140.08	s	140.1	s	141.6	s	140.0	s	141.6	s	141.5	s	141.6	s	141.6	s	140.1	s	140.1	s
6'	98.1	d	98.1	d	99.1	d	98.5	d	99.2	d	99.2	d	99.1	d	99.2	d	98.1	d	98.1	d
7'	161.5	s	161.5	s	162.9	s	161.3	s	162.9	s	162.9	s	163.0	s	162.9	s	161.5	s	161.5	s
8'	108.2	s	108.2	s	109.7	s	108.8	s	109.8	s	109.8	s	109.8	s	109.8	s	108.4	s	108.4	s
9'	155.4	s	155.4	s	156.5	s	154.6	s	156.3	s	154.3	s	156.4	s	156.3	s	155.4	s	155.4	s
9a'	108.4	s	108.5	s	109.4	s	108.2	s	109.3	s	109.2	s	109.3	s	109.3	s	108.2	s	108.2	s
10'	162.87	s	162.87	s	162.9	s	161.0	s	163.3	s	162.9	s	163.4	s	163.3	s	162.9	s	162.82	s
10a'	99.3	s	99.4	s	100.7	s	99.6	s	100.6	s	100.5	s	100.7	s	100.6	s	99.3	s	99.3	s
11'	56.0	q	56.0	q	56.2	q	56.2	q	56.2	q	56.3	q	56.2	q	56.2	q	55.9	q	55.9	q
12'	41.9	t	41.8	t	43.1	t	43.2	t	43.1	t	43.1	t	43.0	t	43.1	t	42.25	t	42.14	t
13'	69.1	d	69.0	d	68.0	d	67.7	d	68.0	d	68.0	d	68.0	d	68.0	d	69.6	d	69.54	d
14'	37.7	t	37.8	t	44.9	t	45.1	t	45.2	t	45.2	t	44.91 ^{d1}	t	44.9	t	42.91	t	42.91	t
15'	25.13	t	25.1	t	69.9	d	69.7	d	70.1	d	70.2	d	71.18 ^{d2}	d	71.32 ^{d5}	d	73.36	d	73.36	d
16'	31.74	t	31.7	t	45.1	t	45.6	t	47.0	t	47.0	t	38.74 ^{d3}	t	38.77 ^{d6}	t	38.38	t	38.4	t
17'	22.58	t	22.6	t	70.1	d	69.8	d	67.3	d	67.3	d	26.23 ^{d4}	t	26.34 ^{d7}	t	24.9	t	24.9	t
18'	14.01	q	14.01	q	46.9	t	46.9	t	23.7	q	23.7	q	27.0	t	27.0	t	31.7	t	31.7	t
19'	—	—	—	—	67.3	d	67.1	d	—	—	—	—	33.6	t	33.6	t	22.6	t	22.6	t
20'	—	—	—	—	23.7	q	24.2	q	—	—	—	—	63.0	t	63.0	t	14.0	q	14.0	q

Abbreviation: DP, dinapinone.

^aChemical shifts are shown as δ values (p.p.m.) relative to CDCl_3 at 77.0 p.p.m. for ^{13}C NMR.^bChemical shifts are shown as δ values (p.p.m.) relative to CD_3OD at 49.0 p.p.m. for ^{13}C NMR.^cChemical shifts are quoted from Uchida *et al.*⁷^dChemical shifts are exchangeable.

Accordingly, **7** was a heterodimer of **11'** and **14** via an 8,8'-linkage (Figure 1), which satisfied the degrees of unsaturation and the molecular formula.

The ^1H and ^{13}C NMR spectra (Tables 2 and 3) and 2D NMR (^1H - ^1H COSY, HSQC and HMBC) data of **8** resembled those of **7**. Accordingly, the planar structure of **8** was also elucidated to be the same as **7**.

Dinapinones AE1 (**9**) and AE2 (**10**): the molecular formula of **9** is $\text{C}_{46}\text{H}_{58}\text{O}_{15}$ on the basis of HRESI-TOF-MS (m/z 873.3668 ($M + \text{Na}$)⁺, $\Delta -0.5$ mmu), indicating that **9** contains one more oxygen atom than **1**. Comparison of the ^1H and ^{13}C NMR spectra (Table 2) between **1** and **9** indicated that the methyl at C-20' (δ_c 14.0, δ_{H_3} 0.90) in **1** are replaced by the oxygenated methylene at C-20' (δ_c 63.0, δ_{H_2}

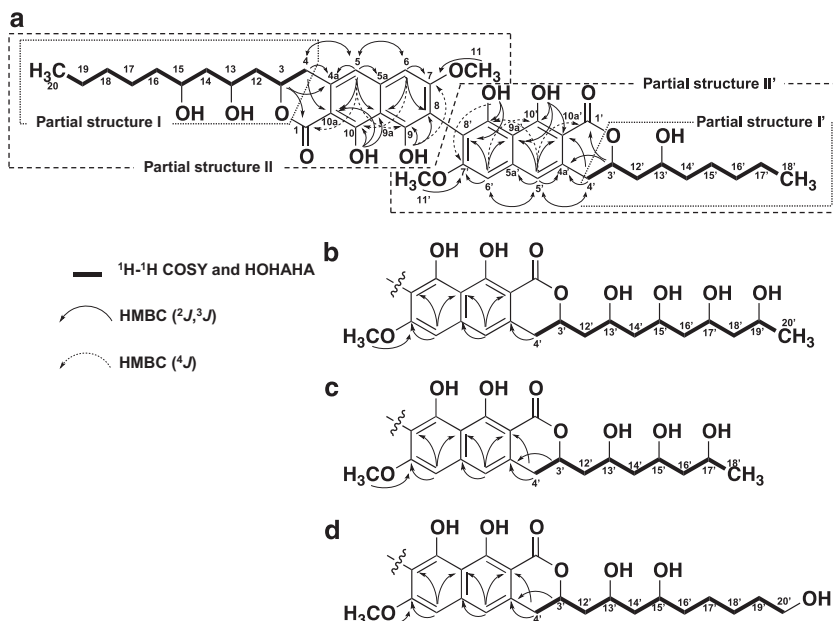


Figure 3 Structure elucidations of **3–10** by ^1H – ^1H COSY, TOCSY and HMBC experiments. (a) Compounds **3** and **4**, (b) **5** and **6**, (c) **7** and **8**, and (d) **9** and **10**.

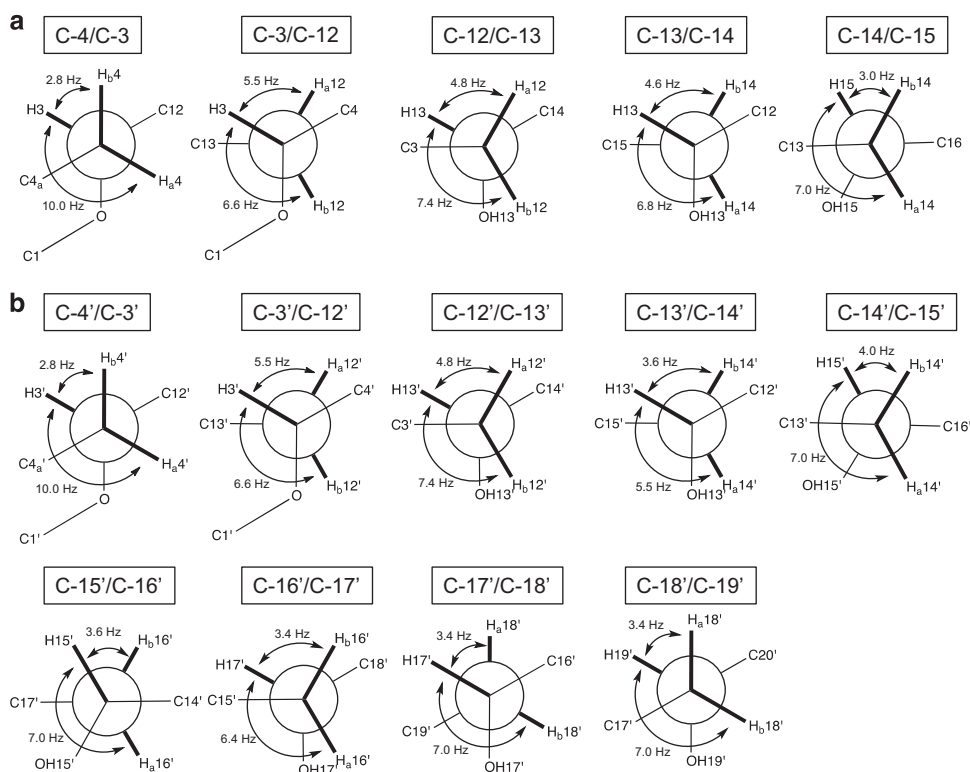


Figure 4 Coupling constants ($^2J_{\text{HH}}$) and the dihedral angles of partial structures I (a) and I' (b) of **5**.

3.56) in **9**, supporting the presence of monapinone E (**15**) substructure. Accordingly, **9** was a heterodimer of **11'** and **15** via an 8,8'-linkage (Figure 1), which satisfied the degrees of unsaturation and the molecular formula.

The ^1H and ^{13}C NMR spectra (Tables 2 and 3) and 2D NMR (^1H – ^1H COSY, HSQC and HMBC) data of **10** resembled those of **9**. Accordingly, the planar structure of **10** was also elucidated to be the same as **9**.

Relative stereochemistries of dinapinones

From the planar structure elucidation, all dinapinones (**3–10**) were heterodimers of one **11'** and one different monapinone (**12'**, **13–15**). Compounds **5** and **6**, consisting of **11'** and **13**, have eight chiral carbons. The relative stereochemistry of the **11'** substructure in **5** was elucidated by analysis of the dihedral angles (Figure 4), ^1H NMR coupling constants (J values) and ROESY experiments (Figure 5). The large value of $J_{3-4\text{axial}}$ (10.0 Hz) indicated that the two protons

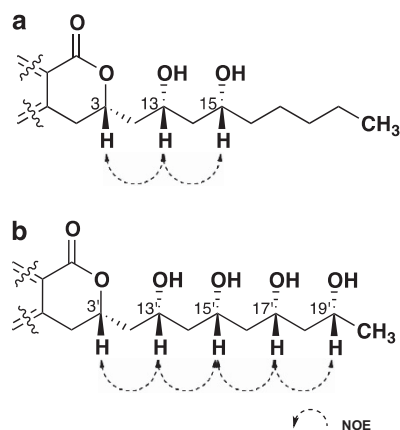


Figure 5 ROESY experiments of partial structures I (a) and I' (b) of 5.

Table 4 Effects of CE and TG synthesis of 1–10 in CHO-K1 cells

Dinapinone	IC_{50} (μM)			Selectivity index CE/TG
	CE	TG	PL	
A1 (1)	>12	>12	>12	—
A2 (2)	2.94	0.87	>12	3.38
AB1 (3)	>12	>12	>12	—
AB2 (4)	10.16	1.17	>12	8.68
AC1 (5)	>12	>12	>12	—
AC2 (6)	>12	>12	>12	—
AD1 (7)	>12	>12	>12	—
AD2 (8)	>12	8.94	>12	>1.34
AE1 (9)	>12	9.19	>12	>1.31
AE2 (10)	>12	9.34	>12	>1.28

Abbreviations: CE, cholesterol ester; PL, phospholipid; TG, triacylglycerol.

were in *trans* diaxial orientation at C-3. As for a dihydroxyalkyl chain at C-13 and C-15 of the 11' substructure in 5, NOEs were observed between H-3 (δ 4.88) and H-13 (δ 4.08) and between H-13 and H-15 (δ 3.79) (Figure 5). Additionally, the coupling constants (Table 2) and the dihedral angles (Figure 4) from C-12 to C-15 were similar to those of 11',⁷ indicating that the 11' substructure in 5 had the same configuration of 11. Thus, the relative stereochemistry of the 11' substructure in 5 was elucidated to be 3*S**13*R**15*R**. The relative stereochemistry of the 13 substructure in 5 was elucidated similarly. The large value of $J_{3'-4'axial}$ (10.0 Hz) indicated that the two protons were also in *trans* diaxial orientation at C-3. As for a tetrahydroxyalkyl chain at C-13', C-15', C-17' and C-19' of the 13 substructure in 5, NOEs were observed between H-3' (δ 4.88) and H-13' (δ 4.10), H-13' and H-15' (δ 4.02) and H-15' and H-17' (δ 3.96) (Figure 5). Additionally, the ¹H NMR coupling constants (Table 2) and the dihedral angles (Figure 4) from C-12' to C-19' supported that the four hydroxy groups were all in *syn* configuration. Thus, the relative stereochemistry of the 13 substructure in 5 was elucidated to be 3'*S**13'*R**15'*R**17'*R**19'*R**. Taking all these findings together, the relative stereochemistry of 5 was elucidated as shown in Figure 1.

The ¹H NMR coupling constants and ROESY experimental data of 6 resembled those of 5 (Table 2). Accordingly, the relative stereochemistry of 6 was elucidated to be 3*S**13*R**15*R**3'*S**13'*R**15'*R**

17'*R**19'*R**. Thus, heterodimers 5 and 6, consisting of 11 and 13, have the same relative stereochemistries, suggesting that they are atropisomers.

The relative stereochemistries of the three pairs of stereoisomers 3 and 4, 7 and 8, and 9 and 10 were also defined to be 3*S**13*R**15*R**3'*S**13'*R**, 3*S**13*R**15*R**3'*S**13'*R**15'*R**17'*R** and 3*S**13*R**15*R**3'*S**13'*R**15'*R**, respectively, from the same analysis of the coupling constants in ¹H NMR and ROESY experiments. Each pair (3 and 4, 5 and 6, 7 and 8, and 9 and 10) was deduced to be atropisomers from the similar relation of 1 and 2 as reported previously.⁷

Absolute axis configurations of dinapinones

To elucidate the absolute axis configuration, the CD data of 3–10 were compared with those of (*M*)-1 and (*P*)-2.⁷ Compounds 3, 5, 7 and 9 exhibited the first negative cotton effect at 284–287 nm and the second positive cotton effect at 247–250 nm in the CD spectrum (Table 1). On the other hand, 4, 6, 8 and 10 exhibited the first positive cotton effect at 275–285 nm and the second negative cotton effect at 245–257 nm in the CD spectrum (Table 1). These spectra were almost identical to those of 1 and 2, respectively. Accordingly, the absolute axis configurations of 3, 5, 7 and 9 were elucidated to be *M* and those of 4, 6, 8 and 10 were *P*.

Inhibition of TG and CE synthesis by dinapinones

The effect of dinapinones (3–10) was evaluated on the synthesis of [¹⁴C]TG and [¹⁴C]CE from [¹⁴C]oleic acid in CHO-K1 cells. Compounds 1 and 2 were also tested for comparative purposes. The IC_{50} values are summarized in Table 4. Among the dinapinones, 2 showed the most potent inhibition of TG synthesis with an IC_{50} value of 0.87 μM , which was comparable with the value (0.65 μM) previously reported.⁷ Compound 4 exhibited the second most potent inhibition (IC_{50} , 1.17 μM), followed by 8, 9 and 10 (IC_{50} , 8.9–9.3 μM). The other dinapinones (1, 3, 5, 6 and 7) showed very weak or no activity at 12 μM . Active dinapinones (2, 4, 8, 9 and 10) were found to inhibit cholesterol ester (CE) synthesis, but to have selectivity toward the inhibition of TG synthesis (selectivity index, 1.3–8.7).

DISCUSSION

In this study, eight new dinapinones (3–10) were purified by LC/UV analysis-guided isolation from the culture broth of *T. pinophilus*. From the structure elucidation, 3–10 were found to be heterodimers consisting of monapinone A (11), and the other monapinones (12–15) and pairs of 3 and 4, 5 and 6, 7 and 8, and 9 and 10 were atropisomers. The relative stereochemistries of monapinone substructures in dinapinones were elucidated by precise analyses of the dihedral angles, *J* values and ROESY experiments (Figures 4 and 5). In the previous study, the stereochemistries of 13–15 were not elucidated, but we confirmed that they are the same as the corresponding substructures in dinapinones (data not shown). These findings indicated that dinapinones are biosynthesized from monapinones.

Considering the axis configuration, there are 2 atropisomers for this series of homodimers. In fact, atropisomers 1 and 2 were obtained as homodimers of monapinone A (11).^{5–7} In the case of a heterodimer, two monomers can link in two different fashions, that is, monomer A–monomer B or monomer B–monomer A, and each heterodimer should have two atropisomers. Thus, there are theoretically four different structures to a heterodimer. Among them, however, a pair of atropisomers was obtained for each dinapinones in this study. This might be due to the characteristics of the

hypothetical dimerization enzyme in this fungal strain. It therefore appeared difficult to elucidate the complete stereochemistry of the two atropisomers, but this could be achieved by X-ray crystallographic analysis.

Regarding the biological activity of dinapinones, **2** and **4** showed potent TG inhibition in CHO-K1 cells and moderate CE inhibition, resulting in low selectivity indexes (3.4 and 8.7, respectively) (Table 4). Other dinapinones are not such potent inhibitors of TG synthesis. In the previous study, we reported that the 1:1 mixture of **1** and **2** increased inhibitory activity against TG synthesis and selectivity toward TG synthesis.⁵ Evaluation of the 1:1 mixture of each atropisomers in this TG synthesis assay using CHO-K1 cells is ongoing, and the results will be described elsewhere.

METHODS

General experimental procedure

SSC-ODS-7515-12 (Senshu Scientific) was used for ODS column chromatography. Various NMR spectra were obtained using NMR System 400 MHz and an INOVA 600 MHz spectrometer (Agilent Technologies, Santa Clara, CA, USA). ESI-MS spectrometry was conducted using a JMS-T100LP spectrometer (JEOL, Tokyo, Japan). Optical rotation and CD were recorded on a JASCO model DIP-181 polarimeter (JASCO, Tokyo, Japan) and J-720 CD spectrometer (JASCO). UV and IR spectra were measured with a Beckman DU640 spectrophotometer (Beckman Coulter, Fullerton, CA, USA) and FT-210 Fourier transform IR spectrometer (HORIBA, Kyoto, Japan), respectively.

Microorganism

Fungal strain *T. pinophilus* FKI-3864 was isolated from a soil sample collected Hilo, Hawaii, USA. This strain was used to produce dinapinones.⁵

Fermentation

A slant culture of strain FKI-3864 grown on Miura's medium (0.10% glycerol, 0.08% KH₂PO₄, 0.02% K₂HPO₄, 0.02% MgSO₄·7H₂O, 0.02% KCl, 0.2% NaNO₃, 0.02% yeast extract and 1.5% agar, adjusted to pH 6.0 before sterilization) was inoculated into a 500-ml Erlenmeyer flask containing 100 ml seed medium (2.0% glucose, 0.5% polypeptone, 0.05% MgSO₄·7H₂O, 0.2% yeast extract, 0.1% KH₂PO₄, 0.10% agar, adjusted to pH 6.0 before sterilization). The flask was incubated on a rotary shaker (210 r.p.m.) for 3 days at 27 °C to obtain the seed culture. The production culture was initiated by transferring 2 ml seed culture into each of the fifty 1 liter Roux flasks containing 200 ml production medium (3.0% sucrose, 3.0% soluble starch, 1.0% malt extract, 0.3% Ebios (Asahi Food & Healthcare, Tokyo, Japan), 0.5% KH₂PO₄ and 0.05% MgSO₄·7H₂O, adjusted to pH 6.0 before sterilization) and fermentation was carried out under static conditions at 27 °C for 14 days.

Cell culture

CHO-K1 cells (a generous gift from Dr Kentaro Hanada, National Institute of Infectious Disease, Tokyo, Japan) were maintained at 37 °C in 5% CO₂ in

Ham's F-12 medium (Sigma-Aldrich, St Louis, MO, USA) supplemented with 10% heat-inactivated FBS using the method described previously.⁸

Assay for TG and CE synthesis in intact CHO-K1 cells

Assays for TG, CE and phospholipid synthesis using CHO-K1 cells were carried out using established methods with some modifications.⁹ CHO-K1 cells (1.25 × 10⁵ cells per 250 μl) were cultured in a 48-well plastic microplate. A sample (2.5 μl in methanol) and [¹⁴C]oleic acid (1 nmol, 1.85 KBq, 5.0 μl in 10% ethanol/phosphate-buffered saline solution) were added to each well of the cell culture. The cells were cultured at 37 °C in 5% CO₂. After 6 h incubation, cells in each well were washed twice with phosphate-buffered saline. The cells were lysed by adding 0.25 ml of 10 mM Tris-HCl (pH 7.5) containing 0.1% (w/v) SDS, and the cellular lipids were extracted by the method of Bligh and Dyer.¹⁰ The total lipids were separated on a TLC plate (silica gel F254, 0.5-mm thick; Merck KGaA, Darmstadt, Germany) and the TLC plate was analysed with a bioimaging analyser (BAS 2000; Fujifilm, Tokyo, Japan) to measure the amount of [¹⁴C] lipids. Lipid synthesis activity (%) was defined as ([¹⁴C]lipid-drug/[¹⁴C]lipid-control) × 100. The IC₅₀ value was defined as the drug concentration causing 50% inhibition of lipid synthesis.

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- 1 Coleman, R. A. & Lee, D. P. Enzymes of triacylglycerol synthesis and their regulation. *Prog. Lipid Res.* **43**, 134–176 (2004).
- 2 Lewin, T. M. & Coleman, R. A. Regulation of myocardial triacylglycerol synthesis and metabolism. *Biochim. Biophys. Acta.* **1634**, 63–75 (2003).
- 3 Choi, S. H. & Ginsberg, H. N. Increased very low density lipoprotein (VLDL) secretion, hepatic steatosis, and insulin resistance. *Trends Endocrinol. Metab.* **22**, 353–363 (2011).
- 4 Mattson, F. H. & Volpenhein, R. A. The digestion and absorption of triglycerides. *J. Biol. Chem.* **239**, 2772–2777 (1964).
- 5 Ohte, S. *et al.* Dinapinones, novel inhibitors of triacylglycerol synthesis in mammalian cells, produced by *Penicillium pinophilum* FKI-3864. *J. Antibiot.* **64**, 489–494 (2011).
- 6 Kawamoto, K. *et al.* Production of monapinones by fermentation of the dinapinone-producing fungus *Penicillium pinophilum* FKI-3864 in a seawater-containing medium. *J. Antibiot.* **64**, 503–508 (2011).
- 7 Uchida, R. *et al.* Structure and absolute stereochemistry of dinapinones A1 and A2, inhibitors of triacylglycerol synthesis, produced by *Penicillium pinophilum* FKI-3864. *J. Antibiot.* **65**, 419–425 (2012).
- 8 Lada, A. T. *et al.* Identification of ACAT1- and ACAT2-specific inhibitors using a novel, cell-based fluorescence assay: individual ACAT uniqueness. *J. Lipid Res.* **45**, 378–386 (2004).
- 9 Ohshiro, T., Rudel, L. L., Ōmura, S. & Tomoda, H. Selectivity of microbial acyl-CoA: cholesterol acyltransferase inhibitors toward isozymes. *J. Antibiot.* **60**, 43–51 (2007).
- 10 Bligh, E. G. & Dyer, W. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37**, 911–917 (1959).