ORGANIC LETTERS

2004
Vol. 6, No. 6
869−872

Novel Dolabellane-Type Diterpene Alkaloids with Lipid Metabolism Promoting Activities from the Seeds of *Nigella sativa*

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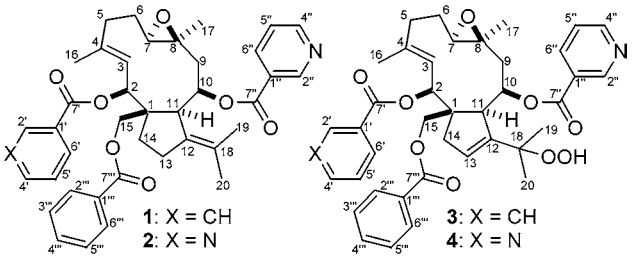
Received November 15, 2003 *(Revised Manuscript Received February 12, 2004)*

ABSTRACT



Four new dolabellane-type diterpene alkaloids, nigellamines A_1 (1), A_2 (2), B_1 (3), and B_2 (4), were isolated from the seeds of *Nigella sativa*. Their absolute stereostructures were determined on the basis of chemical and physicochemical evidence. Nigellamines A_1 (1), B_1 (3), and B_2 (4) were found to show potent lipid metabolism promoting activity in primary cultured mouse hepatocytes, and their activities were equivalent to that of a PPAR-α agonist, clofibrate.

The Ranunculaceae annual plant *Nigella sativa* L. is widely cultivated in Arabian countries, and the seeds are commonly called "black cumin". The seeds of this plant have been used as a food and spice and also prescribed in Egyptian folk medicine for the treatment of asthma, flatulence, polio, kidney stones, abdominal pain, etc.[1]

In the course of our characterization studies on bioactive constituents from Egyptian medicinal herbs,[2] four novel dolabellane-type diterpene alkaloids named nigellamines A_1 (**1**), A_2 (**2**), B_1 (**3**), and B_2 (**4**) were isolated from the methanolic extract of the seeds of *N. sativa*. This paper deals with the absolute stereostructure elucidation and lipid metabolism promoting activities of nigellamines (**1**−**4**).

The seeds of *N. sativa* (purchased in Egypt) were extracted with methanol three times under reflux for 3 h. The methanolic extract (17.4% from this natural medicine) was partitioned into an EtOAc and water mixture to give an EtOAc-soluble fraction (10.1%) and an aqueous phase (7.3%). The EtOAc-soluble fraction was subjected to ordinary-phase [*n*-hexanes−EtOAc (20:1−10:1−5:1−2:1−1:2) to $CHCl_3$−MeOH−H_2O (20:3:1, lower layer 6:4:1) to MeOH] and reversed-phase column chromatographies (MeOH−H_2O), and finally HPLC (YMC-Pack ODS-5-A, 250×20 mm i.d., MeOH−H_2O or CH_3CN−H_2O) to give nigellamines A_1 (**1**, 0.0096% from the natural medicine), A_2 (**2**, 0.0078%), B_1 (**3**, 0.0012%), and B_2 (**4**, 0.0036%).

Nigellamine A_1 (**1**) was isolated as a white powder with negative optical rotation [$[\alpha]^{27}_D$ −23.4 (c = 1.20, $CHCl_3$)]. The positive-ion fast atom bombardment (FAB)-MS of **1** showed a quasimolecular ion peak at m/z 650 $(M + H)^+$, and the molecular formula $C_{40}H_{43}NO_7$ of **1** was determined

(1) (a) Sayed, K. A.; Ross, S. A.; El Sohly, M. A.; Khalafalla, M. M.; Abdel-Halim, O. B.; Ikegami, F. *Saudi Pharm. J.* **2000**, *8*, 175−181. (b) Enomoto, S.; Asano, R.; Iwahori, Y.; Narui, T.; Okada, Y.; Singab, A. N. B.; Okuyama, T. *Biol. Pharm. Bull.* **2001**, *24*, 307−310 and literature cited therein.

(2) (a) Yoshikawa, M.; Xu, F.; Morikawa, T.; Ninomiya, K.; Matsuda, H. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1045−1049. (b) Yoshikawa, M.; Morikawa, T.; Xu, F.; Ando, S.; Matsuda, H. *Heterocycles* **2003**, *60*, 1787−1792 and literature cited therein.

10.1021/ol036239c CCC: $27.50
Published on Web 02/27/2004

by high-resolution MS measurement.[3] The IR (KBr) spectrum of **1** showed absorption bands at 1717, 1647, 1636, 1592, 1541, 1509, 1420, 1277, 1111, 1069, 1026, 947, 756, and 712 cm^{-1} ascribable to ester carbonyl, olefin, and ether functions and aromatic rings. In the UV spectrum of **1** (in MeOH), absorption maxima were observed at 226 (log ϵ 4.61) and 264 (3.73) nm. The ^1H and ^{13}C NMR ($CDCl_3$, Table 1) spectra of **1** showed signals assignable to four methyls, a methylene, and three methine bearing the oxygen function, an olefin, two benzoyl groups, and a nicotinoyl group together with five methylenes (H_2-5, 6, 9, 13, 14), a methine (H-11), and five quaternary carbons (C-1, 4, 8, 12, 18). Treatment of **1** with 0.1% sodium methoxide (NaOMe)−MeOH at room temperature furnished the desacyl derivative nigellanol A (**1a**)[4,5] together with methyl nicotinate and methyl benzoate, which were identified by HPLC analysis.[6] The dolabellane-type diterpene structure and positions of acyl groups in **1** were constructed on the basis of various NMR experiments.[7] Thus, the $^1H-^1H$ COSY experiments on **1** indicated the presence of four partial structures in bold lines as shown in Figure 1 (C-2−3, C-5−7, C-9−11, C-13−14).

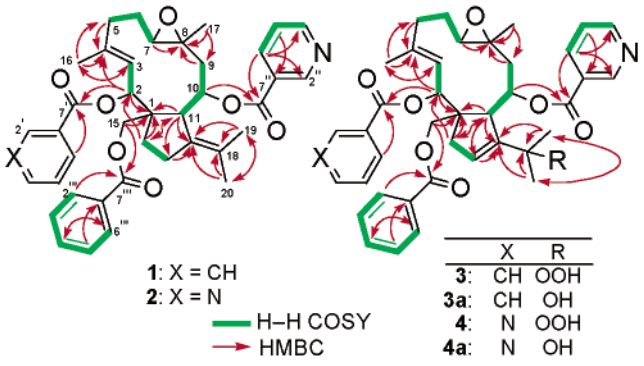


Figure 1. $^1H-^1H$ COSY and HMBC correlations of **1**−**4**.

In the HMBC experiment of **1**, long-range correlations were observed between the following proton and quaternary carbon pairs: H-2, 11, H_2-15, and C-1; H-2, H_2-5, H_3-16, and C-4; H-7, H_2-9, H_3-17, and C-8; H-11, H_2-13, H_3-19, 20, and C-12; H_3-19, 20 and C-18; H-2, H-6′, and C-7′; H-10, H-6″, and C-7″; H_2-15, H-2‴,6‴, and C-7‴ (Figure 1). Next, the relative stereostructures of **1** and **1a** were confirmed by NOESY experiment on **1a**, in which NOE correlations were observed between the following proton pairs: H-2 and H-11, H_3-16; H-3 and H-7, H-15b; Hβ-5 and Hβ-6, H-7; Hα-6 and H_3-17; Hβ-6 and H-7; H-7 and Hβ-9, H-15b; Hα-9 and H-10, H_3-17; H-10 and H-11; H_2-14 and H_2-15 (Figure 2). Finally,

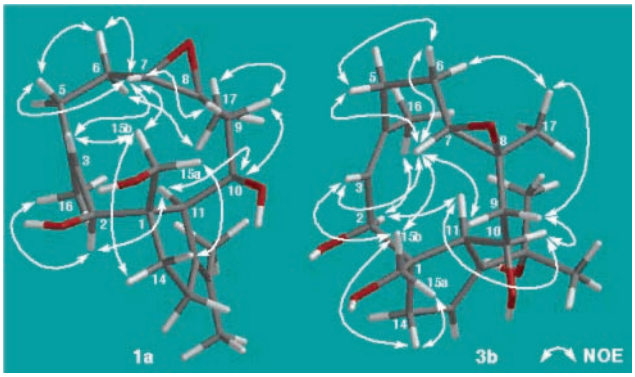


Figure 2. NOE correlations of **1a** and **3b**.

the absolute stereostructure of **1** was determined by application of the CD excitation chirality method for an allylic benzoate.[8] Thus, **1** was treated with 0.1% NaOMe−MeOH at 0 °C to give the partial desacylated derivatives including 2-*O*-benzoylnigellanol A (**1b**).[9] Compound **1b** showed a negative Cotton effect [246 nm ($\Delta\epsilon$ −1.31) in MeOH], which indicated the absolute configuration at the 2-position in **1b** to be *S*. On the basis of this evidence, the absolute stereostructure of **1** was determined.

Nigellamine A_2 (**2**),[10] a white powder, $[\alpha]^{27}_D$ −24.2 (c = 1.00, $CHCl_3$), $C_{39}H_{42}N_2O_7$, and its IR and UV spectra were very similar to those of **1**. The proton and carbon signals in the ^1H and ^{13}C NMR ($CDCl_3$, Table 1) spectra[7] of **2** indicated the presence of nigellanol A part, two nicotinoyl groups, and a benzoyl group. The positions of the acyl groups in **2** were determined by HMBC experiment as shown in Figure 1. Treatment of **2** with 0.1% NaOMe−MeOH at room tem-

(3) **1**: high-resolution positive-ion FAB-MS calcd for $C_{40}H_{44}NO_7$ (M + H)$^+$ 650.3118, found 650.3123; CD (MeOH, $\Delta\epsilon$) −1.63 (259 nm).

(4) **1a**: high-resolution FAB-MS calcd for $C_{20}H_{32}O_4Na$ (M + Na)$^+$ 359.2198, found 359.2202; ^1H NMR (CD_3OD) δ 1.36, 1.70, 1.74 (3H each, all s, H_3-17, 19, 20), 1.51 (1H, dd, J = 12.8, 13.8 Hz, Hβ-9), 1.62 (3H, d, J = 1.2 Hz, H_3-16), 1.72 (1H, m, Hα-6), 1.83 (1H, m, Hβ-6), 2.03 (1H, m, Hβ-13), 2.12 (1H, m, Hα-13), 2.19 (1H, dd, J = 5.5, 13.8 Hz, Hα-9), 2.27 (1H, m, Hα-5), 2.29 (1H, br s, H-11), 2.32 (1H, m, Hβ-14), 2.37 (1H, ddd, J = 5.2, 12.6, 12.6 Hz, Hβ-5), 2.46 (1H, m, Hα-14), 2.96 (1H, d, J = ca.10 Hz, H-7), 3.90 (1H, d, J = 10.4 Hz, H-2), 3.94, 4.00 (1H each, both d, J = 11.0 Hz, H_2-15), 3.99 (1H, br dd, J = ca. 6, 13 Hz, H-10), 5.37 (1H, dd, J = 1.2, 10.4 Hz, H-3); positive-ion FAB-MS m/z 359 (M + Na)$^+$.

(5) Spartan (version '02, Wavefunction, Inc., Irvine, CA) was used to build and optimize the conformations of **1a** and **3b** (Figure 2) using MOPAC (AM1) program. Those conformations were also supported by the NOE correlations in the NOESY experiments, respectively.

(6) Methyl nicotinate (**i**) and methyl benzoate (**ii**) were identified by HPLC analysis through comparison with standard samples obtained by diazomethane methylation of commercial nicotinic acid and benzoic acid. [t_R (**i**) 5.58, (**ii**) 15.80 min, detection: UV (254 nm), column: YMC-Pack ODS-5-A, 250 × 4.6 mm i.d., mobile phase: MeOH−H_2O (60: 40, v/v); flow rate 0.7 mL/min].

(7) The ^1H and ^{13}C NMR spectra of **1**−**4** were assigned with the aid of $^1H-^1H$ and $^{13}C-^1H$ COSY, DEPT, and HMBC experiments.

(8) Harada, N.; Iwabuchi, J.; Yokota, Y.; Uda, H.; Nakanishi, K. *J. Am. Chem. Soc.* **1981**, *103*, 5590−5591.

(9) **1b**: ^1H NMR ($CDCl_3$): δ 1.40, 1.70, 1.76 (3H each, all s, H_3-17, 20, 19), 1.64 (1H, dd, J = 12.8, 13.8 Hz, Hβ-9), 1.67 (1H, m, Hα-6), 1.72 (1H, m, Hβ-14), 1.79 (3H, d, J = 0.9 Hz, H_3-16), 1.93 (1H, m, Hβ-6), 2.28 (1H, m, Hα-14), 2.30 (2H, m, H_2-5), 2.34 (2H, m, H_2-13), 2.37 (1H, dd, J = 5.2, 13.8 Hz, Hα-9), 2.48 (1H, br s, H-11), 2.92 (1H, br d, J = ca. 9 Hz, H-7), 4.11 (1H, m, H-10), 4.14, 4.26 (1H each, both d, J = 11.3 Hz, H_2-15), 5.28 (1H, dd, J = 0.9, 10.4 Hz, H-3), 5.32 (1H, d, J = 10.4 Hz, H-2), 7.45 (2H, dd, J = 7.6, 8.3 Hz, H-3′, 5′), 7.57 (1H, tt, J = 1.2, 7.6 Hz, H-4′), 8.03 (2H, dd, J = 1.2, 8.3 Hz, H-2′, 6′).

(10) **2**: high-resolution FAB-MS calcd for $C_{39}H_{43}N_2O_7$ (M + H)$^+$ 651.3071, found 651.3065; CD (MeOH, $\Delta\epsilon$) −1.42 (256 nm); UV (MeOH, log ϵ) 220 (4.50), 264 (3.82) nm; IR (KBr) 1717, 1647, 1636, 1592, 1541, 1509, 1456, 1279, 1109, 1024, 947, 741, 703 cm^{-1}; Positive-ion FAB-MS m/z 651 (M + H)$^+$.

Table 1. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Data of Nigellamines A_1 (**1**), A_2 (**2**), B_1 (**3**), and B_2 (**4**) in $CDCl_3$[a]

	1		**2**		**3**		**4**	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1		56.5		56.6		54.8		54.9
2	5.45 d (10.4)	73.1	5.48 d (10.3)	73.7	5.57 d (10.4)	73.4	5.59 d (10.7)	74.0
3	5.74 dd (0.9, 10.4)	123.8	5.73 d (10.3)	123.4	5.78 dd (0.9, 10.4)	122.4	5.79 dd (0.9, 10.7)	122.0
4		139.8		140.3		141.7		142.2
5α	2.39 br d (ca. 13)	37.9	2.38 m	37.9	2.35 br d (ca. 13)	38.1	2.36 br d (ca. 13)	38.1
5β	2.47 ddd (4.9, 12.9, 12.9)		2.48 ddd (5.2,12.8,12.8)		2.47 ddd (4.9, 12.9, 12.9)		2.47 ddd (4.9, 12.9, 12.9)	
6α	1.72 m	22.8	1.65 m	22.8	1.67 m	23.0	1.66 m	23.0
6β	2.01 m		2.02 d-like		2.00 m		2.02 m	
7	3.08 br d (ca. 10)	65.5	3.07 br d (ca. 9)	65.4	2.99 br d (ca. 10)	66.9	2.99 br d (ca. 10)	66.9
8		58.6		58.6		58.8		58.7
9α	2.59 dd (5.5, 13.8)	42.0	2.59 dd (5.5, 13.7)	42.0	2.72 dd (5.8, 13.7)	41.0	2.71 dd (5.8, 13.4)	41.0
9β	1.62 dd (12.5, 13.8)		1.61 dd (12.5, 13.7)		1.59 dd (12.2, 13.7)		1.58 dd (12.8, 13.4)	
10	5.70 br dd (ca. 6.13)	75.4	5.70 br dd (ca. 6, 13)	75.4	5.92 br dd (ca. 6, 12)	74.0	5.92 br dd (ca. 6, 13)	73.9
11	2.72 br s	48.1	2.71 br s	48.1	2.85 br s	51.8	2.84 br s	51.8
12		135.8		135.6		145.9		145.9
13	2.31 m (2H)	28.3	2.36 m (2H)	28.2	5.79 br s	128.8	5.81 br s	128.8
14α	2.34 m	31.4	2.34 m	31.4	2.78 br s (2H)	38.3	2.74 dd (3.0, 17.0)	38.4
14β	2.20 m		2.23 m				2.79 br d (ca. 17)	
15	4.93 d (11.0)	67.2	4.90 d (11.0)	67.0	5.12 d (11.0)	66.3	5.11 d (11.0)	66.2
	5.29 d (11.0)		5.31 d (11.0)		5.26 d (11.0)		5.28 d (11.0)	
16	1.87 d (0.9)	16.5	1.87 s	16.7	1.94 d (0.9)	17.3	1.94 d (0.9)	17.3
17	1.54 s	18.4	1.53 s	18.4	1.52 s	17.2	1.52 s	17.2
18		127.1		127.3		82.5		82.6
19	1.66 s	22.6	1.67 s	22.6	*1.48 s	*26.1	*1.49 s	*26.0
20	1.88 s	21.8	1.88 s	21.8	*1.51 s	*27.2	*1.51 s	*27.2
1′		130.0		125.9		129.9		125.9
2′	7.84 dd (1.3, 8.3)	129.7	9.11 br s	151.0	7.81 dd (1.3, 8.3)	129.7	9.09 br s	150.9
3′	7.13 dd (7.6, 8.3)	128.1			7.12 dd (7.6, 8.3)	128.1		
4′	7.44 tt (1.3, 7.6)	132.8	8.65 br d (ca. 5)	153.3	7.44 tt (1.3, 7.6)	132.9	8.66 br s	153.2
5′	7.13 dd (7.6, 8.3)	128.1	6.98 dd (4.9, 8.0)	122.9	7.12 dd (7.6, 8.3)	128.1	6.96 dd (4.9, 8.0)	122.9
6′	7.84 dd (1.3, 8.3)	129.7	7.96 ddd (1.8, 1.8, 8.0)	136.9	7.81 dd (1.3, 8.3)	129.7	7.93 ddd (1.8, 1.9, 8.0)	137.0
7′		166.5		165.1		166.5		165.2
1″		125.9		125.8		126.0		125.8
2″	9.24 br d (ca. 2)	150.8	9.24 br s	150.8	9.21 br s	150.8	9.20 br s	150.8
4″	8.78 br d (ca. 5)	153.6	8.79 br d (ca. 4)	153.7	8.78 br s	153.6	8.74 br s	153.6
5″	7.41 dd (4.9, 8.0)	123.5	7.42 m	123.6	7.42 dd (4.9, 8.0)	123.7	7.43 dd (4.9, 8.0)	123.6
6″	8.31 ddd (1.9, 2.2, 8.0)	137.1	8.31 ddd (1.8, 1.8, 7.9)	137.1	8.32 ddd (1.8, 1.9, 8.0)	137.4	8.31 ddd (1.8, 1.9, 8.0)	137.4
7″		164.4		164.4		165.1		165.1
1‴		130.3		130.1		130.2		130.1
2‴	8.14 dd (1.3, 8.3)	130.1	8.10 br d (ca. 8)	129.9	8.14 dd (1.3, 8.3)	130.1	8.11 dd (1.3, 8.3)	129.9
3‴	7.39 dd (7.6, 8.3)	128.4	7.39 dd (7.8, 7.9)	128.5	7.40 dd (7.7, 8.3)	128.5	7.42 dd (7.6, 8.3)	128.6
4‴	7.58 tt (1.3, 7.6)	133.1	7.58 br t (ca. 8)	133.3	7.60 tt (1.3, 7.7)	133.1	7.60 tt (1.3, 7.6)	133.3
5‴	7.39 dd (7.6, 8.3)	128.4	7.39 dd (7.8, 7.9)	128.5	7.40 dd (7.7, 8.3)	128.5	7.42 dd (7.6, 8.3)	128.6
6‴	8.14 dd (1.3, 8.3)	130.1	8.10 br d (ca. 8)	129.9	8.14 dd (1.3, 8.3)	130.1	8.11 dd (1.3, 8.3)	129.9
7‴		166.6		166.5		166.6		166.4

[a] Entries marked with an asterisk may be interchangeable within the same column.

perature furnished **1a**, methyl nicotinate, and methyl benzoate,[6] so that the absolute stereostructure of **2** was elucidated as the same as that of **1**. Moreover, treatment of **2** with 0.1% NaOMe−MeOH at 0 °C gave the partial desacylated derivatives including 2-*O*-nicotinoylnigellanol A (**2a**).[11] The CD spectrum of **2a** showed a negative Cotton effect [264 nm ($\Delta\epsilon$ −1.76) in MeOH]. This evidence suggested that allylic benzoate rule is also applicable to the allyl nicotinate moiety.

Nigellamine B_1 (**3**), a white powder, $[\alpha]^{27}_D$ +20.2 (c = 0.80, $CHCl_3$), $C_{40}H_{43}NO_9$, showed a quasimolecular ion peak at m/z 682 $(M + H)^+$ in positive-ion FAB-MS.[12] The IR spectrum of **3** showed absorption bands at 1725, 1653, 1592, 1541, 1507, 1420, 1281, 1111, 1024, 943, 741, and 702 cm^{-1} ascribable to ester carbonyl, olefin, and ether functions and aromatic rings. In the UV spectrum of **3** (measured in MeOH), absorption maxima were observed at 227 (log ϵ 4.72) and 264 (3.91) nm. The ^{1}H and ^{13}C NMR ($CDCl_3$,

Table 1) spectra of **3** showed signals assignable to four methyls, a methylene and three methine bearing the oxygen function, two olefins, a nicotinoyl, two benzoyl groups[6] together with four methylenes, a methine, and five quaternary carbons. Treatment of **3** with triphenylphosphine (PPh_3) gave the 18-hydroxyl derivative (**3a**).[13] The 18-carbon signal in the ^{13}C NMR spectrum of **3a** (δ_C 71.5) was observed at a higher field than that of **3** (δ_C 82.5). Treatment of **3a** with 0.1% NaOMe−MeOH at room temperature gave the desacyl derivative (**3b**).[5,14] The relative stereostructures of **3**, **3a**, and **3b** were confirmed by NOESY experiment on **3b**, in which NOE correlations were observed as shown in Figure 2. Fourthermore, the partial desacyl derivatives including the 10,15-desacyl derivative (**3c**) were obtained by treatment of **3a** with 0.1% NaOMe−MeOH at 0 °C.[15] Compound **3c** showed a negative Cotton effect [229 nm ($\Delta\epsilon$ −2.20) in MeOH], which indicated the absolute configuration at the 2-position in **3c** to be *S*. Consequently, the absolute stereostructure of **3** was determined.

Nigellamine B_2 (**4**),[16] colorless fine crystals (mp 139.5−141.5 °C from MeOH), $[\alpha]^{27}_D$ +20.0 (c = 1.10, $CHCl_3$), $C_{39}H_{42}N_2O_9$ and its IR and UV spectra were very similar to those of **3**. The proton and carbon signals in the ^1H NMR ($CDCl_3$) and ^{13}C NMR (Table 1) spectra[7] of **4** indicated the presence of the same functional groups as **3**, except for the signals due to an acyl group.[6] The positions of the acyl groups in **4** were clarified by the HMBC experiment as shown in Figure 1. Treatment of **4** with PPh_3 furnished the 18-hydroxyl derivative (**4a**),[17] and successive treatment of **4a** with 0.1% NaOMe−MeOH at room temperature gave **3b**, so that the absolute stereostructure of **4** was determined to be the same as that of **3**. Previously, several dolabellane-type diterpenes were isolated from the marine soft coral, brown algae, or liverwort;[18] however, the isolation reports of this type of diterpenes from the higher plants were very rare. Until now, only one plant material has been reported on the aerial part of *Chrozophora oblique*.[19] Furthermore, these dolabellane-type diterpenes are the first known to have a nicotinic acid and/or hydroperoxyl group at the 18-position.

Effects of nigellamines (**1**−**4**) on stored triglyceride in primary cultured mouse hepatocytes were examined,[20] and **1** [inhibition (%) at 0.1 μM: 64 ± 4], **3** (70 ± 2%), and **4** (79 ± 2%) were found to show potent reduction of triglyceride levels. Their activities were equivalent to that of a hypolipidemic medicine, clofibrate (64 ± 5%).

Supporting Information Available: ^1H and ^{13}C NMR spectra of nigellamines (**1**−**4**) and experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

OL036239C

(11) **2a**: high-resolution EI-MS calcd for $C_{26}H_{35}NO_5$ (M^+) 441.2515, found 441.2524; ^1H NMR (CD_3OD) δ 1.40, 1.73, 1.81 (3H each, all s, H_3-17, 20, 19), 1.62, 2.40 (1H each, both m, H_2-9), 1.76, 1.87 (1H each, both m, H_2-6), 1.77 (3H, d, J = 0.9 Hz, H_3-16), 2.24−2.35 (4H, m, H_2-13, 14), 2.48, 2.53 (1H each, both m, H_2-5), 2.49 (1H, br s, H-11), 3.01 (1H, d, J = ca. 9 Hz, H-7), 4.04, 4.22 (1H each, both d, J = 11.6 Hz, H_2-15), 4.05 (1H, br dd, J = ca. 6, 12 Hz, H-10), 5.39 (1H, d, J = 10.7 Hz, H-2), 5.46 (1H, br d, J = ca. 11 Hz, H-3), 7.59 (1H, dd, J = 4.5, 7.6 Hz, H-5′), 8.40 (1H, br s, J = ca. 8 Hz, H-6′), 8.76 (1H, br s, H-4′), 9.13 (1H, br s, H-2′); EI-MS m/z 441 (M^+, 2), 423 ($M^+ - H_2O$, 25), 121 (100).

(12) **3**: high-resolution FAB-MS calcd for $C_{40}H_{44}NO_9$ $(M + H)^+$ 682.3016, found 682.3013; CD (MeOH, $\Delta\epsilon$) +4.98 (217 nm), −2.18 (236 nm); positive-ion FAB-MS m/z 682 $(M + H)^+$.

(13) **3a**: ^1H NMR ($CDCl_3$) δ 1.46, 1.51, 1.52 (3H each, all s, H_3-19, 20, 17), 1.67 (1H, m, Hα-6), 1.70 (1H, dd, J = 12.4, 13.5 Hz, Hβ-9), 1.97 (3H, d, J = 1.1 Hz, H_3-16), 2.01 (1H, m, Hβ-6), 2.36 (1H, m, Hα-5), 2.45 (1H, ddd, J = 4.9, 12.9, 12.9 Hz, Hβ-5), 2.55 (1H, dd, J = 5.7, 13.5 Hz, Hα-9), 2.66 (2H, br s, H_2-14), 2.88 (1H, br s, H-11), 2.99 (1H, br d, J = ca.10 Hz, H-7), 5.16, 5.27 (1H each, both d, J = 11.0 Hz, H_2-15), 5.53 (1H, br s, H-13), 5.51 (1H, d, J = 10.3 Hz, H-2), 5.79 (1H, dd, J = 1.1, 10.3 Hz, H-3), 6.29 (1H, br dd, J = ca. 6, 12 Hz, H-10), 7.09 (2H, dd, J = 7.6, 8.3 Hz, H-3′, 5′), 7.40 (2H, dd, J = 7.6, 8.3 Hz, H-3‴, 5‴), 7.42 (1H, tt, J = 1.3, 7.6 Hz, H-4′), 7.43 (1H, dd, J = 4.9, 8.0 Hz, H-5″), 7.60 (1H, tt, J = 1.3, 7.6 Hz, H-4‴), 7.80 (2H, dd, J = 1.3, 8.3 Hz, H-2′, 6′), 8.14 (2H, dd, J = 1.3, 8.3 Hz, H-2‴, 6‴), 8.34 (1H, ddd, J = 1.9, 1.9, 8.0 Hz, H-6″), 8.78 (1H, br d, J = ca. 5 Hz, H-4″), 9.24 (1H, br s, H-2″).

(14) **3b**: ^1H NMR ($CDCl_3$) δ 1.33, 1.45, 1.52 (3H each, all s, H_3-17, 19, 20), 1.49 (1H, dd, J = 12.5, 13.5 Hz, Hβ-9), 1.61 (1H, m, Hα-6), 1.69 (3H, d, J = 0.9 Hz, H_3-16), 1.92 (1H, m, Hβ-6), 2.28 (1H, m, Hα-5), 2.37 (1H, ddd, J = 4.6, 12.2, 12.2 Hz, Hβ-5), 2.35 (1H, dd, J = 5.5, 13.5 Hz, Hα-9), 2.50 (1H, br s, H-11), [2.65 (1H, br d, J = ca. 18 Hz), 2.79 (1H, dd, J = 3.4, 17.4 Hz), H_2-14], 2.83 (1H, br d, J = ca.10 Hz, H-7), 4.05 (1H, d, J = 10.7 Hz, H-2), 4.16, 4.20 (1H each, both d, J = 11.0 Hz, H_2-15), 4.51 (1H, br dd, J = ca. 6, 13 Hz, H-10), 5.48 (1H, dd, J = 0.9, 10.7 Hz, H-3), 5.75 (1H, br s, H-13).

(15) **3c**: ^1H NMR ($CDCl_3$): δ 1.39, 1.50, 1.53, 1.86 (3H each, all s, H_3-17, 19, 20, 16), 1.66 (1H, m, Hα-6), 1.70 (1H, dd, J = 12.8, 13.4 Hz, Hβ-9), 1.94 (1H, m, Hβ-6), 2.29 (1H, m, Hα-5), 2.33 (1H, ddd, J = 5.2, 12.8, 12.8 Hz, Hβ-5), 2.43 (1H, dd, J = 5.2, 13.4 Hz, Hα-9), 2.23 (1H, dd, J = 3.1, 17.6 Hz, Hα-14), 2.73 (1H, br s, H-11), 2.86 (1H, br d, J = ca.10 Hz, H-7), 2.93 (1H, br d, J = ca. 18 Hz, Hβ-14), 4.11, 4.29 (1H each, both d, J = 11.6 Hz, H_2-15), 4.67 (1H, br dd, J = ca. 5, 13 Hz, H-10), 5.32 (1H, d, J = 10.4 Hz, H-3), 5.34 (1H, d, J = 10.4 Hz, H-2), 5.72 (1H, br s, H-13), 7.44 (2H, dd, J = 7.6, 8.3 Hz, H-3′, 5′), 7.57 (1H, tt, J = 1.2, 7.6 Hz, H-4′), 8.01 (2H, dd, J = 1.2, 8.3 Hz, H-2′, 6′).

(16) **4**: high-resolution FAB-MS calcd for $C_{39}H_{43}N_2O_9$ $(M + H)^+$ 683.2969, found 683.2965; CD (MeOH, $\Delta\epsilon$) +6.03 (221 nm), −0.64 (255 nm); UV (MeOH, log ϵ): 221 (4.71), 264 (4.09) nm; IR (KBr) 1717, 1647, 1636, 1592, 1541, 1509, 1456, 1281, 1113, 1026, 941, 743, 714 cm^{-1}; positive-ion FAB-MS m/z 683 $(M + H)^+$.

(17) **4a**: ^1H NMR ($CDCl_3$) δ 1.46, 1.51, 1.52 (3H each, all s, H_3-19, 20, 17), 1.66 (1H, m, Hα-6), 1.69 (1H, dd, J = 12.8, 13.4 Hz, Hβ-9), 1.97 (3H, d, J = 0.9 Hz, H_3-16), 2.02 (1H, m, Hβ-6), 2.37 (1H, br d, J = ca. 13 Hz, Hα-5), 2.48 (1H, ddd, J = 4.9, 12.8, 12.8 Hz, Hβ-5), 2.56 (1H, dd, J = 5.8, 13.4 Hz, Hα-9), 2.65 (2H, br s, H_2-14), 2.88 (1H, br s, H-11), 2.99 (1H, br d, J = ca. 10 Hz, H-7), 5.15, 5.28 (1H each, both d, J = 11.0 Hz, H_2-15), 5.54 (1H, br s, H-13), 5.55 (1H, d, J = 10.7 Hz, H-2), 5.80 (1H, dd, J = 0.9, 10.7 Hz, H-3), 6.29 (1H, br dd, J = ca. 6, 13 Hz, H-10), 6.95 (1H, dd, J = 4.9, 8.0 Hz, H-5′), 7.42 (2H, dd, J = 7.6, 8.3 Hz, H-3‴, 5‴), 7.46 (1H, dd, J = 4.9, 8.0 Hz, H-5″), 7.60 (1H, tt, J = 1.2, 7.6 Hz, H-4‴), 7.92 (1H, ddd, J = 1.8, 1.9, 8.0 Hz, H-6′), 8.11 (2H, dd, J = 1.2, 8.3 Hz, H-2‴, 6‴), 8.35 (1H, ddd, J = 1.8, 1.9, 8.0 Hz, H-6″), 8.66 (1H, br d, J = ca. 5 Hz, H-4′), 8.82 (1H, br d, J = ca. 5 Hz, H-4″), 9.09 (1H, br s, H-2′), 9.26 (1H, br s, H-2″).

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(20) (a) Hepatocytes were isolated from male ddY mice (ca. 40 g) using the collagenase perfusion method.[20b] A cell suspension of 8×10^4 cells in 200 μL William's E medium containing fetal calf serum (FCS, 10%), penicillin (100 units/μL), and streptomycin (100 μg/mL) was incubated on a 48-well tissue culture plate, and pre-cultured for 2 h at 37 °C under a 5% CO_2 atmosphere. An aliquot (200 μL) of the medium containing test sample was added to the each well, and the cells were cultured for 20 h. The plate was centrifuged (2000 rpm, 4 °C, 10 min), the supernatant was removed, and 120 μL of distilled water was added to each well. The hepatocytes were broken with sonication, and the suspension was centrifuged. To determine the concentration of triglyceride in the supernatant using a commercial kit (Triglyceride G-test wako), each test compound was dissolved in DMSO, and the solution was added to the medium (final DMSO concentration was 0.5%). (b) Seglen P. O. *Methods Cell Biol.* **1976**, *13*, 29−83.