Nigellamines A₃, A₄, A₅, and C, New Dolabellane-Type Diterpene Alkaloids, with Lipid Metabolism-Promoting Activities from the Egyptian Medicinal Food Black Cumin

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Received January 9, 2004; accepted February 23, 2004

New dolabellane-type diterpene alkaloids, nigellamines A₃, A₄, A₅, and C, were isolated from the methanolic extract of an Egyptian medicinal food, black cumin (the seeds of *Nigella sativa*). Their absolute configurations were determined on the basis of chemical and physicochemical evidence. Nigellamines were found to lower triglyceride levels in primary cultured mouse hepatocytes, and in particular, the activity of nigellamine A₅ was equivalent to that of the hypolipidemic agent, clofibrate.

Key words *Nigella sativa*; black cumin; nigellamine; dolabellane-type diterpene; lipid metabolism-promoting activity; Egyptian medicinal food

In the course of our studies on the bioactive constituents of Egyptian folk medicines,^{1–4} we previously reported the structures of four novel dolabellane-type diterpene alkaloids, nigellamines A₁ (5), A₂ (6), B₁ (7), and B₂ (8), from the methanolic extract of a medicinal food, the seeds of *Nigella sativa* L. (common name “black cumin”, Ranunculaceae).⁵ As a continuing study of this medicinal food, we additionally isolated four new dolabellane-type diterpene alkaloids designated nigellamines A₃ (1), A₄ (2), A₅ (3), and C (4). This communication deals with elucidation of the absolute stereostructures of nigellamines (1–4) as well as their lipid metabolism-promoting activities.

The ethyl acetate (EtOAc)-soluble fraction obtained from the seeds of *N. sativa*, which was described previously,⁵ was subjected to ordinary-phase [*n*-hexane–EtOAc (20:1–10:1–5:1–2:1–1:2)–CHCl₃–MeOH–H₂O (10:3:1,

lower layer–6:4:1)–MeOH] and reverse-phase column chromatographies [MeOH–H₂O], and finally to HPLC [YMC-Pack ODS-5-A, 250×20 mm i.d., MeOH–H₂O] to give nigellamines A₃ (1, 0.0005% from the natural medicine), A₄ (2, 0.0002%), A₅ (3, 0.0002%), and C (4, 0.0003%).

Nigellamine A₃ (1) was isolated as a white powder with negative optical rotation $\{[\alpha]_D^{27} - 11.3^\circ (c=0.50, \text{CHCl}_3)\}$. The positive-ion fast atom bombardment (FAB)-MS of 1 showed quasimolecular ion peak at m/z 645 ($M+H$)⁺ and the molecular formula C₃₈H₄₈N₂O₇ of 1 was determined by high-resolution MS measurement. In the UV spectrum of 1 (measured in MeOH), absorption maxima were observed at 217 (log ϵ 4.35) and 264 (3.85) nm. The IR (KBr) spectrum of 1 showed absorption bands at 1725, 1647, 1636, 1509, 1420, and 1024 cm^{−1} ascribable to ester carbonyl, olefin, and ether functions and the aromatic ring. Treatment of 1 with 0.1% sodium methoxide (NaOMe)–MeOH at room temperature yielded a desacyl derivative (nigellanol A) together with methyl nicotinate and methyl hexanoate, which were identified by HPLC analysis.⁷ The ¹H- and ¹³C-NMR (CDCl₃, Table 1) spectra⁸ of 1 showed signals assignable to four methyls [δ 1.51, 1.65, 1.86 (3H each, all s, 17, 19, 20-H₃), 1.85 (3H, d, $J=0.9$ Hz, 16-H₃)], a methylene, and three methines bearing an oxygen function [δ 2.99 (1H, br d, $J=ca.$ 9 Hz, 7-H), 4.79, 4.83 (1H each, both d, $J=11.0$ Hz, 15-H₂), 5.39 (1H, d, $J=10.4$ Hz, 2-H), 5.65 (1H, br dd, $J=ca.$ 6, 13 Hz, 10-H)], an olefin [δ 5.57 (1H, dd, $J=0.9, 10.4$ Hz, 3-H)], an *n*-hexanoyl group [δ 0.86 (3H, t, $J=7.0$ Hz, 6''-H₃), 1.27, 1.30, 1.66 (2H each, all m, 5'', 4'', 3''-H₂), 2.44 (2H, t, $J=7.0$ Hz, 2''-H₂)], two nicotinoyl groups [δ 7.41, 7.43 (1H each, both dd, $J=4.9, 8.0$ Hz, 5', 5''-H), 8.31, 8.33 (1H each, both ddd, $J=1.8, 1.9, 8.0$ Hz, 6', 6''-H), 8.80 (2H, br s, 4', 4''-H), 9.23, 9.28 (1H each, both br s, 2', 2''-H)] together with five methylenes (5, 6, 9, 13, 14-H₂), a methine (11-H), and five quaternary carbons (1, 4, 8, 12, 18-C). The ¹H–¹H COSY experiment on 1 indicated the presence of the partial structures shown as bold lines in Fig. 1. The positions of quaternary carbons in 1 were constructed on the basis of the HMBC experiment. Thus long-range correlations were observed between the following proton and carbon pairs: 2-H

Chart 1

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Table 1. ^1H - and ^{13}C -NMR Data on Nigellamines A₃ (**1**), A₄ (**2**), A₅ (**3**), and C (**4**) in CDCl_3

	1		2		3		4	
	δ (J in Hz)	δ_{C}	δ (J in Hz)	δ_{C}	δ (J in Hz)	δ_{C}	δ (J in Hz)	δ_{C}
1		56.1		56.1		56.1		52.8
2	5.39 (d, 10.4)	73.5	5.39 (d, 10.4)	73.5	5.39 (d, 10.4)	73.5	5.32 (d, 9.8)	74.0
3	5.57 (dd, 0.9, 10.4)	123.4	5.57 (dd, 1.2, 10.4)	123.4	5.54 (dd, 0.9, 10.4)	123.3	5.51 (dd, 0.9, 9.8)	124.8
4		140.2		140.2		140.2		138.7
5 α	2.35 (br d, <i>ca.</i> 13)	37.9	2.34 (m)	37.9	2.34 (br d, <i>ca.</i> 13)	37.8	2.35 (br d, <i>ca.</i> 13)	37.9
5 β	2.39 (ddd, 5.2, 12.5, 12.5)		2.39 (ddd, 5.2, 12.5, 12.5)		2.37 (ddd, 5.2, 12.5, 12.5)		2.38 (ddd, 5.2, 12.5, 12.5)	
6 α	1.70 (m)	22.7	1.69 (m)	22.7	1.68 (m)	22.6	1.72 (m)	22.6
6 β	1.99 (m)		1.98 (m)		1.96 (m)		1.96 (m)	
7	2.99 (br d, <i>ca.</i> 9)	65.2	2.99 (br d, <i>ca.</i> 9)	65.2	2.97 (br d, <i>ca.</i> 9)	65.1	3.01 (br d, <i>ca.</i> 9)	65.2
8		58.6		58.6		58.5		59.0
9 α	2.52 (dd, 5.5, 13.7)	41.8	2.52 (dd, 5.5, 13.4)	41.8	2.50 (dd, 5.5, 13.7)	41.8	2.46 (dd, 5.5, 13.5)	41.3
9 β	1.54 (dd, 12.5, 13.7)		1.54 (dd, 12.5, 13.4)		1.51 (dd, 12.5, 13.7)		1.82 (dd, 12.5, 13.5)	
10	5.65 (br dd, <i>ca.</i> 6, 13)	75.3	5.65 (br dd, <i>ca.</i> 6, 13)	75.3	5.65 (br dd, <i>ca.</i> 6, 13)	75.3	5.65 (br dd, <i>ca.</i> 6, 13)	76.4
11	2.66 (br s)	47.7	2.66 (br s)	47.8	2.66 (br s)	47.7	2.60 (br s)	46.4
12		135.7		135.8		135.7		137.1
13	2.28 (m) (2H)	28.0	2.28 (m) (2H)	28.0	2.27 (m) (2H)	28.0	2.21 (m) (2H)	28.0
14 α	2.16 (m)	31.1	2.15 (m)	31.1	2.15 (m)	31.2	1.97 (m)	36.0
14 β	2.04 (m)		2.04 (m)		2.01 (m)		1.88 (m)	
15	4.79 (d, 11.0)	66.3	4.79 (d, 11.0)	66.3	4.84 (br s) (2H)	67.1	1.63 (s) (3H)	19.6
	4.83 (d, 11.0)		4.84 (d, 11.0)					
16	1.85 (d, 0.9) (3H)	16.4	1.85 (d, 1.2) (3H)	16.4	1.84 (d, 0.9) (3H)	16.4	1.81 (d, 0.9) (3H)	16.4
17	1.51 (s) (3H)	18.4	1.51 (s) (3H)	18.4	1.49 (s) (3H)	18.4	1.51 (s) (3H)	18.5
18		127.2		127.2		127.3		126.5
19	1.65 (s) (3H)	22.5	1.65 (s) (3H)	22.5	1.65 (s) (3H)	22.5	1.63 (s) (3H)	22.3
20	1.86 (s) (3H)	21.8	1.86 (s) (3H)	21.8	1.85 (s) (3H)	21.8	1.84 (s) (3H)	21.9
1'		126.3		126.3		126.2		126.4
2'	9.23 (br s)	150.8	9.23 (br s)	150.9	9.33 (br s)	151.0	9.27 (br s)	150.9
4'	8.80 (br s)	153.6	8.81 (dd, 1.9, 4.9)	153.6	8.81 (br d, <i>ca.</i> 5)	153.6	8.80 (br s)	153.5
5'	7.41 (dd, 4.9, 8.0)	123.3	7.40 (dd, 4.9, 8.0)	123.3	7.40 (dd, 4.9, 8.0)	123.4	7.42 (dd, 4.9, 8.0)	123.5
6'	8.33 (ddd, 1.8, 1.9, 8.0)	137.0	8.33 (ddd, 1.8, 1.9, 8.0)	137.0	8.32 (ddd, 1.8, 1.9, 8.0)	137.0	8.31 (ddd, 1.8, 1.9, 8.0)	137.0
7'		164.9		164.9		164.9		164.8
1''		125.8		125.8		125.8		126.1
2''	9.28 (br s)	151.0	9.28 (br s)	151.0	9.21 (br s)	150.8	9.21 (br s)	150.7
4''	8.80 (br s)	153.7	8.79 (dd, 1.9, 4.9)	153.7	8.79 (br d, <i>ca.</i> 5)	153.7	8.80 (br s)	153.6
5''	7.43 (dd, 4.9, 8.0)	123.5	7.42 (dd, 4.9, 8.0)	123.5	7.38 (dd, 4.9, 8.0)	123.5	7.42 (dd, 4.9, 8.0)	123.5
6''	8.31 (ddd, 1.8, 1.9, 8.0)	137.1	8.31 (ddd, 1.8, 1.9, 8.0)	137.1	8.26 (ddd, 1.8, 1.9, 8.0)	137.1	8.26 (ddd, 1.8, 1.9, 8.0)	136.9
7''		164.4		164.4		164.3		164.5
1'''		173.8		173.7		133.6		
2'''	2.44 (t, 7.0) (2H)	34.6	2.43 (br t, <i>ca.</i> 7) (2H)	36.4	7.27 (br d, <i>ca.</i> 8)	129.1		
3'''	1.66 (m) (2H)	24.8	1.71 (m) (2H)	18.6	7.29 (dd, 7.6, 8.2)	128.6		
4'''	1.30 (m) (2H)	31.4	0.96 (t, 7.4) (3H)	13.7	7.25 (br t, <i>ca.</i> 8)	127.3		
5'''	1.27 (m) (2H)	22.3			7.29 (dd, 7.6, 8.2)	128.6		
6'''	0.86 (t, 7.0) (3H)	13.9			7.27 (br d, <i>ca.</i> 8)	129.1		
7'''					3.77 (s) (2H)	41.4		
8'''						171.8		

* May be interchangeable within the same column.

and 4, 15, 7'-C; 3-H and 16-C; 5-H₂ and 4-C; 7-H and 8-C; 9-H₂ and 8-C; 10-H and 7''-C; 11-H and 12-14-C; 13-H₂ and 12-C; 14-H₂ and 11-C; 15-H₂ and 1, 2, 14, 1'''-C; 16-H₃ and 3-5-C; 17-H₃ and 8, 9-C; 19-H₃ and 12, 18, 20-C; 20-H₃ and 12, 18, 19-C; 6'-H and 7'-C; 6''-H and 7''-C; and 2'''-H and 1'''-C (Fig. 1). This evidence led us to construct the planar structure of a dolabellane-type diterpene. The relative stereostructure of **1** including the geometry of the 3-double bond was elucidated using a nuclear Overhauser enhancement spectroscopy (NOESY) experiment, which showed NOE correlations between the following proton pairs: 2-H and 11-H, 16-H₃; 7-H and 3-H, 5 β -H, 6 β -H, 9 β -H, 15-H₂; 9 α -H and 17-H₃; 10-H and 11-H, 17-H₃; and 14 β -H and 15-H₂ (Fig. 1). The absolute configuration of **1** was determined using the modified allylic benzoate rule.^{5,9} The circular dichronic (CD) spectrum of **1** showed a negative Cotton effect [246 nm

Fig. 1

($\Delta\epsilon - 1.84$) in MeOH], so that the orientation of the 2-position in **1** was determined to be *S*. On the basis of the above evidence, the absolute stereostructure of **1** was determined as shown.

Nigellamine A₄¹⁰ (**2**), a white powder, $[\alpha]_D^{26} - 13.4^\circ$ ($c=0.20$, CHCl₃), C₃₆H₄₄N₂O₇, and its UV and IR spectra were very similar to those of **1**. Treatment of **2** with 0.1% NaOMe–MeOH at room temperature gave nigellanol A, methyl nicotinate, and methyl butylate.⁷ The ¹H- and ¹³C-NMR (CDCl₃, Table 1) spectra⁸ of **2** indicated the presence of the following functions: a nigellanol A part {four methyls [δ 1.51, 1.65, 1.86 (3H each, all s, 17, 19, 20-H₃), 1.85 (3H, d, $J=1.2$ Hz, 16-H₃)], a methylene and three methines bearing an oxygen function [δ 2.99 (1H, br d, $J=ca.$ 9 Hz, 7-H), 4.79, 4.84 (1H each, both d, $J=11.0$ Hz, 15-H₂), 5.39 (1H, d, $J=10.4$ Hz, 2-H), 5.65 (1H, br dd, $J=ca.$ 6, 13 Hz, 10-H)], an olefin [δ 5.57 (1H, dd, $J=1.2$, 10.4 Hz, 3-H)] and acyl group parts {an *n*-butyloyl group [δ 0.96 (3H, t, $J=7.4$ Hz, 4''-H₃), 1.71 (2H, m, 3''-H₂), 2.43 (br t, $J=ca.$ 7 Hz, 2''-H₂)], and two nicotinoyl groups [δ 7.40, 7.42 (1H each, both dd, $J=4.9$, 8.0 Hz, 5', 5''-H), 8.31, 8.33 (1H each, both ddd, $J=1.8$, 1.9, 8.0 Hz, 6'', 6'-H), 8.79, 8.81 (1H each, both dd, $J=1.9$, 4.9 Hz, 4'', 4'-H), 9.23, 9.28 (1H each, both br s, 2', 2''-H)]}. The positions of the acyl groups in **2** were determined in an HMBC experiment, as shown in Fig. 1. NOE correlations in a NOESY experiment with **2** were observed, as shown in Fig. 1. Finally, the CD spectrum of **2** showed a similar Cotton effect to that of **1** [247 nm ($\Delta\epsilon - 1.43$) in MeOH]. Consequently, the absolute stereostructure of **2** was elucidated.

Nigellamine A₅¹¹ (**3**), a white powder, $[\alpha]_D^{28} - 14.8^\circ$ ($c=0.20$, CHCl₃), C₄₀H₄₄N₂O₇, showed a molecular ion peak at m/z 664 (M^+ , 3%) and fragment ion peaks at m/z 541 ($M^+ - C_6H_5NO_2$, 11%) and 124 ($C_6H_5NO_2^+$, 100%) in electron ionization (EI)-MS. Nigellanol A, methyl nicotinate, and methyl phenylacetate were obtained by treatment of **3** with 0.1% NaOMe–MeOH at room temperature.⁷ The ¹H- and ¹³C-NMR (CDCl₃, Table 1) spectra⁸ of **3** showed signals assignable to four methyls [δ 1.49, 1.65, 1.85 (3H each, all s, 17, 19, 20-H₃), 1.84 (3H, d, $J=0.9$ Hz, 16-H₃)], a methylene and three methines bearing an oxygen function [δ 2.97 (1H, br d, $J=ca.$ 9 Hz, 7-H), 4.84 (2H, br s, 15-H₂), 5.39 (1H, d, $J=10.4$ Hz, 2-H), 5.65 (1H, br dd, $J=ca.$ 6, 13 Hz, 10-H)], an olefin [δ 5.54 (1H, dd, $J=0.9$, 10.4 Hz, 3-H)], a phenyl acetyl group [δ 3.77 (2H, s, 7'''-H₂), 7.25 (1H, br t, $J=ca.$ 8 Hz, 4'''-H), 7.27 (2H, br d, $J=ca.$ 8 Hz, 2''', 6'''-H), 7.29 (2H, dd, $J=7.6$, 8.2 Hz, 3''', 5'''-H)], two nicotinoyl groups [δ 7.38, 7.40 (1H each, both dd, $J=4.9$, 8.0 Hz, 5'', 5'-H), 8.26, 8.32 (1H each, both ddd, $J=1.8$, 1.9, 8.0 Hz, 6'', 6'-H), 8.79, 8.81 (1H each, both br d, $J=ca.$ 5 Hz, 4'', 4'-H), 9.21, 9.33 (1H each, both br s, 2'', 2'-H)] together with five methylenes (5, 6, 9, 13, 14-H₂), a methine (11-H), and five quaternary carbons (1, 4, 8, 12, 18-C). The positions of the acyl groups in **3** were clarified by the HMBC experiment and the relative stereostructure of **3** was also elucidated by NOE correlations in a NOESY experiment, as shown in Fig. 1. The CD spectrum of **3** showed a negative Cotton effect at 246 nm ($\Delta\epsilon - 1.65$ in MeOH) and the absolute stereostructure of **3** was determined as shown.

Nigellamine C¹² (**4**), a white powder, $[\alpha]_D^{27} - 23.6^\circ$ ($c=0.30$, CHCl₃), C₃₂H₃₈N₂O₅, showed a quasimolecular ion

peak at m/z 531 ($M+H$)⁺ in positive-ion FAB-MS. In the UV spectrum of **4** (measured in MeOH), absorption maxima were observed at 217 (log ϵ 4.35) and 264 (3.79) nm. The IR spectrum of **4** showed absorption bands at 1723, 1647, 1636, 1592, 1509, 1420, and 1024 cm⁻¹ ascribable to ester carbonyl, olefin, and ether functions and aromatic rings. Treatment of **4** with 0.1% NaOMe–MeOH at room temperature gave methyl nicotinate.¹³ The proton and carbon signals in the ¹H- and ¹³C-NMR (CDCl₃, Table 1) spectra⁸ of **4** were superimposable on those of **1**, **2**, and **3**, except for the signals due to the 15-methyl group. That is, they showed signals assignable to five methyls [δ 1.51, 1.63, 1.63, 1.84 (3H each, all s, 17, 15, 19, 20-H₃), 1.81 (3H, d, $J=0.9$ Hz, 16-H₃)], five methylenes (5, 6, 9, 13, 14-C), a methine [δ 2.60 (1H, br s, 11-H)], three methines bearing an oxygen function [δ 3.01 (1H, br d, $J=ca.$ 9 Hz, 7-H), 5.32 (1H, d, $J=9.8$ Hz, 2-H), 5.65 (1H, br dd, $J=ca.$ 6, 13 Hz, 10-H)], an olefin [δ 5.51 (1H, dd, $J=0.9$, 9.8 Hz, 3-H)], two nicotinoyl groups [δ 7.42 (2H, dd, $J=4.9$, 8.0 Hz, 5', 5''-H), 8.26, 8.31 (1H each, both ddd, $J=1.8$, 1.9, 8.0 Hz, 6'', 6'-H), 8.80 (2H, br s, 4', 4''-H), 9.21, 9.27 (1H each, both br s, 2'', 2'-H)], and five quaternary carbons (1, 4, 8, 12, 18-C). The ¹H–¹H COSY data of **4** indicated the presence of the partial structures shown as bold lines and the planar structure of **4** was determined in an HMBC experiment, in which long-range correlations were observed, as shown in Fig. 2. The relative stereostructure of **4** was elucidated using a NOESY experiment, which showed NOE correlations between the following proton pairs: 2-H and 11-H, 16-H₃; 7-H and 3-H, 5 β -H, 6 β -H, 9 β -H, 15-H₃; 9 α -H and 17-H₃; 10-H and 11-H, 17-H₃; and 14 β -H and 15-H₃ (Fig. 2). The CD spectrum of **4**, which showed a negative Cotton effect at 245 nm ($\Delta\epsilon = -1.73$ in MeOH), was very similar to those of **1**, **2**, and **3**, so that the absolute configuration of **4** was the same as those of **1**, **2**, and **3**. Consequently, the stereostructure of **4** was determined as shown.

Fig. 2

Table 2. Inhibitory Effects on Nigellamines (**1**–**4**) from *N. sativa* on Stored Triglyceride in Primary Cultured Mouse Hepatocytes

	% of control		
	0 μ M	0.1 μ M	1 μ M
Nigellamine A ₃ (1)	100 $\pm$ 1	97 $\pm$ 4	85 $\pm$ 6*
Nigellamine A ₄ (2)	100 $\pm$ 6	88 $\pm$ 1	82 $\pm$ 3**
Nigellamine A ₅ (3)	100 $\pm$ 2	67 $\pm$ 5**	66 $\pm$ 2**
Nigellamine C (4)	100 $\pm$ 4	97 $\pm$ 3	81 $\pm$ 1*

Each value represents the mean $\pm$ S.E.M. ($n=4$). Significantly different from the control, * $p < 0.05$, ** $p < 0.01$.

We examined the effect of nigellamines (**1**–**4**) on stored triglyceride in primary cultured mouse hepatocytes.⁵⁾ Among them, nigellamine A₅ (**3**) was found to show potent reduction of triglyceride levels in primary cultured mouse hepatocytes and its activity was equivalent to that of the hypolipidemic agent, clofibrate [inhibition (%) at 0.1 μ M: 64 $\pm$ 5].

References and Notes

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- 1**: High resolution FAB-MS: Calcd for C₃₈H₄₉N₂O₇ (M+H)⁺: 645.3540. Found: 645.3549. CD [MeOH, nm ($\Delta\epsilon$)]: 246 (–1.84). IR (KBr): 1725, 1647, 1636, 1592, 1541, 1509, 1420, 1281, 1165, 1111, 1024, 945, 741, 702 cm^{–1}.
- A solution of **1**–**3** (2.0 mg each) in 0.1% NaOMe–MeOH (1.0 ml) was stirred at room temperature for 7 h. Evaporation of the solvent from the filtrate under reduced pressure yielded a residue, which was purified by HPLC [detection, RI; column, YMC-Pack ODS-5-A, 250 $\times$ 20 mm i.d.; mobile phase, MeOH–H₂O (80:20, v/v)] to give nigellanol A⁵⁾ [(0.8 mg, 77% from **1**), (0.8 mg, 73% from **2**), and (0.7 mg, 69% from **3**)]. In addition, methylated acyl groups, methyl nicotinate (**i**, *t*_R 5.58 min, from **1**–**3**), methyl hexanoate (**ii**, *t*_R 27.19 min, from **1**), methyl butyrate (**iii**, *t*_R 8.41 min, from **2**), and methyl phenylacetate (**iv**, *t*_R 13.34 min, from **3**), were identified by HPLC analysis [detection, RI; column, YMC-Pack ODS-5-A, 250 $\times$ 4.6 mm i.d.; mobile phase, MeOH–H₂O (60:40, v/v), flow rate 0.7 ml/min]. Methyl nicotinate (**i**) was identified through comparison with standard samples obtained by diazomethane methylation of commercial nicotinic acid. Methyl hexanoate (**ii**), methyl butyrate (**iii**), and methyl phenylacetate (**iv**) were also identified through comparison with commercial samples.
- The ¹H- and ¹³C-NMR spectra of **1**–**4** were assigned with the aid of homo- and heterocorrelation spectroscopy (¹H–¹H, ¹³C–¹H COSY), distortionless enhancement by polarization transfer (DEPT), and heteronuclear multiple-bond connectivity (HMBC) experiments.
- The CD spectra of 2-*O*-benzoylnigellanol A and 2-*O*-nicotinoylnigellanol A showed a similar negative Cotton effect at 246 nm ($\Delta\epsilon$ –1.31) and 264 nm ($\Delta\epsilon$ –1.76), respectively (both in MeOH), so that the allylic benzoate rule was found to be applicable to the allylic nicotinate derivative.
- 2**: High-resolution EI-MS: Calcd for C₃₆H₄₄N₂O₇ (M⁺): 616.3148. Found: 616.3132. UV [MeOH, nm (log ϵ)]: 218 (4.45), 264 (3.88). IR (KBr): 1725, 1647, 1636, 1592, 1541, 1509, 1420, 1281, 1111, 1024, 945, 741, 704 cm^{–1}. EI-MS (%): *m/z* 616 (M⁺, 2), 493 (M⁺–C₆H₅NO₂, 13), 124 (C₆H₆NO₂⁺, 100), 106 (C₆H₄NO⁺, 24), 71 (C₄H₇O⁺, 8).
- 3**: High-resolution EI-MS: Calcd for C₄₀H₄₄N₂O₇ (M⁺): 664.3148. Found: 664.3141. UV [MeOH, nm (log ϵ)]: 218 (4.38), 264 (3.79). IR (KBr): 1717, 1647, 1636, 1592, 1541, 1509, 1420, 1281, 1111, 1024, 942, 741, 702 cm^{–1}. EI-MS (%): *m/z* 664 (M⁺, 3), 541 (M⁺–C₆H₅NO₂, 11), 124 (C₆H₆NO₂⁺, 100), 119 (C₈H₇O⁺, 12), 106 (C₆H₄NO⁺, 24), 91 (C₇H₇⁺, 27).
- 4**: High-resolution FAB-MS: Calcd for C₃₂H₃₉N₂O₅ (M+H)⁺: 531.2859. Found: 531.2853. IR (KBr): 1723, 1647, 1636, 1592, 1541, 1509, 1420, 1281, 1103, 1024, 941, 741, 702 cm^{–1}.
- A solution of **4** (0.2 mg) in 0.1% NaOMe–MeOH (0.5 ml) was stirred at room temperature for 7 h. Through a similar procedure,⁷⁾ methyl nicotinate (**i**, *t*_R 5.58 min) was identified by HPLC analysis of the reaction mixture.