New Amide Alkaloids from the Roots of *Piper nigrum*

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Seven new amide alkaloids, named *N*-isobutyl-4-hexanoyl-4-hydroxypyrrolidin-1-one (**1**), (±)-*erythro*-1-(1-oxo-4,5-dihydroxy-2*E*-decaenyl)piperidine (**2**), (±)-*threo*-1-(1- oxo-4,5-dihydroxy-2*E*-decaenyl)piperidine (**3**), (±)-*threo*-*N*-isobutyl-4,5-dihydroxy-2*E*-octaenamide (**4**), 1-(1,6-dioxo-2*E*,4*E*-decadienyl)piperidine (**5**), 1-[1-oxo-3(3,4-methylenedioxy-5-methoxyphenyl)-2*Z*-propenyl]piperidine (**6**), and 1-[1-oxo-5(3,4-methyl-enedioxyphenyl)-2*Z*,4*E*-pentadienyl]pyrrolidine (**7**), were isolated from the roots of *Piper nigrum*, together with 32 known amides. Their structures were elucidated on the basis of spectroscopic analysis and chemical evidence.

Piper nigrum L. (Piperaceae) is widely distributed in the tropical and subtropical regions of the world. Pepper (fruits of *P. nigrum)* is one of the most popular spices in the world and has been also used as a folk medicine due to its many physiological activities, e.g., stimulation of the central nervous system, analgesic, and antipyretic activities.[1] Phytochemical investigations of the fruits of this plant resulted in the isolation of 35 amides.[2] However, very little is known on the chemical constituents of the roots of *P. nigrum* with only three amides reported.[3,4] In our study, the root of *P. nigrum* was extracted with 70% MeOH. The residue was suspended in water and successively extracted with CHCl₃, EtOAc, and *n*-BuOH. The CHCl₃ fraction was found to increase amobarbital-induced sleeping time in mice. Phytochemical investigation of this fraction resulted in the isolation of seven new amides, *N*-isobutyl-4-hex-anoyl-4-hydroxypyrrolidin-1-one (**1**), (±)-*erythro*-1-(1-oxo-4,5-dihydroxy-2*E*-decaenyl)piperidine (**2**), (±)-*threo*-1-(1- oxo-4,5-dihydroxy-2*E*-decaenyl)piperidine (**3**), (±)-*threo*-*N*-isobutyl-4,5-dihydroxy-2*E*-octaenamide (**4**), 1-(1,6-dioxo-2*E*,4*E*-decadienyl)piperidine (**5**), 1-[1-oxo-3(3,4-methylenedi-oxy-5-methoxyphenyl)-2*Z*-propenyl]piperidine (**6**), and 1-[1-oxo-5(3,4-methylenedioxyphenyl)-2*Z*,4*E*-pentadienyl]pyrro-lidine (**7**), together with 32 known amides. The known amides were identified as 1-[1-oxo-3-phenyl-2*E*-propenyl]-piperidine (**8**),[5] 1-[1-oxo-3-(3,4-methylenedioxyphenyl)pro-pyl]piperidine (**9**),[6] 1-[1-oxo-3-(3,4-methylenedioxyphenyl)-2*E*-propenyl]piperidine (**10**),[3] 1-[1-oxo-3-(3,4-methylenedioxy-phenyl)-2*Z*-propenyl]piperidine (**11**),[7] 1-[1-oxo-5-(3,4-meth-ylenedioxyphenyl)-2*E*-pentenyl]piperidine (**12**),[5] piperine (**13**),[8] 1-[1-oxo-5-(3,4-methylenedioxyphenyl)-2*Z*,4*E*-penta-dienyl]piperidine (**14**),[9] 1-[1-oxo-5-(3,4-methylenedioxyphen-yl)-2*E*,4*Z*-pentadienyl]piperidine (**15**),[9] 1-[1-oxo-7-(3,4-methylenedioxyphenyl)-2*E*,4*E*,6*E*-heptatrienyl]piperidine (**16**),[8] 1-[1-oxo-9-(3,4-methylenedioxyphenyl)-2*E*,8*E*- nona-dienyl]piperidine (**17**),[10] 1-[1-oxo-9(3,4-methylenedioxyphen-yl)-8*E*-nonenyl])piperidine (**18**),[8] 1-[1-oxo-3-phenyl-2*E*-propenyl]-pyrrolidine (**19**),[11] 1-[1-oxo-3-(3,4-methylenedioxy-phenyl)-2*E*-propenyl]pyrrolidine (**20**),[12] 1-[1-oxo-5-(3,4-meth-ylene dioxyphenyl)-2*E*-pentenyl]pyrrolidine (**21**),[13] 1-[1-oxo-5-(3,4-methylenedioxyphenyl)-2*E*,4*E*-pentadienyl]pyrroli-dine (**22**),[8] 1-[1-oxo-5-(3,4-methylenedioxyphenyl)-2*E*,4*Z*-

Chart 1. Structures of New Amides from the Roots of *P. nigrum*
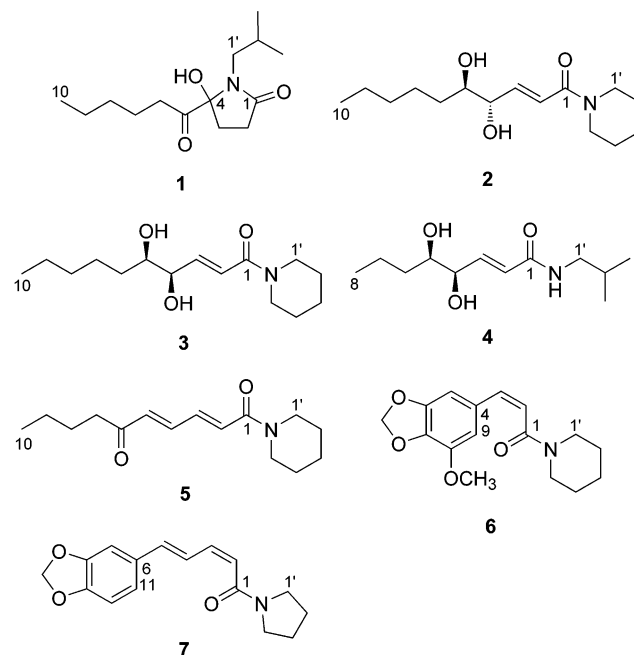
pentadienyl]pyrrolidine (**23**),[13] 1-[1-oxo-7-(3,4-methylenedioxy-phenyl)-2*E*,6*E*-heptadienyl]pyrrolidine (**24**),[8] 1-[1-oxo-7(3,4-methylenedioxyphenyl)-2*E*,4*E*,6*E*-heptatrienyl]pyrro-lidine (**25**),[14] 1-[1-oxo-9(3,4-methylenedioxyphenyl)-8*E*-non-enyl]pyrrolidine (**26**),[8] 1-[1-oxo-9-(3,4-methylenedioxy-phenyl)-2*E*,8*E*-nonadienyl]pyrrolidine (**27**),[8] 1-[1-oxo-9(3,4-methylenedioxyphenyl)-2*E*,4*E*,8*E*-nonatrienyl]pyrroli-dine (**28**),[8] *N*-isobutyl-3(3,4-methylenedioxyphenyl)-2*E*-tri-enamide (**29**),[12] 1-(1-oxo-2*E*,4*E*-dodedienyl)pyrrolidine (**30**),[8] 1-(1-oxo-2*E*,4*E*-decadienyl)pyrrolidine (**31**),[8] 1-(1-oxo-2*E*-decaenyl)piperidine (**32**),[15] 1-(1-oxo-2*E*,4*E*-decadienyl)pip-eridine (**33**),[16] *N*-isobutyl-2*E*,4*E*-decadienamide (**34**),[17] *N*-isobutyl-2*E*,4*E*-octadienamide (**35**),[18] *N*-isobutyl-2*E*,4*E*-dodedienamide (**36**),[19] *N*-isobutyl-4,5-dihydroxy-2*E*-decae-namide (**37**),[20] *N*-isobutyl-4,5-epoxy-2*E*-decaenamide (**38**),[21] and *N*-isobutyl-2*E*,4*E*,12*Z*-octadecatrienamide (**39**)[22] by comparison of their spectroscopic data with the reported values (see Supporting Information). Among the known amides, **9** and **11** are reported for the first time as natural products.

Table 1. Spectral Data for **1** (500/125 MHz, CDCl$_3$, δ in ppm, J in Hz)

C/H	δ_H	δ_C	DEPT
1		175.1	C
2a	2.71, ddd (17.2, 10.8, 5.0)	29.3	CH$_2$
2b	2.50, m		
3a	2.30, ddd (14.2, 10.5, 5.0)	30.4	CH$_2$
3b	2.10, ddd (14.2, 10.5, 7.0)		
4		92.8	C
5		209.3	C
6	2.48, m	35.0	CH$_2$
7a	1.67, m	23.4	CH$_2$
7b	1.62, m		
8a	1.30, m	31.3	CH$_2$
8b	1.28, m		
9a	1.35, m	22.4	CH$_2$
9b	1.30, m		
10	0.90, t (6.9)	13.8	CH$_3$
1′a	3.22, dd (13.8, 7.5)	47.9	CH$_2$
1′b	2.49, m		
2′	1.87, tsept (7.5, 6.4)	27.9	CH
3′ or 4′	0.87, d (6.4)	20.3	CH$_3$
3′ or 4′	0.86, d (6.4)	20.5	CH$_3$
OH	4.90, s		

Results and Discussion

Compound **1** was isolated as a colorless oil. Its molecular formula of C$_{14}$H$_{25}$NO$_3$ was determined by HRFABMS (*m/z* 256.1933 [M + H]$^+$, calcd 256.1913). The ^{1}H and ^{13}C NMR spectra, in combination with the data from DEPT and HMQC experiments, revealed the presence of three methyl groups, including one primary (C-10) and two secondary (C-3′ and C-4′), seven methylenes (C-2, C-3, C-6, C-7, C-8, C-9, and C-1′), one sp^3-hybridized methine (C-2′), one sp^3-hybridized quaternary carbon (C-4), and two quaternary sp^2-carbons (C-1 and C-5) (Table 1). In the ^{1}H NMR spectrum, the presence of an isobutylamino moiety was indicated by signals of the methylene protons at δ 3.22 (1H, dd, J = 13.8, 7.5 Hz, Ha-1′) and 2.49 (1H, m, Hb-1′), the methine proton at δ 1.87 (1H, tsept, J = 7.5, 6.4 Hz, H-2′), and six protons due to two methyls at δ 0.87 (3H, d, J = 6.4 Hz, H-3′ or H-4′) and 0.86 (3H, d, J = 6.4 Hz, H-3′ or H-4′), and in the ^{13}C NMR by signals at δ 47.9, 27.9, 20.3, and 20.5 (Table 1). ^{1}H−^{1}H COSY correlations defined three spin systems: one involving the protons of the isobutyl-amino group, one involving the protons of an *n*-amyl group from H-6 to H-10, and one involving the protons due to two coupled methylenes at δ 2.71 (Ha-2) and 2.50 (Hb-2) and δ 2.30 (Ha-3) and 2.10 (Hb-3). In the HMBC spectrum, cross-peaks were observed for ^3J-correlations between δ 2.49 (Hb-1′) and 92.8 (C-4), 175.1 (C-1), δ 2.30 (Ha-3), 2.10 (Hb-3) and 175.1 (C-1), 209.3 (C-5), and δ 2.71 (Ha-2), 2.51 (Hb-2) and 92.8 (C-4), 175.1 (C-1). Thus, the positions of the two carbonyl groups at C-1 and C-5 and the sp^3-hybridized quaternary carbon at C-4 were determined (Figure 1). In the NOESY spectrum, NOEs were observed between the C-4 hydroxy and H-3b, H-1′, H-2′, and H-3′. On the basis of the above evidence, the structure of **1** was determined as *N*-isobutyl-4-hexanoyl-4-hydroxypyrrolidin-1-one.

Compound **2** was obtained as a colorless oil and analyzed for C$_{15}$H$_{27}$NO$_3$ by HRFABMS (*m/z* 270.2069 [M + H]$^+$, calcd 270.2069). The ^{1}H NMR spectrum of **2** showed signals for a piperidine ring at δ 3.50 (2H, t, J = 5.3 Hz, H-1′), 1.57 (2H, m, H-2′), 1.65 (2H, m, H-3′), 1.57 (2H, m, H-4′), and 3.60 (2H, t, J = 5.5 Hz, H-5′). Further analysis of ^{1}H−^{1}H COSY correlations defined another spin system, involving the protons from H-2 to H-10. Among these, one *trans*-α,β-olefinic double bond at δ 6.55 (1H, dd, J = 15.2, 1.7 Hz, H-2) and 6.77 (1H, dd, J = 15.2, 4.7 Hz, H-3) could also be
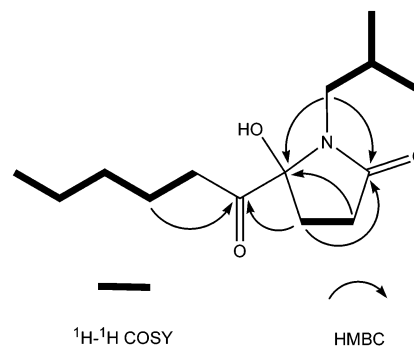
Figure 1. Important ^{1}H−^{1}H COSY and HMBC correlations for **1**.

observed. The signals appearing at δ 4.26 and 3.71 in the ^{1}H NMR spectrum and their ^{13}C NMR resonances at δ 74.6 and 74.5 suggested the presence of a vicinal diol (Tables 2 and 3). The relative configuration of the 4,5-diol was determined to be *erythro* by formation of its 4,5-acetonide (**2a**) and subsequent NOE difference experiments on this derivate.[23] Upon irradiation of the methyl signal at δ 1.38 of **2a**, NOEs were observed at δ 4.22 and 4.67, corresponding to H-5 and H-4, respectively. However, no NOEs at H-4 and H-5 were observed upon irradiation of the second methyl group at δ 1.51. This result suggested the *erythro* configuration of the 4,5-diol functionality (Scheme 1). Furthermore, the HMBC experiments established connections of the two spin systems by ^3J-correlations between H-3/C-1 and H-1′/C-1. Thus, the structure of **2** was determined to be ($\pm$)-*erythro*-1-(1-oxo-4,5-dihydroxy-2*E*-deca-enyl)piperidine.

Compound **3**, isolated as a colorless oil, showed the same molecular formula of C$_{15}$H$_{27}$NO$_3$ as **2** by HRFABMS (*m/z* 270.2084 [M + H]$^+$). When comparing the ^{1}H NMR spectrum of **3** with that of **2**, the signals were superimposable except the signals due to H-4 and H-5 (δ 4.26 and 3.71 in **2**; δ 4.08 and 3.55 in **3**), which suggested these two compounds may be stereoisomers sharing the same structural features. The relative configuration in **3** was determined by applying the same methodology as in **2** (Scheme 2). Namely, upon irradiation of the methyl signal at δ 1.41 of the 4,5-acetonide (**3a**) of **3**, an NOE was observed at δ 3.74, corresponding to H-5. Upon irradiation of the methyl signal at δ 1.44 of **3a**, an NOE was observed at δ 4.16 corresponding to H-4. These results demonstrated the *threo* configuration of the vicinal diol at C-4/C-5 in **3**. Thus, the structure of **3** was determined as ($\pm$)-*threo*-1-(1-oxo-4,5-dihydroxy-2*E*-decaenyl)piperidine.

Compound **4** was obtained as a colorless oil and confirmed to have a molecular formula of C$_{12}$H$_{23}$NO$_3$ by HRFABMS ([M + Na]$^+$ (*m/z*) 252.1587, calcd 252.1576). The ^{1}H NMR spectrum showed the presence of an isobutylamino group with proton signals at δ 3.15 (2H, t, J = 6.8 Hz), 1.80 (1H, nonet, J = 6.8 Hz), 0.93 (3H, d, J = 6.8 Hz), and 0.93 (3H, d, J = 6.8 Hz). Besides the above-mentioned moiety, the ^{1}H−^{1}H COSY also defined another spin system involving the protons from H-2 to H-8, which included the signals due to a *trans*-α,β-olefinic double bond at δ 6.12 (1H, d, J = 15.4 Hz) and 6.82 (1H, dd, J = 15.4, 4.6 Hz) and a vicinal diol at δ 4.12 and 3.56 (Table 2). The relative configuration of the 4,5-diol was determined by the NOE difference spectrum experiment on the 4,5-acetonide (**4a**) (Scheme 3). Namely, upon irradiation of the methyl signal at δ 1.41 of the 4,5-acetonide (**4a**) of **4**, an NOE was observed at δ 3.73, corresponding to H-5. Upon irradiation of the methyl signal at δ 1.44 of **4a**, an NOE was observed at δ 4.15, corresponding to H-4. The above NOE data

Table 2. ¹H NMR Spectral Data of **2**−**7** (400 MHz, CDCl₃, δ in ppm, J in Hz)

H	2	3	4a[a]	5a[a]	6	7
H-1						
H-2	6.55, dd (15.2, 1.7)	6.54, dd (15.3, 1.7)	6.12, d (15.4)	6.72, d (14.4)	5.94, d (12.5)	5.90, d (11.2)
H-3	6.77, dd (15.2, 4.7)	6.72, dd (15.3, 4.5)	6.82, dd (15.4, 4.6)	7.27, dd (14.4, 11.4)	6.49, d (12.5)	6.56, dd (11.2, 11.2)
H-4	4.26, br s	4.08, br s	4.12, br s	7.22, dd (14.9, 11.4)		8.03, dd (15.6, 11.2)
H-5	3.71, m	3.55, m	3.56, br s	6.42, d (14.9)	6.62, d (1.4)	6.65, d (15.6)
H-6	1.46, m	1.47, m	1.52, m			
H-7	1.29, m	1.27, m	1.52, m	2.58, t (7.3)		7.09, d (1.7)
H-8	1.29, m	1.27, m	0.92, t (6.9)	1.60, m		
H-9	1.29, m	1.27, m		1.35, h (7.3)	6.59, d (1.4)	
H-10	0.88, t (6.6)	0.88, t (6.6)		0.92, t (7.3)		6.75, d (8.1)
H-11						6.93, dd (8.1, 1.7)
H-1′	3.50, t (5.5)	3.47, t (5.5)	3.15, t (6.8)	3.50, br s	3.34, t (5.7)	3.51, t (6.8)
H-2′	1.57, m	1.54, m	1.80, nonet (6.8)	1.59, m	1.55, m	1.97, m
H-3′	1.65, m	1.63, m	0.93, d (6.8)	1.67, m	1.29, m	1.89, m
H-4′	1.57, m	1.54, m	0.93, d (6.8)	1.59, m	1.55, m	3.56, t (6.8)
H-5′	3.60, t (5.5)	3.55[b]		3.63, br s	3.61, t (5.7)	
OCH₂O					5.97, s	5.96, s
OCH₃					3.87, s	
NH			5.74, br s			

[a] Spectra of **4** and **5** were recorded at 500 MHz. [b] Overlapped signal.

Table 3. ¹³C NMR Spectral Data of **2**−**7** (100 MHz, CDCl₃, δ in ppm)

C	2	3	4[a]	5[a]	6	7
C-1	165.8	165.6	165.6	164.3	167.4	165.6
C-2	121.8	121.4	124.7	129.0	122.5	118.5
C-3	142.8	144.3	142.8	139.2	132.4	139.1
C-4	74.6	74.3	74.2	139.1	130.3	124.2
C-5	74.5	74.0	74.0	133.8	108.4	140.9
C-6	32.2	33.0	35.2	200.4	148.9	131.4
C-7	25.7	25.4	18.9	41.2	143.5	106.4
C-8	31.8	31.8	14.0	26.2	135.4	148.2
C-9	22.6	22.6		22.4	102.4	148.1
C-10	14.1	14.0		13.8		108.3
C-11						122.6
C-1′	47.1	47.1	47.0	47.1	47.3	46.9
C-2′	26.6	26.5	28.6	26.8	26.2	26.3
C-3′	24.5	24.5	20.2	24.6	24.5	24.4
C-4′	25.6	25.5	20.2	25.6	25.3	45.6
C-5′	43.2	43.2		43.4	42.0	
OCH₂O					101.6	101.0
OCH₃					56.5	

[a] Spectra of **4** and **5** were recorded at 125 MHz.

suggested that the vicinal diol at the C-4 and C-5 positions also has a *threo* configuration. Thus, the structure of **4** was defined as (±)-*threo*-*N*-isobutyl-4,5-dihydroxy-2*E*-octaen-amide.

Compound **5** was isolated as a colorless oil, and its molecule formula of C₁₅H₂₃NO₂ was determined by HREIMS (*m/z* 249.1729, calcd 249.1729). Its ¹H NMR spectrum, coupled with a detailed analysis of the ¹H−¹H COSY data, showed the presence of three separate spin systems, including the signals due to a piperidine ring at δ 3.63 (2H, br s), 3.50 (2H, br s), 1.67 (2H, m), 1.59 (2H, m), and 1.59 (2H, m), an *n*-butyl group from H-7 to H-10, and a conjugated diene moiety at δ 6.72 (1H, d, J = 14.9 Hz, H-2), 7.22 (1H, dd, J = 14.9, 11.4 Hz, H-3), 7.27 (1H, dd, J = 14.4, 11.4 Hz, H-4), and 6.42 (1H, d, J = 14.4 Hz, H-5), which were both determined to be *trans* configured

from their coupling constants of 14.9 and 14.4 Hz (Table 2). Furthermore, in the ¹³C NMR spectrum, the signal for a carbonyl carbon at δ 200.4 was assigned to C-6 on the basis of analysis of the HBMC correlations between δ_H 7.22 (H-4) and δ_C 200.4 and between δ_H 1.60 (H-8) and δ_C 200.4. Thus, the structure of **5** was elucidated to be 1-(1,6-dioxo-2*E*,4*E*-decadienyl)piperidine.

Compound **6** was isolated as a colorless oil, and its molecular formula of C₁₆H₁₉NO₄ was determined by HREIMS (*m/z* 289.1334, calcd 289.1314). The ¹H NMR spectrum showed signals due to a piperidine ring at δ 3.61 (2H, t, J = 5.7 Hz, H-5′), 3.34 (2H, t, J = 5.7 Hz, H-1′), 1.55 (2H, m, H-2′), 1.55 (2H, m, H-4′), and 1.29 (2H, m, H-3′) and two *meta*-coupled aromatic doublets at δ 6.62 (1H, d, J = 1.4 Hz) and 6.59 (1H, d, J = 1.4 Hz), indicating the presence of a 1,3,4,5-tetrasubstituted benzene ring, together with the signals due to an O-methyl group at δ 3.87 and a methylenedioxy group at δ 5.97. Furthermore, it also showed signals due to two olefinic protons at δ 5.94 (1H, d, J = 12.5 Hz, H-2) and 6.49 (1H, d, J = 12.5 Hz, H-3), indicating an α,β-unsaturated carbonyl system (Table 2). The coupling constant indicated that the double bonds possess *Z* geometry. The attribution of this configuration was corroborated by the shielded signals of H-2 and H-3 in the *Z* isomer when compared with the *E* isomer.[24] The signal at δ 3.87 was assigned to the O-methyl group at C-8 on the basis of its correlation with the carbon signals at δ 135.4 (C-8) in the HMBC spectrum. This conclusion was further supported by the NOE difference experiment. Upon irradiation of the methyl signal, an NOE was observed at δ 6.59, corresponding to H-9. Thus, the structure of **6** was determined as 1-[1-oxo-3(3,4-methylenedioxy-5-methoxy-phenyl)-2*Z*-propenyl]piperidine.

Compound **7** was isolated as a colorless oil and shown to have a molecular formula of C₁₆H₁₇NO₃ on the basis of the HREIMS (*m/z* 271.1219, calcd 271.1209). The ¹H NMR spectrum showed the presence of a pyrrolidine ring with
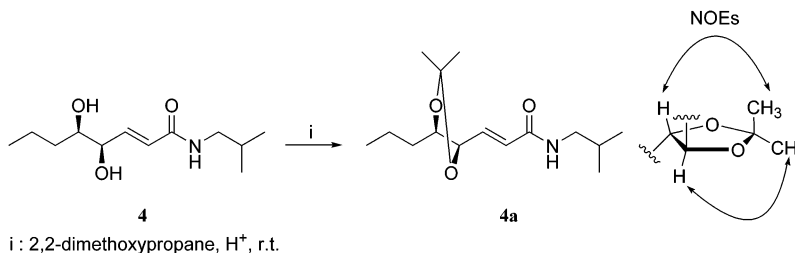
Scheme 1

i : 2,2-dimethoxypropane, H⁺, r.t.

Scheme 2
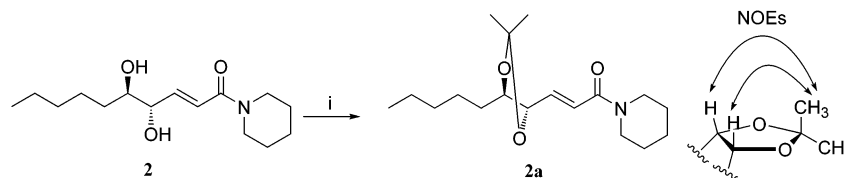
i : 2,2-dimethoxypropane, H⁺, r.t.

Scheme 3

i : 2,2-dimethoxypropane, H⁺, r.t.
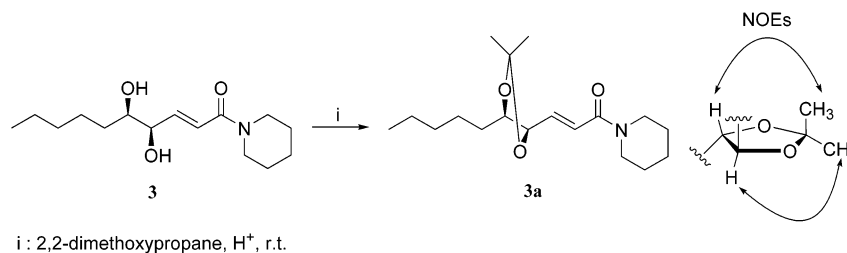
proton signals at δ 3.56 (2H, t, $J = 6.8$ Hz, H-4′), 3.51 (2H, t, $J = 6.8$ Hz, H-1′), 1.97 (2H, m, H-2′), and 1.89 (2H, m, H-3′), a 1,3,4-trisubstituted aromatic group at δ 7.09 (1H, d, $J = 1.7$ Hz, H-7), 6.75 (1H, d, $J = 8.1$ Hz, H-10), and 6.93 (1H, dd, $J = 8.1, 1.7$ Hz, H-11), a methylenedioxy at δ 5.96 (2H, s), and a diene system with signals at δ 5.90 (1H, d, $J = 11.2$ Hz, H-2), 6.56 (1H, dd, $J = 11.2, 11.2$ Hz, H-3), 8.03 (1H, dd, $J = 15.6, 11.2$ Hz, H-4), and 6.65 (1H, d, $J = 15.6$ Hz, H-5) (Table 2). The geometry of the double bonds (Δ^2 and Δ^4) was determined to be Z and E from their coupling constants of 11.2 and 15.6 Hz.[22] Therefore, compound **7** was determined as 1-[oxo-5(3,4-methylene-dioxyphenyl)-2Z,4E-pentadienyl]pyrrolidine.

It is noted that as the chloroform-soluble fraction of the methanol extract of the roots of *P. nigrum* was found to increase amobarbital-induced sleeping time in mice. Work assessing the in-vivo activity of the compounds isolated in the present study is in progress.

Experimental Section

General Experimental Procedures. The UV spectra were obtained with a Shimadzu UV-160 spectrophotometer, whereas the IR spectra were measured with a JASCO FT/IR-300E (by a KBr disk method) spectrometer. Optical rotations were measured with a JASCO DIP-370 digital polarimeter in a 0.5 dm cell. The EIMS and HREIMS were taken on a JEOL JMS-AX505HA spectrometer. The FABMS and HRFABMS were taken on a JEOL JMS-700 MStation spectrometer. The ^{1}H and ^{13}C NMR spectra were measured with a JEOL ECP-500 and a JEOL AL-400 spectrometer in CDCl$_3$ solution with TMS as the internal reference, and chemical shifts are expressed in δ (ppm). Reversed-phase HPLC separations were carried out using a JASCO PU-2080 HPLC system, equipped with a Shodex RI-101 differential refractometer detector and a Senshu Pak C$_{18}$ column (20 × 150 mm i.d.) at a flow rate of 5.0 mL/min. Reversed-phase column chromatography (RP-CC) was accomplished with RP-C$_{18}$ silica gel (100−200 mesh, Chromatorex DM1020T ODS, Fuji Silysia Chemical Ltd.). Silica gel CC was carried out using Kieselgel 60 (200−300 mesh, E. Merck). TLC was performed on Kieselgel 60 F$_{254}$ plates (E. Merck).

Plant Material. The roots of *P. nigrum* L. used in this study were collected in Hainan Island, People's Republic of China, in April 2001, and identified by Y.C. A voucher specimen (TH04001) is deposited in the herbarium of Toho University, Japan.

Extraction and Isolation. The dried powdered roots (7 kg) were extracted repeatedly with 70% methanol (3 L × 4) at room temperature. The aqueous methanol extracts were combined and evaporated under vacuum to give a residue (508 g). The residue was dispersed in H$_2$O (1 L), then extracted successively with chloroform (1 L × 3), ethyl acetate (1 L × 3), and *n*-BuOH saturated with H$_2$O (1 L × 3). The solvents were evaporated in vacuo. The chloroform extract (150 g) was chromatographed by silica gel CC (2500 g) with a gradient of petroleum ether and acetone to give 10 fractions, A−J. Fraction E (10 g) was subjected to silica gel CC (100 g), eluting with petroleum ether and acetone (10:1, 800 mL; 4:1, 600 mL), to afford two subfractions, E1 and E2. Fraction E1 (1.5 g) was further purified by RP-CC (7.5 g, MeOH−H$_2$O, 3:1) and RP-HPLC (MeOH−H$_2$O, 4:1) to yield **30** (81 mg, t_R 50.4 min), **31** (238 mg, t_R 36.6 min), **32** (17 mg, t_R 53.3 min), and **33** (2 mg, t_R 43.6 min). Fraction E2 (2.8 g) was subjected to RP-CC (15 g, MeOH−H$_2$O, 2:1) and RP-HPLC (MeOH−H$_2$O, 7:3) to furnish **34** (10 mg, t_R 44.3 min), **35** (20 mg, t_R 38.4 min), and **36** (22 mg, t_R 46.0 min). The eluate of the RP-CC with MeOH was crystallized to afforded **39** (8 mg) by MeOH−H$_2$O. Fraction F (13 g) was purified by silica gel CC (130 g), eluting with petroleum ether and acetone (8:1, 800 mL; 4:1, 800 mL; 1:1, 800 mL). Fractions were grouped according to TLC into three subfractions, F1−F3. Fraction F1 (0.8 g) was chromatographed successively with RP-CC (5 g, MeOH−H$_2$O, 2:1) and RP-HPLC (MeOH−H$_2$O, 7:3) to yield **8** (15 mg, t_R 32.5 min), **9** (48 mg, t_R 21.0 min), and **12** (17 mg, t_R 40.9 min). Fraction F2 (1.2 g) was further fractionated by RP-CC (6 g, MeOH−H$_2$O, 2:1) and RP-HPLC (MeOH−H$_2$O, 7:3) to afford **1** (39 mg, t_R 44.3 min) and **5** (22 mg, t_R 33.1 min). Fraction F3 (2 g) was chromatographed by RP-CC (10 g, MeOH−H$_2$O, 2:1) and RP-HPLC (MeOH−H$_2$O, 7:3) to yield **2** (19 mg, t_R 26.6 min), **3** (12 mg, t_R 27.7 min), **4** (3 mg, t_R 19.9min), **37** (56 mg, t_R 25.9 min), and **38** (22 mg, t_R 42.5 min). Fraction G (36 g) was subjected to a silica gel CC (360 g), eluting with petroleum ether and acetone (4:1, 20000 mL; 2:1, 2000 mL; 1:1, 2000 mL), to give three subfractions (G1, G2, and G3). Fraction G1 (5.5 g) was subjected to RP-CC (30 g) with MeOH−H$_2$O (4:1) and RP-HPLC (MeOH−H$_2$O, 4:1) to give **17** (22 mg, t_R 50.1 min), **18** (34 mg, t_R 56.2 min), **21** (11 mg, t_R 22.6 min), **24** (5 mg, t_R 27.8 min), **26** (13 mg, t_R 47.7 min), **27** (4 mg, t_R 41.6 min), and **28** (8 mg, t_R 36.5 min). Crystallization of fractions G2 (7.2 g) and G3 (6.3 g) afforded **10** (3.2 g) and **13** (2.5 g) by acetone and hexane, respectively. Fraction H (8 g) was purified by silica gel CC (80 g), eluting with petroleum ether and acetone (8:1, 800 mL; 4:1, 800 mL), to give two subfractions, H1 and H2. Fraction H1 (1.5 g) was subjected to RP-CC (7.5 g, MeOH−H$_2$O, 4:1) and RP-HPLC (MeOH−H$_2$O, 7:3) to afford **19** (4 mg, t_R 26.5 min), **20** (6 mg, t_R 25.6 min), and **29** (6 mg, t_R 27.1 min).

Fraction H2 (1.2 g) was purified by RP-CC (6 g) using MeOH−H$_2$O (3:1) and RP-HPLC (MeOH−H$_2$O, 7:3) to yield **6** (7 mg, t_R 26.1 min), **7** (4 mg, t_R 33.3 min), **11** (25 mg, t_R 27.3 min), **14** (36 mg, t_R 41.6 min), **15** (94 mg, t_R 42.2 min), **22** (39 mg, t_R 30.2 min), and **23** (27 mg, t_R 34.4 min). Fraction I (0.6 g) was chromatographed by RP-CC (3 g, MeOH−H$_2$O, 2:1) and RP-HPLC (MeOH−H$_2$O, 7:3) to yield **16** (1 mg, t_R 32.1 min) and **25** (4 mg, t_R 40.6 min).

Pipercycliamide (1): colorless oil; [α]^{25}$_D$ ±0° (*c* 1.0, CHCl$_3$); IR (KBr) ν_{max} 3439, 1675, 1457, 1268, 1123, 874 cm^{-1}; ^{1}H NMR (CDCl$_3$, 500 MHz) and ^{13}C NMR (CDCl$_3$, 125 MHz) (see Table 1); FABMS *m/z* 256 [M + H]$^+$; HRFABMS *m/z* 256.1933 [M + H]$^+$ (calcd for C$_{14}$H$_{26}$NO$_3$, 256.1913).

(±)-*erythro*-1-(1-Oxo-4,5-dihydroxy-2*E*-decaenyl)piperidine (2): colorless oil; [α]^{25}$_D$ ±0° (*c* 0.8, CHCl$_3$); IR (KBr) ν_{max} 3414, 1656, 1598, 1456, 1266, 1130, 988 cm^{-1}; ^{1}H NMR (CDCl$_3$, 400 MHz) and ^{13}C NMR (CDCl$_3$, 100 MHz) (see Table 2 and Table 3, respectively); FABMS *m/z* 270 [M + H]$^+$; HRFABMS *m/z* 270.2069 [M + H]$^+$ (calcd for C$_{15}$H$_{28}$NO$_3$, 270.2069).

(±)-*threo*-1-(1-Oxo-4,5-dihydroxy-2*E*-decaenyl)piperidine (3): colorless oil; [α]^{25}$_D$ ±0° (*c* 0.9, CHCl$_3$); IR (KBr) ν_{max} 3423, 1642, 1600, 1449, 1266, 1130, 989 cm^{-1}; ^{1}H NMR (CDCl$_3$, 400 MHz) and ^{13}C NMR (CDCl$_3$, 100 MHz) (see Table 2 and Table 3, respectively); FABMS *m/z* 270 [M + H]$^+$; HRFABMS *m/z* 270.2084 [M + H]$^+$ (calcd for C$_{15}$H$_{28}$NO$_3$, 270.2069).

(±)-*threo*-*N*-Isobutyl-4,5-dihydroxy-2*E*-octaenamide (4): colorless oil; [α]^{25}$_D$ ±0° (*c* 0.3, CHCl$_3$); IR (KBr) ν_{max} 3430, 1628, 1456, 1267, 1148, 990, 816 cm^{-1}; ^{1}H NMR (CDCl$_3$, 500 MHz) and ^{13}C NMR (CDCl$_3$, 125 MHz) (see Table 2 and Table 3, respectively); FABMS *m/z* 252 [M + Na]$^+$; HRFABMS *m/z* 252.1587 [M + Na]$^+$ (calcd for C$_{12}$H$_{23}$NO$_3$Na, 252.1576).

1-(1,6-Dioxo-2*E*,4*E*-decadienyl)piperidine (5): colorless oil; UV (MeOH) λ_{max} (log ϵ) 275.4 nm (4.32); IR (KBr) ν_{max} 3453, 1629, 1600, 1449, 1258 cm^{-1}; ^{1}H NMR (CDCl$_3$, 500 MHz) and ^{13}C NMR (CDCl$_3$, 125 MHz) (see Table 2 and Table 3, respectively); EIMS *m/z* 249 [M]$^+$ (23), 149 (9), 137 (14), 109 (10), 70 (100); HREIMS *m/z* 249.1729 (calcd for C$_{15}$H$_{23}$NO$_2$, 249.1729).

1-[1-Oxo-3-(3,4-methylenedioxy-5-methoxyphenyl)-2*Z*-propenyl]piperidine (6): colorless oil; UV (MeOH) λ_{max} (log ϵ) 281.8 nm (3.95); IR (KBr) ν_{max} 3439, 1618, 1517, 1448, 1261, 1125, 1039 cm^{-1}; ^{1}H NMR (CDCl$_3$, 400 MHz) and ^{13}C NMR (CDCl$_3$, 100 MHz) (see Table 2 and Table 3, respectively); EIMS *m/z* 289 [M]$^+$ (87), 206 (100), 178 (41), 149 (27); HREIMS *m/z* 289.1334 (calcd for C$_{16}$H$_{19}$NO$_4$, 289.1314).

1-[1-Oxo-5-(3,4-methylenedioxyphenyl)-2*Z*,4*E*-pentadienyl]pyrrolidine (7): colorless oil; UV (MeOH) λ_{max} (log ϵ) 262.8 (3.92), 308.8 (4.01), 345.6 nm (4.12); IR (KBr) ν_{max} 3429, 1628, 1498, 1448, 1251, 1037, 661 cm^{-1}; ^{1}H NMR (CDCl$_3$, 400 MHz) and ^{13}C NMR (CDCl$_3$, 100 MHz) (see Table 2 and Table 3, respectively); EIMS *m/z* 271 [M]$^+$ (100), 201 (99), 173 (39), 149 (29), 114 (30); HREIMS *m/z* 271.1219 (calcd for C$_{16}$H$_{17}$NO$_3$, 271.1209).

Preparation of the Acetonide (2a) from Compound 2. A solution of **2** (1.6 mg, 6.02 μmol) in 2,2-dimethoxypropane (0.5 mL) was treated with Dowex 50W-X8 (H$^+$ form, 20 mg), and the mixture was stirred at room temperature for 3 h. The resin was removed by filtration. Removal of the solvent from the filtrate under reduced pressure yielded **2a** (1.5 mg).

2a: colorless oil; ^{1}H NMR (400 MHz, CDCl$_3$) δ (ppm) 6.52 (1H, dd, *J* = 15.0, 1.5 Hz, 2-H), 6.74 (1H, dd, *J* = 15.0, 5.8 Hz, 3-H), 4.67 (1H, td, *J* = 5.8, 1.5 Hz, 4-H), 4.22 (1H, m, 5-H), 1.26−1.48 (8H, overlapped, 6-H, 7-H, 8-H, and 9-H), 0.88 (3H, t, *J* = 6.8 Hz, 10-H), 3.49 (2H, br s, 1′-H), 1.58 (2H, m, 2′-H), 1.65 (2H, m, 3′-H), 1.58 (2H, m, 4′-H), 3.60 (2H, br s, 5′-H), 1.51 (3H, s, (CH$_3$)$_2$−C−), 1.38 (3H, s, (CH$_3$)$_2$−C−).

Preparation of the Acetonide (3a) from Compound 3. A solution of **3** (1.4 mg, 5.27 μmol) in 2,2-dimethoxypropane (0.5 mL) was treated with Dowex 50W-X8 (H$^+$ form, 20 mg), and the mixture was stirred at room temperature for 3 h. The resin was removed by filtration. Removal of the solvent from the filtrate under reduced pressure yielded **3a** (1.4 mg).

3a: colorless oil; ^{1}H NMR (500 MHz, CDCl$_3$) δ (ppm) 6.58 (1H, dd, *J* = 15.0, 1.4 Hz, 2-H), 6.76 (1H, dd, *J* = 15.0, 5.4 Hz, 3-H), 4.16 (1H, ddd, *J* = 8.4, 5.4, 1.4 Hz, 4-H), 3.74 (1H, m, 5-H), 1.25−1.49 (8H, overlapped, 6-H, 7-H, 8-H, and 9-H), 0.89 (3H, t, *J* = 6.8 Hz, 10-H), 3.49 (2H, br s, 1′-H), 1.58 (2H, m, 2′-H), 1.65 (2H, m, 3′-H), 1.58 (2H, m, 4′-H), 3.61 (2H, br s, 5′-H), 1.41 (3H, s, (CH$_3$)$_2$−C−), 1.44 (3H, s, (CH$_3$)$_2$−C−).

Preparation of the Acetonide (4a) from Compound 4. A solution of **4** (1.5 mg, 5.27 μmol) in 2,2-dimethoxypropane (0.5 mL) was treated with Dowex 50W-X8 (H$^+$ form, 20 mg), and the mixture was stirred at room temperature for 2 h. The resin was removed by filtration. Removal of the solvent from the filtrate under reduced pressure yielded **4a** (1.5 mg).

4a: colorless oil; ^{1}H NMR (400 MHz, CDCl$_3$) δ (ppm) 6.09 (1H, dd, *J* = 15.2, 1.5 Hz, 2-H), 6.78 (1H, dd, *J* = 15.2, 5.3 Hz, 3-H), 4.15 (1H, ddd, *J* = 8.4, 5.3, 1.5 Hz, 4-H), 3.73 (1H, m, 5-H), 1.52 (2H, overlapped, 6-H), 1.50 (2H, overlapped, 7-H), 0.94 (3H, t, *J* = 8.0 Hz, 8-H), 3.17 (2H, t, *J* = 7.0 Hz, 1′-H), 1.81 (1H, nonet, *J* = 6.8 Hz, 2′-H), 0.93 (6H, d, *J* = 6.6 Hz, 3′-H, 4′-H), 1.41 (3H, s, (CH$_3$)$_2$−C−), 1.44 (3H, s, (CH$_3$)$_2$−C−).

Supporting Information Available: Figures of structures and tables of complete ^{1}H and ^{13}C NMR data for all known compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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