HETEROCYCLES, Vol. 57, No. 9, 2002, pp. 1653-1658, Received, 20th May, 2002

AMIDES FROM THE SEEDS OF *PIPER NIGRUM* LINN AND THEIR INSECTICIDAL ACTIVITY

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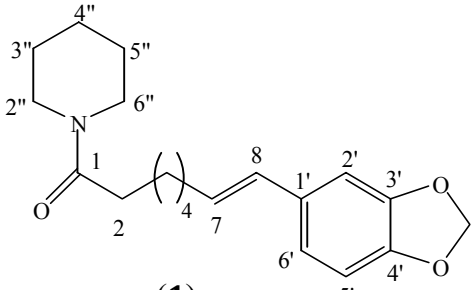
Abstract–Two new amides pipertipine (**1**) and pipercitine (**2**) have been isolated from the dried seeds of *Piper nigrum* along with eight known constituents of β-sitosterol, (2*E*, 4*E*, 8*Z*)-*N*-*iso*butyleicosatrienamide, pellitorine, guineensine, piperine, piperettine, pipericine, and (3,4-methylenedioxyphenyl)cinnamaldehyde. The structures of **1** and **2** have been elucidated as 1-[8-(3´, 4´-methylenedioxyphenyl)-(7*E*)-octaenoyl]piperidine and 1-[(2*E*)-octadecenoyl]piperidine, through extensive spectral studies including 2D NMR. New compounds **1** and **2** and most of the known exhibited toxicity against fourth- instar larvae of *Aedes aegypti*.

Piper nigrum (black pepper) belonging to the Piperaceae family has over 700 species distributed in both hemispheres. It is acrid, pungent and is the most widely used spice in the world being rich in amides. Some of *Piper* species also show insecticidal properties.[1-3]

In view of attributed medicinal and biological properties[1] of this genus, studies on the seeds of *P. nigrum* were undertaken in the present work, which furnished ten compounds including two new amides pipertipine (**1**) and pipercitine (**2**), along with eight known constituents, β-sitosterol,[4] (2*E*, 4*E*, 8*Z*)-*N*-*iso*butyleicosatrienamide,[5] pellitorine,[6] guineensine,[7] piperine,[8] piperettine,[7] (3, 4-methylenedioxyphenyl)-cinnamaldehyde[3] and pipericine.[8] These known compounds were identified by comparison of their physical and spectral data with those of the respective compounds reported in literature.

The molecular formula of pipertipine (**1**), $C_{20}H_{27}NO_3$, was obtained by HREIMS, at *m/z* 329.1990. Its IR spectrum showed bands at 1640 cm^{-1} for amide C=O and four absorptions between 1610 and 1400 cm^{-1} for aromatic and aliphatic C=C. Characteristic absorption bands at 263 (ε=13, 400) and 310 nm (ε=5, 850)[9] in the UV spectrum suggested the presence of a 3´, 4´-methylenedioxystyrene moiety, which was confirmed from the NMR spectra. The ^{1}H-NMR spectrum (Table 1) of **1** showed signals for three aromatic protons at δ 6.74 (dd, *J* = 8.0, 1.5 Hz, H-6´), 6.87 (d, *J* = 1.5 Hz, H-2´) and 6.71 (d, *J* = 8.0 Hz, H-5´) assigned to the 1, 3, 4-trisubstituted benzene ring. Two olefinic protons were observed at δ 6.25 (dt,

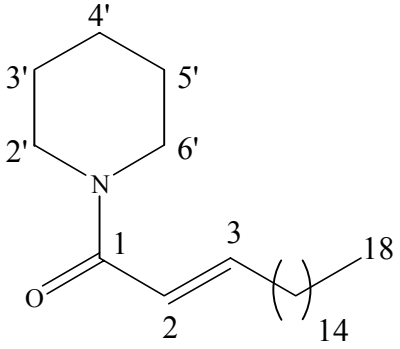
J = 16.0, 1.5 Hz, H-8) and 6.03 (dt, *J* = 16.0, 7.0 Hz, H-7), while a two-proton singlet at δ 5.91 manifested the presence of a methylenedioxy moiety. The respective carbons for these functionalities were observed at δ 120.0 (C-6′), 105.2 (C-2′), 108.0 (C-5′), 129.2 (C-8), 129.0 (C-7) and 100.7



(1)

(methylenedioxy carbon) in the ^{13}C-NMR spectra (BB, DEPT, HMQC; Table 1). The other key features of the ^{13}C-NMR spectrum were the signals at δ 172.6 for carbonyl carbon, and at δ 147.7, 146.4, and 132.3 for three aromatic quaternary carbons (C-3′, C-4′ and C-1′, respectively). Further, the ^{1}H-NMR spectrum showed a two-proton triplet at δ 2.20 (2H, *J* = 7.5 Hz, H-2) and a two-proton doublet of quartet at δ 2.15 (2H, *J* = 7.0, 1.5 Hz, H-6), the small *J* value of the latter was due to its allylic coupling with H-8. Six protons at δ 1.64 - 1.70 were assigned to methylene protons at position 3 to 5. Further, two triplets at δ 3.44 (2H, *J* = 6.7 Hz, H-2″) and 3.38 (2H, *J* = 6.7 Hz, H-6″), two two-proton quintets at δ 1.59 (2H, *J* = 6.7 Hz, H-3″) and 1.57 (2H, *J* = 6.7 Hz, H-5″) and a quintet at δ 1.62 (2H, *J* = 6.7 Hz, H-4″) were attributable to piperidine ring protons. The presence of piperidine ring was substantiated through HREIMS fragment at *m/z* 84.0808 ($C_5H_{10}N^+$). Analysis of the ^{1}H-^{1}H COSY and HMQC spectra fully supported the assigned structure. These observations led to elucidate the structure of **1** as 1-[8-(3′, 4′-methylenedioxyphenyl)-(7*E*)-octaenoyl]piperidine. It may be noted that **1** resembles piperolein-B except for one less CH_2 group. [7]

The molecular formula of pipercitine (**2**), $C_{23}H_{43}NO$, was obtained by HREIMS peak at *m/z* 349.3344. The IR spectrum showed absorptions at 1660 and 1600 cm^{-1} for amide C=O and C=C. The ^{1}H-NMR spectrum of **2** revealed the presence of a piperidine ring by three multiplets at δ 3.55 (4H, m, H -2′



(2)

and H-6′), 1.57 (4H, m, H-3′ and H-5′) and 1.64 (2H, m, H-4′). Two double triplets at δ 6.21 (*J* = 14.0,

1.3 Hz) and 6.80 (J = 14.0 and 7.0 Hz) indicated the presence of a *trans* double bond conjugated with amido moiety in analogy with the data for similar compounds.[10] A doublet of quartet at δ 2.18 (2H, J = 7.0, 1.3 Hz) showing allylic coupling (1.3 Hz) with H-2 was assigned to H-4. The resonances in the ^{1}H- and ^{13}C-NMR spectra of **2** (Table 1) were confirmed by ^{1}H-^{1}H COSY, HMQC and HMBC experiments. The spectral data discussed above led to elucidate the structure of pipercitine as 1-[(2*E*)-octadecenoyl]piperidine (**2**).

The compounds (**1**), (**2**), (2*E*, 4*E*, 8*Z*)-*N-iso*butyleicosatrienamide, pellitorine, guineensine, piperine, and pipericine exhibited toxicity at 5, 60, 24, 20, 25, 10, and 25 ppm concentration, respectively, against fourth-instar larvae of *Aedes aegypti* by WHO method.[11]

Table 1. ^{1}H- and ^{13}C-NMR Data of **1** and **2** ($CDCl_3$)

1			**2**		
Position	δ_C	δ_H	Position	δ_C	δ_H
1	172	-	1	165	-
2	34	2.20 t (7.5)	2	120	6.21 dt (14.0, 1.3)
3-5	28-29	1.64-1.70 m	3	145	6.80 dt (14.0, 7.0)
6	32.6	2.15 dq (7.0,1.5)	4	32	2.18 dq (7.0,1.3)
7	129	6.03 dt (16.0, 7.0)	5-15	28-29	1.2-1.3 m
8	129	6.25 dt (16.0, 1.5)	16	32	1.2-1.3 m
1′	132	-	17	22	1.2-1.3 m
2′	105	6.87 d (1.5)	18	14	0.87 t (7.0)
3′	147[a]	-	2′	46[a]	3.5-3.6 m
4′	146[a]	-	3′	26[b]	1.57 m
5′	108	6.71 d (8.0)	4′	24	1.64 m
6′	120	6.74 dd (8.0, 1.5)	5′	25[b]	1.57 m
2″	46[b]	3.44 t (6.7)	6′	43[a]	3.5-3.6 m
3″	25[c]	1.59 quint (6.7)			
4″	24	1.62 quint (6.7)			
5″	24[c]	1.57 quint (6.7)			
6″	46[b]	3.38 t (6.7)			
	100	5.91 s			

Chemical shifts are in δ (ppm) and J values (in Hz) are presented in parenthesis.
[a, b, c] Assignments in a vertical column are interchangeable

EXPERIMENTAL

General Methods

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. UV and IR spectra were recorded on Hitachi U-3200 and JASCO A-302 spectrophotometers respectively. The EI and

HREIMS were recorded on Finnigan Mat-311 and JMS HX-110 spectrometers respectively. The ^{1}H- and ^{13}C-NMR spectra of compounds **1** and **2** were recorded on Bruker AMX 500 NMR spectrometer operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C nuclei. The spectra were referenced to the residual solvent signals. The chemical shifts are in ppm (δ) and coupling constants (*J*) are in Hz. For TLC, preparative TLC and vacuum liquid chromatography (VLC), silica gel PF_{254} (Merck)was used. Column chromatography was performed on Kieselgel 60 (0.063-0.200 mm) (Merck). The seeds of *P. nigrum* were purchased from the local market in Karachi.

Extraction and Isolation

Air-dried, ground seeds of *P.nigrum* (5 kg) were collected from the local market of Karachi and extracted with hexane (10 L x 3) at rt for 72 h. Evaporation of combined extracts *in vacuo* afforded a dark brown viscous residue (102.12 g). A portion (10 g) of this extract was directly subjected to preparative thin layer chromatography using a solvent system of hexane-EtOAc (9.5:0.5), which furnished four major bands. The upper band was pure and was identified as *β*-sitosterol (2.9 mg), while other bands by further PTLC purification with solvent system of hexane- EtOAc (7:3) afforded three known compounds, (2*E,* 4*E,* 8*Z)-N-iso*butyleicosatrienamide (5.1 mg), pellitorine (6.4 mg) and guineensine (5.2 mg). The remaining portion of the hexane extract (91.8 g) was partitioned between hexane and 90% MeOH. The 90% MeOH phase was extracted with EtOAc after saturation with saline water. The EtOAc extract on usual work up yielded a brownish syrup (24.78 g), a part (13.15 g) of which was subjected to VLC (1 L) on silica gel and eluted with hexane, hexane-EtOAc, EtOAc, $CHCl_3$, and $CHCl_3$ - MeOH to give 17 fractions. Fr-13 (304 mg), which was eluted with (hexane-EtOAc, 6.7:3.3 – 6.0:4.0), was subjected to column chromatography on silica gel, which was eluted with hexane, hexane-EtOAc, EtOAc, $CHCl_3$, and $CHCl_3$ - MeOH furnishing 20 fractions. Fr-7 (hexane-EtOAc, 9.3:0.7 eluate) of this column was pure (3,4-methylenedioxyphenyl)cinnamaldehyde (1.9 mg). Fr -11, which was eluted with hexane- EtOAc (8.5:1.5), furnished pellitorine (1.5 mg) on purification over PTLC in solvent system hexane-EtOAc (8.0:2.0), while fr-12 (hexane-EtOAc, 6.0:4.0 eluate) gave piperine (4.9 mg) as a major compound on purification over PTLC in solvent system hexane-EtOAc (7:3). Fr-13, (hexane-EtOAc, 1:1 eluate) could be separated into two bands on PTLC in solvent system hexane-EtOAc (6.5:3.5). The faster running band was identified as piperine (2.3 mg) while the slower moving was characterized as **1** (2.1 mg). Fr-14 (hexane –EtOAc, 3:7 eluate) yielded the major quantity of **1** (15.3 mg) as a pure constituent on PTLC in the solvent system hexane- EtOAc (6.5:3.5). Fr-19 (40 mg) was purified on a small silica gel column which was eluted with hexane, hexane-EtOAc, EtOAc, to give pure piperettine (6 mg) (hexane- EtOAc, 4.5: 5.5 eluate).

The seeds residue left after extraction with hexane was further extracted with MeOH (10 L x 6) at rt for

168 h. Evaporation of the solvent from the combined methanolic extracts under reduced pressure gave a solid mass (800 g), which was treated with ether to obtain ether soluble and ether insoluble fractions. The ether soluble fraction was freed of the solvent to give a residue (164 g). A portion (50 g) of this residue was separated on silica gel column by successive elution with hexane, hexane-EtOAc, EtOAc, $CHCl_3$, $CHCl_3$-MeOH and MeOH. Total 81 fractions were obtained. Purification of fr- 54 (hexane-EtOAc, 8.6:1.4 eluate) yielded two major bands 54A and 54B on repeated chromatography (PTLC) in solvent system hexane-EtOAc (8:2). 54A (3.3 mg) was identified as pipericine while 54B (18.9 mg) was characterized as **2**.

Pipertipine (1): Yellow wax; IR ν_{max} (KBr) cm^{-1}: 3050, 2950, 1640, 1610-1400 (4 peaks), 1245, 1037, 965; UV λ_{max} (MeOH) nm: 322 (sh), 310 (ε=5, 850), 270 (ε=12, 800), 263 (ε=13, 400); ^{1}H- and ^{13}C-NMR data: see Table 1; HREIMS *m/z*; 329.1990 [M^+](calcd for $C_{20}H_{27}NO_3$, 329.1991), 245.1173 ($C_{15}H_{17}O_3^+$), 194.1543 ($C_{12}H_{20}NO^+$), 168.1351 ($C_{10}H_{18}NO^+$), 161.0600 ($C_{10}H_9O_2^+$), 148.0626 ($C_9H_7O_2^+$), 135.0444 ($C_8H_7O_2^+$), 131.0159 ($C_9H_7O^+$), 126.0881 ($C_7H_{12}NO^+$), 112.0724 ($C_6H_{10}NO^+$), 103.0545 ($C_8H_7^+$), 98.0722 ($C_6H_{10}O^+$), 84.0808 ($C_5H_{10}N^+$).

Pipercitine (2): Amorphous powder; IR ν_{max} (KBr) cm^{-1}: 2930, 2840, 1660, 1600; UV λ_{max} (MeOH) nm: 210 (ε=6,450); ^{1}H- and ^{13}C-NMR data: see Table 1; EIMS *m/z* (rel. int., %) 349 ($M^{+\bullet}$, 38), 321 (38), 306 (7), 292 (9), 278 (9), 264 (12), 222 (15), 208 (21), 194 (26) 180 (30), 166 (68), 152 (30), 138 (100), 127 (81), 112 (52), 84 (70), 85 (63), 71 (24), 57 (63); HREIMS *m/z* 349.3344 [M^+](calcd for $C_{23}H_{43}NO$ 349.3344).

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