Asian Journal of Chemistry Vol. 20, No. 8 (2008), 5931-5940

Alkaloids and Carboxylic Acids from *Piper nigrum*

G.C.L. EE*, S.K. LIM, C.M. LIM and K. DZULKEFLY
Chemistry Department, Faculty of Science, University Putra Malaysia
43400, Serdang, Selangor, Malaysia
Fax: (603)89435380; Tel: (603)89466785; E-mail: gwen@fsas.upm.edu.my

Detailed chemical studies were carried out on the roots of *Piper nigrum*. Six alkaloids and four carboxylic acids were isolated and identified from this study. The alkaloids were piperolactam A (**1**), piperolactam D (**2**), cepharadione A (**3**), piperine (**4**), sylvamide (**5**) and 2,4-tetradecadienoic acid isobutyl amide (**6**). The carboxylic acids were tetracosanoic acid, *p*-hydroxy-*m*-methoxycinnamate ester (**7**), 3,4-methylenedioxycinnamic acid (**8**), 2-butenedioic acid, mono-(2-methylpropyl) ester (**9**) and 3,4-methylenedioxy benzoic acid (**10**). Larvicidal assays on *Aedes aegypti* were carried out on the crude extracts of these plants as well as the pure compounds. The larvae were found to be susceptible to some of these extracts and compounds. This paper reports the isolation and characterization of these compounds as well as bioassay data. This is the first report on the presence of piperolactam A (**1**), piperolactam D (**2**) cepharadione A (**3**) and sylvamide (**5**) in *Piper nigrum*.

Key Words: Alkaloids, Carboxylic acid, *Piper nigrum*, Larvicidal.

INTRODUCTION

The genus *Piper* belongs to the Piperaceae family and has over 700 species. The *Piper* species have high commercial, economical and medicinal importance. These plants are reputed in the Indian Ayurvedic system of medicine for their medicinal properties and in folklore medicine[1,2]. *Piper nigrum*, more commonly known as black pepper is used as a stimulant and carminative and prescribed for cholera, dyspepsia, flatulence, diarrhoea and various gastric ailments[3]. Several alkaloids and non-alkaloidal constituents have been reported from this plant[4-6]. Several alkaloids isolated from *Piper nigrum* have been shown to exhibit CNS depressant property[7] as well as insecticidal activity against adzuki bean weevil[8] and larvicidal activity against larvae of *Toxocara canis*[9]. This paper reports the isolation and characterization of the six alkaloids as well as bioassay data.

EXPERIMENTAL

The roots of *Piper nigrum* were collected from Sri Aman Sarawak, Malaysia.

Infrared spectra were measured in KBr/NaCl pellet on a Perkin-Elmer FTIR Spectrum BX spectrometer. EIMS were recorded on a Shimadzu GCMS-QP5050A spectrometer. NMR spectra were obtained using a Unity INOVA 500 MHz NMR/Jeol 400 MHz FT NMR spectrometer using tetramethylsilane (TMS) as internal standard. Ultra violet spectra were recorded in $CHCl_3$ on a Shimadzu UV-160A, UV-Visible recording spectrophotometer.

Extraction and isolation: 2.5 kg of dried ground roots were extracted twice with distilled hexane for 48 h at room temperature. This gave 19.5 g of crude hexane extract. The crude extract was then subjected to gravity column chromatography using Merck SiO_2 and eluted with various solvent systems comprising hexane/chloroform, chloroform/ethyl acetate and ethyl acetate/methanol. Further purification of the crude fraction using AlO_2 in a mini-column gave sylvamide (**5**). The roots were then extracted with distilled chloroform for more than 48 h, twice to give 70.1 g of an oily extract. The extract was then purified by SiO_2 vacuum column chromatography using hexane/chloroform, chloroform/ethyl acetate and ethyl acetate/methanol. This gave 2-butenedioic acid, mono-(2-methylpropyl) ester (**9**) and piperine (**4**). A similar extraction procedure was repeated using ethyl acetate to produce 16.4 g of a gummy solid. The extract was purified by SiO_2 vacuum column chromatography using various percentages of hexane/chloroform, chloroform/ethyl acetate and ethyl acetate/methanol as the eluting solvents. Further purification of the 15th fraction using column chromatography yielded five pure compounds: 3,4-methylenedioxy benzoic acid (**10**), tetracosanoic acid, *p*-hydroxy-*m*-methoxycinnamate ester (**7**), 3,4-methylenedioxycinnamic acid (**8**), piperolactam D (**2**), piperolactam A (**1**). A similar extraction method was repeated using methanol solvent to yield 15.3 g of a crude gummy solid. 20 Fractions were collected from the extraction using SiO_2 vacuum column chromatography. Further purification of the 13th fraction using a small column produced cepharadione A (**3**). Another sample of the dried roots (2.5 kg) was defatted in distilled petroleum ether. The defatted plant material was then extracted with distilled ethanol and heated in a water bath. The alcoholic extract was then concentrated to about 1/20th of its original volume under reduced pressure before a large quantity of aqueous acid solution (5 % HCl) was added slowly to the solution. The acidic solution was then filtered through kieselghur to remove the non-alkaloidal substances. The filtrate was then basified with concentrated ammonia solution to pH 10. The liberated alkaloids were extracted exhaustively with chloroform. The chloroform extract was then washed with distilled water and dried over anhydrous sodium sulphate. Finally, the solvent was removed by evaporation

under reduced pressure to furnish the crude alkaloid. This yielded *ca.* 4.3 g of oily extract. The extract was purified using SiO_2 mini column and gave 2,4-tetradecadienoic acid isobutyl amide (**6**).

Piperolactam A (1): Yellowish needle crystals with melting point 300-301 °C (Lit.[10] 303-306 °C). UV λ_{max} nm (acetone, log ε): 234 (4.16), 263 (4.50), 276 (4.56), 286 (4.56), 320 (4.01), 384 (3.96). IR ν_{max} (cm^{-1}, NaCl disc): 3474, 3188 (2 sharp broad peak, OH), 1656 (C=O), 1502, 1448, 1412, 1380, 1324, 1280, 1184, 1130, 1108, 1054, 1032, 884.MS m/z (% intensity): 265 (89), 264 (100), 251 (27), 222 (20), 206 (2), 193 (9), 180 (6), 166 (43), 151 (5), 139 (15), 130 (1), 111 (4), 92 (11), 83 (53), 75 (16), 69 (33), 63 (5), 51 (2), 40 (1). ^{1}H NMR, δ (400 MHz, D-acetone) and ^{13}C NMR, δ (100 MHz, D-acetone): Table-1.

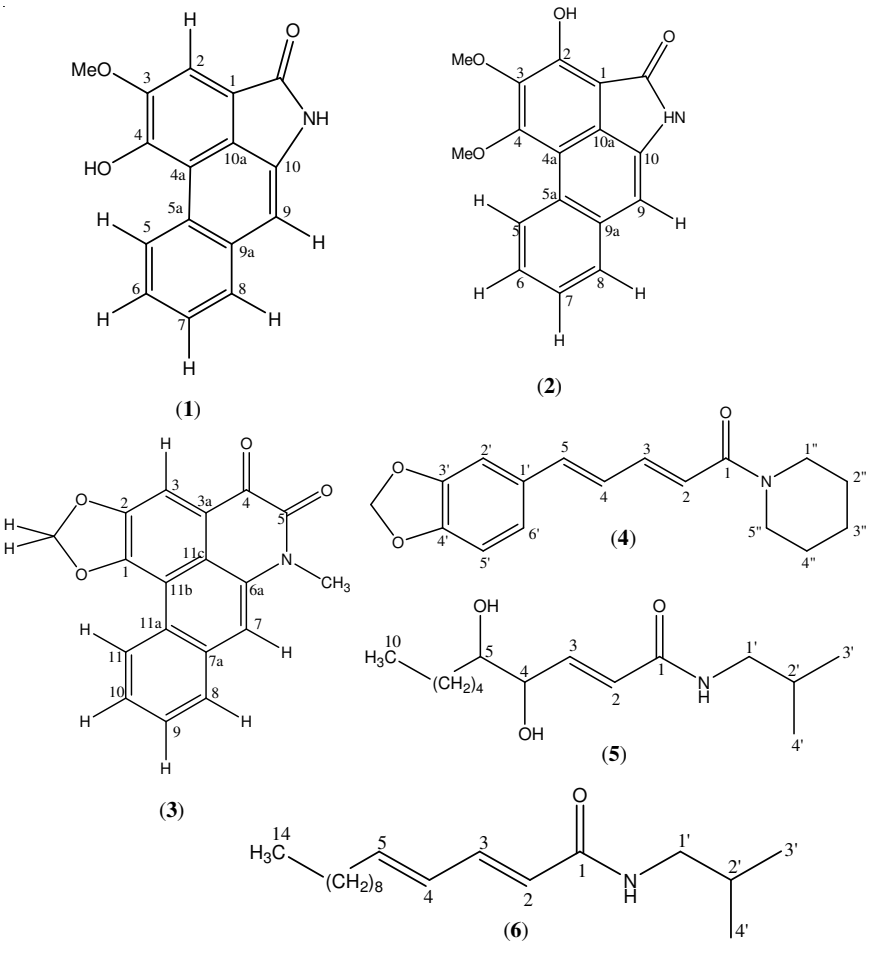


Fig. 1. Structures of alkaloids isolated from *Piper nigrum*

TABLE-1
ONE AND TWO-DIMENSIONAL NMR DATA FOR PIPEROLACTAM A (**1**)

Position	^{1}H NMR (δ)	^{13}C NMR (δ)	HMQC (C-H correlation)	HMBC (C-H correlation)
1		117.28		7.75 (H-2) (^{2}J)
2	7.75 (1H, s)	108.76	7.75 (H-2)	
3		150.32		
4		149.05		4.09 (OMe) (^{3}J)
4a		115.49		
5a		135.49		9.31 (H-5) (^{2}J)
				7.15 (H-9) (^{3}J)
5	9.31 (1H, m)	128.73	9.31 (H-5)	7.25 (H-7) (^{3}J)
6	7.52 (1H, m)	127.49	7.52 (H-6)	9.31 (H-5) (^{2}J)
7	7.25 (1H, m)	125.79	7.25 (H-7)	7.86 (H-8) (^{2}J)
8	7.86 (1H, m)	129.44	7.86 (H-8)	7.25 (H-7) (^{2}J)
				7.15 (H-9) (^{3}J)
9a		127.95		7.15 (H-9) (^{2}J)
9	7.15 (1H, s)	105.46	7.15 (H-9)	
10		136.11		7.15 (H-9) (^{2}J)
10a		125.79		7.75 (H-2) (^{3}J)
NH	9.83 (br, s)			
C=O		169.88		7.75 (H-2) (^{3}J)
OMe	4.09 (3H, s)	57.57		

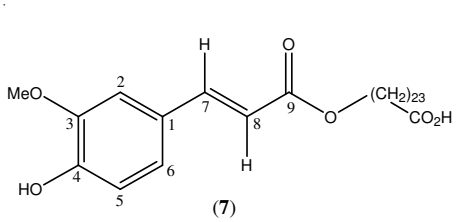
Piperolactam D (2): Yellow crystalline solid with melting point 227-229 °C (Lit.[11] 226-227 °C). UV λ_{max} nm (acetone, log ε): 235 (4.83), 266 (4.15), 294 (4.81), 329 (4.27), 362 (4.33), 381 (4.30). IR ν_{max} (cm^{-1}, NaCl disc): 3262 (broad, OH), 2924 (NH), 1678 (C=O), 1610, 1482, 1460, 1400, 1316, 1196, 1152, 1120, 1080. MS m/z (% intensity): 295 (M^+, 100), 280 (70), 277 (16), 252 (45), 249 (17), 234 (22), 209 (48), 193 (8), 180 (18), 166 (14), 153 (43), 137 (12), 126 (42), 118 (19), 100 (8), 90 (57), 76 (45), 63 (31), 43 (17). ^{1}H NMR, δ (400 MHz, D-acetone) 3.93 (3H, s, C3-OMe), 4.45 (3H, s, C4-OMe), 7.25 (1H, s, H-9), 7.49 (2H, m, H-6, H-7), 7.88 (1H, m, H-8), 9.28 (1H, m, H-5), 9.81 (1H, brs, NH). ^{13}C NMR, δ (100 MHz, D-acetone) 61.89 (C3-OMe), 62.90 (C4-OMe), 105.84 (C-9), 106.37 (C-1), 112.23 (C-10a), 125.69 (C-7), 126.71 (C-6), 127.26 (C-4a), 127.64 (C-5a), 127.83 (C-5), 129.21 (C-8), 134.24 (C-9a), 135.52 (C-10), 140.04 (C-3), 153.75 (C-4), 154.67 (C-2), 167.53 (C=O).

Cepharadione A (3): Orange needle crystals with melting point 353-355°C (Lit.[12] 350 °C). UV λ_{max} nm (MeOH, log ε): 219 (4.6), 238 (4.2), 238 (4.2), 265 (4.2), 279 (4.2), 290 (4.2), 303 (4.24), 315 (4.3), 439 (4.3). IR ν_{max} (cm^{-1}, NaCl disc): 2950, 1690, 1554, 1412, 1330, 1250. MS m/z (% intensity): 305 (M^+, 87), 277 (100), 263 (10), 260 (21), 248 (20), 232 (2),

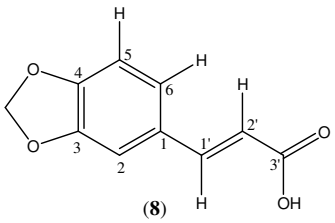
219 (12), 204 (3), 190 (14), 175 (4), 163 (34), 150 (11), 138 (48), 123 (7), 109 (16), 95 (22), 81 (30), 74 (13), 69 (1), 63 (5), 49 (3), 44 (6). ^{1}H NMR, δ (400 MHz, D-MeOH) and ^{13}C NMR, δ (100 MHz, D-MeOH): Table-2.

TABLE-2
ONE AND TWO-DIMENSIONAL NMR DATA FOR CEPHARADIONE A (**3**)

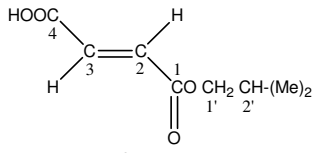
Position	^{1}H NMR (δ)	^{13}C NMR (δ)	HMQC (C-H correlation)	HMBC (C-H correlation)
1		151.38		8.06 (H-3) (^{3}J)
2		147.83		8.06 (H-3) (^{2}J)
3	8.06 (1H, s)	108.97	8.06 (H-3)	
3a		125.48		
4		174.68		
5		156.55		N-CH_3 (^{3}J)
6a		131.56		N-CH_3 (^{3}J)
				7.43 (H-7) (^{2}J)
7	7.43 (1H, s)	114.25	7.43 (H-7)	
7a		129.05		7.43 (H-7) (^{2}J)
				8.93 (H-11)(^{3}J)
8	7.82 (1H, m)	128.26	7.82 (H-8)	7.43 (H-7) (^{3}J)
9	7.62 (2H, m)	127.49	7.62 (H-9)	7.82 (H-8) (^{2}J)
10	7.62 (2H, m)	126.69	7.62 (H-10)	
11	8.93 (1H, m)	128.60	8.93 (H-11)	
11a		127.73		7.43 (H-7) (^{3}J)
				7.82 (H-8) (^{3}J)
11b		115.13		
11c		121.04		8.06 (H-3) (^{3}J)
				7.43 (H-7) (^{3}J)
O-CH_2-O	6.38 (2H, s)	103.03	6.38 (-CH_2)	
N-CH_3	3.77 (3H, s)	29.69	3.77 (-CH_3)	



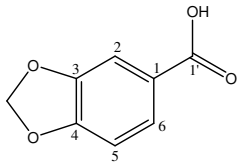
(**7**)



(**8**)



(**9**)



(**10**)

Fig. 2. Structures of carboxylic acids isolated from *Piper nigrum*

Piperine (4): Yellow crystalline solid with melting point 84-85 °C (Lit.[13] 85 °C). UV λ_{max} nm ($CHCl_3$, log ε): 344 (2.3), 310 (1.4), 278 (0.7), 222 (0.3), 216 (0.5). IR ν_{max} (cm^{-1}, NaCl disc): 2936, 2854, 1638, 1590, 1502, 1490, 1444, 1362, 1318, 1292, 1252, 1196, 1134, 1120, 1038, 998. MS m/z (% intensity): 285 (M^+, 60), 286 (2), 256 (2), 242 (1), 229 (1), 216 (0.3), 201 (86), 187 (2), 173 (61), 159 (10), 143 (46), 137 (14), 115 (100), 100 (6), 84 (28), 77 (3), 56 (8), 55 (12), 41 (23). ^{1}H NMR, δ (400 MHz, $CDCl_3$) and ^{13}C NMR, δ (100 MHz, $CDCl_3$): spectral data are in agreement with published data[13].

Sylvamide (5): Colourless needle crystals, m.p. 142-144 °C (Lit.[14] 143-144 ºC) UV λ_{max} nm (MeOH, log ε): 214 (4.15). IR ν_{max} (cm^{-1}, NaCl disc): 3282 (combination of broad, sharp peak, OH, NH), 2954, 1634 (C=O), 1558, 1084. MS m/z (% intensity): 257 (M^+, 0.2), 241 (02), 224 (01), 196 (0.1), 186 (5), 168 (2), 158 (100), 140 (2), 129 (8), 115 (2), 101 (9), 85 (92), 83 (85), 75 (25), 55 (78), 41 (78). ^{1}H NMR, δ (400 MHz, D-MeOH) 0.85 (4H, d, *J* = 6.44 Hz, H-3' & H-4'), 0.87 (3H, d, *J* = 7.32 Hz, H-10), 1.28 (6H, m, H-7, H-8, H-9), 1.52 (2H, m, H-6), 1.75 (1H, m, H-2'), 3.01 (2H, d, *J* = 7.36 Hz, H-1'), 3.45 (1H, m, H-5), 4.02 (1H, m, H-4), 6.13 (1H, dd, *J* = 1.84, 15.6 Hz, H-2), 6.81 (1H, dd, *J* = 4.56, 15.6 Hz, H-3). ^{13}C NMR, δ (100 MHz, D-MeOH) 14.39 (C-10), 20.49 (C-3', C-4'), 23.7 (C-9), 26.58 (C-7), 29.71 (C-2'), 33.05 (C-8), 33.74 (C-6), 48.00 (C-1'), 75.41 (C-5), 75.75 (C-4), 125.09 (C-2), 144.45 (C-3), 168.49 (C-1).

2,4-Tetradecadienoic acid isobutyl amide (6): Oily yellow crystals with melting point 88-90 °C (Lit.[15] 90 ºC). UV λ_{max} nm ($CHCl_3$, log ε): 240(1.2), 214 (1.4). IR ν_{max} (cm^{-1}, NaCl disc): 3250 (NH), 2950, 1655, 1554, 1410, 1340, 1255, 988. MS m/z (% intensity): 279 (M^+, 0.7), 259 (0.4), 235 (9), 223 (36), 208 (16), 192 (19), 180 (10), 164 (19), 151 (100), 138 (17), 123 (28), 110 (18), 96 (65), 81 (79), 67 (32), 55 (27). ^{1}H NMR, δ (400 MHz, $CDCl_3$) 0.92-0.86 (3H, m, H-14), 0.92- 0.86 (6H, m, H-3', H-4'), 1.33-1.25 (12H, m, H-8-H-13), 1.41 (2H, m, H-7), 1.81 (1H, m, H-2'), 2.14 (2H, q, *J* = 6.4, 7.36, 12.84 Hz, H-6), 3.15 (2H, t, *J* = 6.4, 12.84 Hz, H-1'), 5.76 (1H, brs, NH), 5.80 (1H, d, *J* = 14.86 Hz, H-2), 6.08 (1H, m), 6.13 (1H, m), 7.18 (1H, dd, *J* = 10.12, 14.86 Hz, H-3). ^{13}C NMR, δ (100 MHz, $CDCl_3$) 20.07 (C-14), 20.07 (C-3', C-4"), 22.42 (C-7), 28.42, 29.68, 31.31 (C-8, C-9, C-10, C-11, C-12, C-13), 28.56 (C-2'), 32.86 (C-6), 46.87 (C-1'), 121.75 (C-2), 128.17 (C-4), 141.17 (C-3), 143.07 (C-5), 166.41 (C-1).

Tetracosanoic acid, *p*-hydroxy-*m*-methoxycinnamate ester (7): Colourless needle crystals with melting point 78-80 °C (Lit.[16] 75 °C). UV λ_{max} nm (MeOH, log ε): 320 (0.4), 240 (0.3), 214 (0.4). IR ν_{max} (cm^{-1}, NaCl disc): 3424 (broad, OH), 2916, 2850, 2354, 1706, 1634, 1598, 1516, 1470, 1430, 1274, 1160, 1126, 1030, 980. MS m/z (% intensity): 560 (M^+, 0.7), 542 (1), 532 (0.4), 514 (3), 498 (1), 486 (2), 470 (1), 332 (0.1), 259 (0.1),

221 (0.4), 196 (2), 194 (27), 177 (53), 163 (2), 145 (14), 137 (8), 117 (9), 97 (11), 89 (11), 73 (32), 69 (51), 60 (44), 55 (100), 43 (90), 41 (78). ^{1}H NMR and ^{13}C NMR spectral data are in agreement with published data[16].

3,4-Methylenedioxycinnamic acid (8): Off-white colour crystals with melting point 198-200 °C (Lit.[17] 200 °C). UV λ_{max} nm (acetone, log ε): 316 (2.3), 224 (0.7) IR ν_{max} (cm^{-1}, NaCl disc): 3432 (broad, OH), 1678, 1608 (C=O), 1498, 1452, 1414, 1368, 1302, 1264, 1166, 1114, 1040. MS m/z (% intensity): 192 (M^+, 100), 175 (31), 165 (32), 145 (36), 135 (7), 117 (18), 105 (5), 89 (34), 77 (7), 73 (8), 63 (13), 53 (10), 51 (18), 50 (13). ^{1}H NMR and ^{13}C NMR spectral data are in agreement with published data[17].

2-Butenedioic acid, mono(2-methylpropyl) ester (9): Colourless needle crystals with melting point 164-165 °C. UV λ_{max} nm (MeOH, log ε): 215 (2.8). IR λ_{max} (cm^{-1}, NaCl disc): 3298, 2956, 1704, 1650, 1554, 1412, 1334, 1248, 988. MS m/z (% intensity): 172 (M^+, 10), 156 (50), 153 (32), 128 (78), 116 (90), 99 (100), 84 (7), 81 (40), 72 (59), 56 (78), 55 (86), 53 (71), 45 (70), 43 (75), 41 (93). ^{1}H NMR, δ (400 MHz, D-MeOH): 0.89 (6H, d, *J* = 6.44 Hz, H-3', H-4'), 1.77 (1H, m, H-2'), 3.06 (2H, d, *J* = 7.32 Hz, H-1'), 6.65 (1H, d, *J* = 15.12 Hz, H-3), 6.96 (1H, d, *J* = 15.12 Hz, H-2). ^{13}C NMR, δ (100 MHz, D-MeOH) 19.83 (C-3' & C-4'), 28.99 (C-2'), 48.36 (C-1'), 130.85 (C-3), 136.96 (C-2), 165.72 (C-1), 168.04 (C-4).

3,4-Methylenedioxy benzoic acid (10): Off-white crystals with melting point 223-225 °C. UV λ_{max} nm (MeOH, log ε): 322 (0.7), 245 (0.7). IR ν_{max} (cm^{-1}, NaCl disc): 3432 (broad, OH), 1670 (C=O), 1504, 1454, 1414, 1368, 1300, 1262, 1242, 1168, 1114, 1074, 1038, 932. MS m/z (% intensity):166 (M^+, 100), 149 (38), 119 (14), 91 (10), 74 (8), 65 (7), 63 (8), 53 (11), 50 (8). ^{1}H NMR, δ (400 MHz, D-MeOH): 5.94 (2H, s, O-CH_2-O), 6.79 (1H, d, *J* = 8.24 Hz, H-5), 7.31 (1H, d, *J* = 1.84 Hz, H-2), 7.55 (1H, d, *J* = 8.24 Hz, H-6). ^{13}C NMR, δ (100 MHz, D-MeOH): 103.31 (O-CH_2-O), 108.83 (C-5), 110.29 (C-2), 125.97 (C-1), 126.5 (C-6), 149.22 (C-3), 153.11 (C-4), 169.44 (C-1').

RESULTS AND DISCUSSION

Piperolactam A (**1**) was isolated as pale yellow crystals from the ethyl acetate and methanol extracts of the roots of *Piper nigrum*. The EI mass spectrum establishes the molecular formula for the compound to be $C_{16}H_{11}NO_3$, ($[M]^+$ m/z 265) and with a melting point of 300-301°C [Lit.[10] 303-306 °C]. The IR spectrum indicated the presence of OH (3474 cm^{-1}), NH (3188 cm^{-1}) and C=O (1656 cm^{-1}) groups.

The ^{1}H NMR spectrum of piperolactam A (**1**) in acetone-D_6 revealed only 9 out of the total 11 hydrogen because two hydrogen probably had exchanged with the deuterium from the solvent (NH and OH). Six rather low field aromatic proton signals at δ7.75 (1H, s), δ9.31 (1H, m), δ7.52

(1H, m), δ7.25 (1H, m), δ7.86 (1H, m) and δ7.15(1H, s) which were for H-2, H-5, H-6, H-7, H-8 and H-9, respectively were observed. These protons were assigned based on their correlations with their respective carbons in the HMBC spectrum. These correlations are summarized in Table-2 below. The ^{1}H-^{1}H COSY spectrum was also used to assign the relevant proton signals to their respective protons. COSY gave couplings between the signals at δ9.31 (H-5) and δ7.52 (H-6) and between the signal at δ7.25 (H-7) and δ7.52 (H-6). The H-5 proton signal appeared rather lowfield at δ9.31 (1H, m), which is a characteristic of H-5 of phenanthrenes[13]. The 3 proton singlet signal at δ4.09 was attributed to the aromatic methoxy group at C-4. The OMe proton signal (δ4.09) was seen to have a correlation with 149.05 (C-4) in the HMBC spectrum *via* a ^{3}J coupling. Thus, the OMe group was assigned to C-4. The ^{1}H NMR values are shown in Table-1. The ^{13}C NMR spectrum showed a total of 16 carbons. DEPT experiments indicated the presence of 9 quaternary, 6 methines and one methyl signal. The ^{13}C NMR values are given in Table-2. These assignments were carried out based on information from the HMQC spectrum. The compound was thus assigned to be piperolactam A (**1**) previously isolated from *Piper longum*[10].

Cepharadione A (**3**) was isolated from the methanol extract of *Piper nigrum*, as orange needle crystals; m.p. 253-355 °C (Lit.[12] 350 °C). This compound gave a positive response to dragendroff reagent. EIMS gave an M^{+} of 305 which is consistent with the molecular formula of $C_{18}H_{11}NO_4$. This alkaloid exhibited two carbonyl bands in its IR spectrum at 1690 and 1554 cm^{-1}.

The ^{1}H NMR spectrum indicated the presence of a methylenedioxy group which was assigned to the C-1 and C-2 position of the aporphine skeleton. Two one proton singlet at δ 8.06 (H-3) and δ 7.43 (H-7) were observed together with four one proton multiplet (δ 7.43, δ 7.61, δ 7.82 and δ 8.93) in the aromatic region. Two singlets at δ 6.38 (2H) and δ 3.77 (3H) were attributed to the methylenedioxy and N-methyl protons, respectively. In addition, a singlet at δ3.77 was attributed to N-methyl. The ^{1}H NMR values are listed in Table-2.

The ^{13}C NMR spectrum, showed the presence of 18 carbons. The ketone and amide carbonyl carbons in the dioxoaporphine resonated at δ174.68 and δ156.55, respectively. This structure was supported by the spectroscopic data from HMQC and HMBC experiments. A ^{3}J correlation was observed between δ151.38 (C-1) and δ8.06 (H-3). Meanwhile, the signal at δ8.06 (H-3) was correlated to the signal at δ147.83 which is C-2. Both C-1 and C-2 gave no HMQC correlations with any protons indicating they did not carry any protons. Hence, the OCH_2 group was assigned to C-1 and C-2. The chemical shifts of each carbon in the ^{13}C NMR spectra (Table-2) was in good agreement with those reported for 1,2-methylenedioxy-4,5-

dioxo-6-methyl-6a,7-dehydroaporphine or better known as cepharadione A (**3**)[18]. Table-2 summarized the ^{1}H, ^{13}C, HMBC and HSQC correlations observed for the compound cepharadione A (**3**).

The other alkaloids piperolactam D (**2**), piperine (**4**) sylvamide (**5**) and 2,4-tetradecadienoic acid isobutyl amide (**6**) and the carboxylic acids tetracosanoic acid, *p*-hydroxy-*m*-methoxycinnamate ester (**7**), 3,4-methylenedioxycinnamic acid (**8**), 2-butenedioic acid, mono(2-methylpropyl) ester (**9**) and 3,4-methylenedioxy benzoic acid (**10**) gave spectral data which are in agreement with published data.

The crude extracts and pure piperine from *Piper nigrum* L. indicated significant activities against the larvae of *Aedes aegypti*. The crude hexane, chloroform, ethyl acetate and methanol extracts of *Piper nigrum* showed very high toxicity towards the larvae by giving LC_{50} values of less then 4.0 µg/mL. The crude hexane extract gave an LC_{50} value of 0.45 µg/mL, the crude chloroform extract an LC_{50} value of 0.37 µg/mL, the crude ethyl acetate an LC_{50} value of 3.96 µg/mL while the methanol extract an LC_{50} value of 1.66 µg/mL. Pure piperine also exhibited a strong activity against the larvae with an LC_{50} value of 4.46 µg/mL. These extracts and piperine may be said to be potential larvicides against the larvae of *Aedes aegypti*.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Jegak Uli for collection of plant samples, Mr. Johadi Iskandar for recording NMR spectra and the Malaysian Government for financial support.

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(*Received*: 19 September 2007; *Accepted*: 20 June 2008) AJC-6631