

Alkaloids from *Piper nigrum* and *Piper betle*

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ABSTRACT

An investigation, on the roots of *Piper nigrum* and the aerial parts of *Piper betle*, has yielded several alkaloids. The dried root sample of *Piper nigrum* was extracted using various solvents in increasing polarity. The dried aerial part of *Piper betle* was extracted using the Soxhlet extraction method. The alkaloids isolated were pellitorine(1), (*E*)-1-[3',4'-(Methylenedioxy)cinnamoyl]piperidine(2), piperine(3), piperolactam D(4), cepharadione A(5), and 2,4-tetradecadienoic acid isobutyl amide(6). These compounds were isolated using chromatographic methods, while the elucidation of the structures was carried out using MS, IR and NMR techniques. The extracts of *Piper nigrum* and *Piper betle* were also tested for cytotoxicity activities. This is the first report on (*E*)-1-[3',4'-(Methylenedioxy)cinnamoyl]piperidine(2) from *Piper nigrum* as a natural product.

Keywords: *Piper nigrum*, *Piper betle*, alkaloids, cytotoxicity

INTRODUCTION

The genus *Piper* belongs to the Piperaceae family and it has over 700 species distributed in both hemispheres. The Piperaceae family is a source of many biologically active phytochemicals with great potential for medicinal and agricultural uses. Species in the genus *Piper* have a wide array of secondary metabolite compounds, particularly alkaloids and amides (Scott *et al.*, 2005). *Piper nigrum* is one of the more well-known species because of its high commercial, economical, and medicinal properties. It is known that *Piper nigrum* has biological activities such as CNS stimulant, analgesic, antipyretic and antifeedent activities (Miyakado *et al.*, 1979). Meanwhile, *Piper betle* possesses a variety of medicinal properties. The leaves of *Piper betle* can be used as a traditional remedy to treat stomach ailments and infections, as well as a general tonic. The leaves of *Piper betle* can also be used to stop bleeding by applying the leaves directly onto the wound. This paper reports the isolation of the alkaloid components pellitorine (1) and (*E*)-1-[3', 4'-(Methylenedioxy)cinnamoyl]piperidine (2), as well as the discovery of bioactive extracts from both plant samples being studied.

MATERIALS AND METHODS

Plant Material

The roots of *Piper nigrum* were collected from Sri Aman, in Sarawak, Malaysia. The aerial parts of *Piper betle* were obtained from Kedah, Malaysia.

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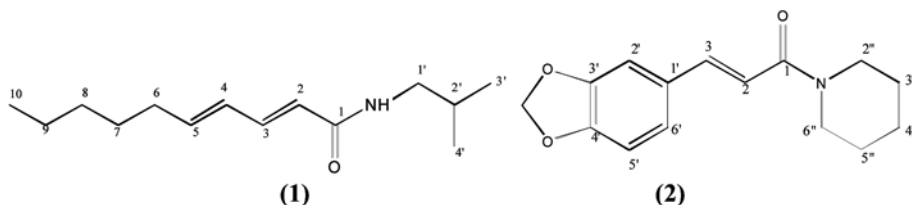
Infrared spectra were measured in NaCl pellet on a Perkin-Elmer FTIR Spectrum BX spectrometer. EIMS were recorded on a Shidmazu GCMS-QP5050A spectrometer. The NMR spectra were obtained using Unity INOVA 500 MHz NMR/ JEOL 400 MHz FTNMR spectrometer using tetramethylsilane (TMS) as internal standard. The ultra violet spectra were recorded in CHCl_3 on a Shidmazu UV-160A, UV-Visible Recording Spectrophotometer.

Extraction and Isolation

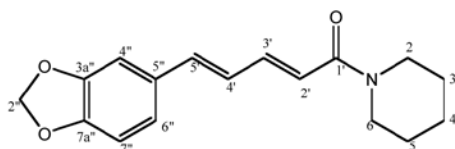
The dry powdered roots of *Piper nigrum* (4.8kg) were extracted repeatedly with distilled petroleum ether thrice, for seventy two hours at room temperature. This was followed by the extraction in chloroform and finally in ethanol. All the extracts were dried in vacuo to obtain crude extracts. The ethanol extract was then added to a large quantity of 5% aqueous hydrochloric acid. After that, the acidic solution was filtered using 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. Then, the chloroform extract was washed with distilled water and dried over anhydrous sodium sulphate. The acid-base treated ethanol extract was obtained by removing the solvent under reduced pressure. The extract after evaporation yielded an oily extract (0.53g). The crude extract was chromatographed over silica gel (120-230 mesh) column and fractions were collected in 100 ml aliquots. Fractions 10-26 gave pellitorine (**1**), and (*E*)-1-[3', 4'-(Methylenedioxy) cinnamoyl] piperidine (**2**). The dried sample of the aerial parts of *Piper betle* underwent the Soxhlet extraction using hexane, chloroform and methanol. All extracts were dried under reduced pressure.

Pellitorine (**1**). White crystals with melting point 60-62°C (Lit. 69°C, Rosario, *et al.*, 1996). UV (CHCl_3) λ_{max} nm (log ϵ): 292(1.87), 236(0.48), 212(0.53), 264(0.27), 220(0.31). IR ν_{max} cm^{-1} (NaCl): 3300 (N-H group), 2926, 2864, 1656 (C=O), 1624, 1550, 1460, 1368, 1260, 1160, 994. EIMS m/z (rel. int.): 223(43), 208(10), 180(7), 166(8), 151(100), 110(12), 96(55), 81(50), 67(21), 53(18). For NMR data, *see* Table 1).

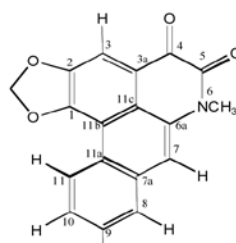
(*E*)-1-[3', 4'-(Methylenedioxy)cinnamoyl] piperidine (**2**). White crystals with melting point 74-76°C (Lit. 83°C, Schobert *et al.*, 2001). UV (CHCl_3) λ_{max} nm (log ϵ): 325(2.46), 235(0.82), 397(0.01), 251(0.60). IR ν_{max} cm^{-1} (NaCl): 3460, 2934, 2858, 1642, 1598, 1494, 1444, 1354, 1248, 1134, 1034, 978, 930, 810. EIMS m/z (rel. int.): 259(57), 175(73), 148(30), 145(100), 138(17), 117(33), 89(66), 84(86), 63(31). For NMR data, *see* Table 2).



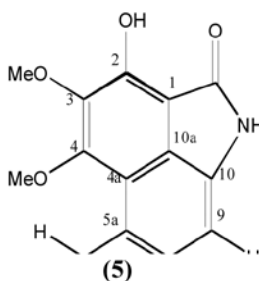
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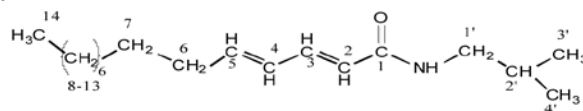
(3)



(4)



(5)



(6)

TABLE 1
NMR data for pellitorine (1)

Position	¹ H NMR, δ	¹³ C NMR, δ	HMBC
1	-	166.45	7.19 (H-3) (³ J), 5.79 (H-2) (² J), 3.16 (H-1') (³ J)
2	5.79 (1H, d, <i>J</i> =15.0Hz)	121.70	6.09 (H-4) (³ J)
3	7.19 (1H, dd, <i>J</i> =15.0,11.0Hz)	141.26	5.79 (H-2) (² J)
4	6.09 (1H, m)	128.16	2.13 (H-6) (³ J)
5	6.12 (1H, m)	128.16	2.13 (H-6) (² J)
6	2.13 (2H, m)	32.87	1.38 (H-7) (² J), 1.29 (H-8) (³ J)
7	1.38 (2H, m)	28.44	2.13 (H-6) (² J)
8	1.29 (2H, m)	31.31	1.38 (H-7) (² J), 2.13 (H-6) (³ J)
9	1.29 (2H, m)	22.42	0.89 (H-10) (² J), 1.29 (H-8) (² J)
10	0.89 (3H, m)	13.96	1.29 (H-9) (² J)
1'	3.16 (2H, dd, <i>J</i> =6.4, 12.8 Hz)	46.89	1.76 (H-2') (² J), 0.91 (H-3') (³ J), 0.93 (H-4') (⁴ J)
2'	1.76 (1H, m)	28.58	0.91 (H-3') (² J), 0.93 (H-4') (² J)
3'	0.91 (3H, d, 6.4 Hz)	20.08	1.76 (H-2') (² J), 3.16 (H-1') (³ J)
4'	0.93 (3H, d, 6.4 Hz)	20.08	1.76 (H-2') (² J), 3.16 (H-1') (³ J)
N-H	5.76 (br s)	-	-

TABLE 2
NMR data for (*E*)-1-[3',4'-(Methylenedioxy)cinnamoyl]piperidine (**2**)

Position	¹ H NMR, δ	¹³ C NMR, δ	HMBC
2''	3.57 (2H, br s)	43.30	1.58-1.69 (H-3'', H-4'') (² <i>J</i> , ³ <i>J</i>)
3''		25.56	1.58-1.69 (H-4'', H-5'') (² <i>J</i> , ³ <i>J</i>)
4''	1.58 - 1.69 (6H, m)	24.60	1.58-1.69 (H-3'', H-5'') (² <i>J</i>)
5''		26.69	1.58-1.69 (H-4'', H-3'') (² <i>J</i> , ³ <i>J</i>)
6''	3.64 (2H, br s)	46.92	1.58-1.69 (H-5'') (² <i>J</i>), (H-4'') (³ <i>J</i>)
1	-	165.38	7.03 (H-2') (² <i>J</i>), 7.54(H-3) (³ <i>J</i>)
2	7.03 (1H, d, <i>J</i> =13.3 Hz)	123.52	7.54 (H-3) (² <i>J</i>)
3	7.54 (1H, d, <i>J</i> =13.3 Hz)	141.89	6.97 (H-2') (³ <i>J</i>), 7.03 (H-2) (² <i>J</i>)
1'	-	129.87	6.78 (H-6') (² <i>J</i>), 7.54 (H-3) (² <i>J</i>)
2'	6.97 (1H br d, <i>J</i> =1.8 Hz)	106.27	7.54 (H-3) (³ <i>J</i>)
3'		148.12	5.98 (OCH ₂ O) (³ <i>J</i>), 6.97 (H-2') (² <i>J</i>), 6.71 (H-5') (³ <i>J</i>)
4'	-	148.76	5.98 (OCH ₂ O) (³ <i>J</i>), 6.97 (H-2') (³ <i>J</i>), 6.71 (H-5') (² <i>J</i>)
5'	6.71 (1H, br d, <i>J</i> =8.2 Hz)	115.63	
6'	6.78 (1H, br d, <i>J</i> =8.2 Hz)	108.41	7.54 (H-3) (³ <i>J</i>)
OCH ₂ O	5.98 (2H, s)	101.33	-

RESULTS AND DISCUSSION

Pellitorine (**1**), white crystals, and with molecular formula C₁₄H₂₅NO exhibited a parent molecular ion peak at *m/z* 223 in the EI-MS spectrum. The infrared spectrum showed a strong absorption band at 3300 cm⁻¹, which accounted for the NH group. Another strong absorption band, observed at 1656 cm⁻¹, belong to the C=O of the amide group. The ¹H NMR spectrum of pellitorine showed three doublet signals at δ5.79 (*J*=14.7 Hz) for H-2, 0.91 (*J*=6.4 Hz) for H-3' and 0.93 (*J*=6.4 Hz) for H-4'. Two multiplet signals, which appeared at δ 6.09 and 6.12, were due to the olefinic protons at C-4 and C-5. Meanwhile, a multiplet occurring at a very upfield region at δ1.76, was due to the proton at C-2', coupling with the adjacent protons in the isobutyl moiety. A broad singlet at δ5.76 was attributed to NH. The ¹³C NMR spectrum gave a total of 14 peaks and most of the carbon peaks appeared at the upfield region. The amide carbonyl carbon resonating at δ166.45(C-1) was further confirmed by DEPT spectra. A ³*J* correlation between δ166.45 (C-1) and δ3.16 (H-1') was observed and this validated the position of the isobutyl group. The location of olefinic protons was confirmed by a ³*J* correlation of the carbonyl carbon at δ166.45 with the proton

at δ 5.79. The chemical shifts of the proton and carbon NMR spectra were compared with the values in the literature, and all the values for the ^1H and ^{13}C NMR spectra were found to be in agreement. Hence, this compound was identified as deca-2*E*,4*E*-dienoic acid isobutyl amide, which is also known as pellitorine, previously isolated from *Cissampelos glaberrima* (Rosario *et al.*, 1996).

(*E*)-1-[3',4'-(Methylenedioxy)cinnamoyl]piperidine (**2**) was isolated as white crystals, and its molecular formula of $\text{C}_{15}\text{H}_{17}\text{NO}_3$ was determined by EIMS (m/z 259). The infrared spectrum showed absorptions at 3460 cm^{-1} and 1642 cm^{-1} , indicating the presence of NH and C=O. The ^1H NMR spectrum displayed signals for the equivalent methylene groups, α to the nitrogen atom at δ 3.57, which was for H-2" and δ 3.64 for H-6." Three downfield signals, observed at δ 6.97 (1H, brd, $J=1.8\text{ Hz}$), δ 6.71 (1H, brd, $J=8.2\text{ Hz}$) and δ 6.78 (1H, brd, $J=8.2\text{ Hz}$) were assigned to H-2', H-5' and H-6', respectively. The ^1H - ^1H correlation was determined using a COSY spectrum. The COSY gave 4J coupling between H-2' and H-6.' At the same time, the 3J coupling between H-2 and H-3 was also observed in the COSY spectrum. The ^{13}C NMR spectrum was assigned using a combination of the DEPT, HMQC and HMBC experiments. From the HMQC spectrum, no correlations were observed for C-1, C-1', C-3' and C-4'. Hence, these carbons are not attached to any protons and are quaternary carbons. Meanwhile, a total of 6 methylene carbons, 5 methine carbons and 4 quaternary carbons were observed from the DEPT spectrum. Long range couplings were also observed between the cinnamoyl carbonyl carbon at δ 165.38 and protons H-3 and H-2. C-4' was found to correlate with the $-\text{OCH}_2\text{O}-$ group protons. A 2J correlation was observed between C-1 and H-2. Moreover, a 3J correlation between C-1 and H-3 was observed in the HMBC spectrum. All the ^{13}C NMR and ^1H NMR values are given in Table 2. As a result, this compound was assigned as (*E*)-1-[3',4'-(Methylenedioxy)cinnamoyl]piperidine(**2**), which was previously synthesized by Schobert *et al.* (2001).

The structures of the other four alkaloids were determined by making comparisons of the spectral data with that of the published data - piperine(**3**) (Park *et al.*, 2002), piperolactam D(**4**) (Olsen *et al.*, 1993), cepharadione A(**5**) (Desai *et al.*, 1988; Wijeratne *et al.*, 1995) and 2,4-tetradecadienoic acid isobutyl amide(**6**) (Greger *et al.*, 1981).

The extracts of *Piper nigrum* and *Piper betle* were also tested for cytotoxic activities. For this purpose, the extracts of *Piper nigrum* were tested on HL60 (Human promyelocytic leukemia cells). The petroleum ether and chloroform extracts were bioactive against HL 60 cell line, with a high inhibitory concentration of less than $30\text{ }\mu\text{g/ml}$. The ethyl acetate extract gave no activity. The petroleum ether extract gave an IC_{50} value of $11.2\text{ }\mu\text{g/ml}$, and the IC_{50} value of chloroform extract was found to be $9.8\text{ }\mu\text{g/ml}$. Meanwhile, the crude extracts of *Piper betle* were tested on HeLa cell line (the human epithelial cells derived from the cervical cancer cells). The crude hexane extract gave an IC_{50} value of $17.6\text{ }\mu\text{g/ml}$, while the chloroform and methanol extracts were found to be not bioactive towards the HeLa cell line.

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