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Research Article

PHYTOCHEMICAL STUDY OF *CANANGA ODORATA* (LAM) HOOK.F. & THOMSON & THOMS (ANNONACEAE)

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ABSTRACT

A phytochemical study of the leaves of *Cananga odorata* (Lam) Hook.F. & Thomson (Annonaceae) yielded four compounds, comprising of two steroids, β-sitosterol (**1**) and stigmasterol (**2**), and two oxoaporphine alkaloids: liriodenine (**3**) and lysicamine (**4**). The compounds were isolated using various chromatographic methods and structural elucidation was accomplished by spectroscopic techniques (i.e NMR, MS, UV and IR) and comparison with literature values. The structure elucidation of the alkaloids using high field NMR (500 MHz) is reported for the first time.

Keywords: *Cananga odorata*, Annonaceae, Leaves, Steroids, Alkaloids.

INTRODUCTION

Cananga is a small genus, belonging to the family Annonaceae and consists of only two species viz. *Cananga odorata* and *Cananga latifolia* that are distributed throughout the tropical and subtropical regions of the world[1,2]. *Cananga odorata* (Lam) Hook.F. & Thomson (Annonaceae) is an evergreen tree that can grow up to 100 feet. It is widespread in the lowland forests in Malaysia and has been grown as a garden tree particularly for its attractive fragrant flowers. In Malaysia, *C. odorata* is locally known as kenanga, chenanga and ylang-ylang. Traditionally, the plant parts have been used for the treatment of malaria, asthma and scabies[1,3]. Previous phytochemical works have been carried out on the leaves and seeds of *C. odorata*, resulting to the isolation of (+)-ushinsunine-β-*N*-oxide, cleistopholine, liriodenine, (-)-anonaine, (+)-nornuciferine, (+)-*N*-acetylnornuciferine, (-)-ushinsunine, (-)-nornuciferine, (-)-asimilobine, (+)-reticuline, *N*-trans-feruloyltyramine, β-sitosterol, stigmasterol, lyscamine, (-)-anaxagoreine, trans-cinamic acid, isosiphonodin and canangone[4,6]. On the other hand, the fruits have been found to contain cananodine, cryptomeridiol-11-α-L-rhamnoside, γ-eudesmol-11-α-*L*-rhamnoside and γ-eudesmol, isosiphonodin[7]. The flowers of *C. odorata* contained essential oils, mainly monoterpenes and sesquiterpenes such as α-cubebene, β-caryophyllene, α-humulene, β-elemene, geraniol, α-pinene oxide, globulol, cedrol, elemol and β-bisabolol[8]. This paper deals with the isolation and identification of four compounds from the leaves of *C. odorata*, comprising of steroids and alkaloids. In total, two steroids were isolated, β-sitosterol **(1)** and stigmasterol **(2)**, in addition to two oxoaporphine alkaloids, namely liriodenine **(3)** and lysicamine **(4)**. All the compounds were identified mainly based on their NMR (500 MHz) spectral data using various 2D techniques and by comparison with the literature data.

MATERIALS AND METHODS

General experimental procedures

The UV spectra were obtained on a Shimadzu UV-160A spectrophotometer. The IR spectra were measured on Perkin Elmer RX I FT-IR spectophotometer. The mass spectra were recorded on Direct Induction Probe (DIP) using a Shimadzu GCMS-QP5050 spectrometer with ionization induced by electron impact at 70 eV. ^{1}H-NMR and ^{13}C-NMR spectra were recorded using a Varian SCNMR VXR 500, operating at 500 and 125 MHz, respectively. The NMR spectra were obtained in $CDCl_3$ with chemical shifts expressed in δ and coupling constant (*J*) in Hertz with tetramethylsilane (TMS) as an internal standard.

Plant material

The leaves of *Cananga odorata* were collected in 2007 from Ulu Bendul Forest, Negeri Sembilan, and a voucher specimen (SM 858) was deposited at the Pharmacognosy Laboratory, Faculty of Pharmacy, Universiti Kebangsaan Malaysia.

Extraction and isolation

The air-dried leaves (0.4 kg) of *Cananga odorata* were ground. The powder was sequentially extracted twice with *n*-hexane by soaking at least 48 hours at room temperature for each time. Subsequent extractions were conducted using ethyl acetate and methanol in a similar manner. The resultant extracts were filtered and evaporated to dryness *in vacuo* to yield crude extracts of hexane (5.1 g), ethyl acetate (20.1 g) and methanol (10.4 g). The crude ethyl acetate extract (20 g) was subjected to flash chromatography (silica gel, Merck 230-400 mesh ASTM) and eluted in a polarity gradient manner with hexane, enriched with increasing percentages of ethyl acetate and methanol. Every 250 ml eluents were collected and these were combined according to their TLC pattern to afford six major combined fractions. Further isolation was performed on fraction 1 by column chromatography, eluted with solvent mixtures of hexane and ethyl acetate to yield β-sitosterol (**1,** 10.0 mg) and stigmasterol (**2**, 10.0 mg). Fraction 3 was further separated by column chromatography and eluted with mixtures of dichloromethane and chloroform with gradual increase in ethyl acetate and methanol to give 30 fractions of 25-ml each. Then, fractions 10-13 were combined and further separated with column chromatography using n-hexane-EtOAc (7:3), yielding liriodinine (**3**, 7.8 mg). Fraction 4 from the flash chromatography was rechromatographed on a column chromatography (sephadex LH 20 using $CHCl_3$:MeOH (4:1)) to afford lysicamine (**4**, 2.8 mg) The spectral data of **1-4** were in agreement with those reported in the literature.

β-Sitosterol (1)

The compound was obtained as colorless needle-shaped crystals with R_f value of 0.75 (30% ethyl aceatate:n-hexane). IR (KBr) ν_{max} cm^{-1}: 3433, 2960, 2866, 1464, 1381, 1053; EIMS *m/z* (relative intensity %): 414 ([M]$^+$, 45), 396 (11), 329 (15), 303 (6), 273 (11), 255 (21), 171 (22), 159 (37), 145 (46), 143 (39), 105 (65), 91 (100), 79 (62); ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.69 (3H, *s*, H-18), 0.82 (3H, *s*, H-29), 0.83 (3H, *d*, *J* = 4 Hz, H-26), 0.86 (3H, *s*, H-27), 0.93 (3H, *d*, *J* = 6.5, H-21), 1.02 (3H, *s*, H-19), 3.53 (*m*, H-3), 5.36 (1H, *br s*, H-6)[4,5].

Stigmasterol (2)

The compound was obtained as colorless needle-shaped crystals with R_f value of 0.75 (30% ethyl aceatate:n-hexane). IR (KBr) ν_{max} cm^{-1}: 3433, 2960, 2866, 1464, 1381, 1053; EIMS *m/z* (relative intensity %): 412 ([M]$^+$, 6), 396 (11), 329 (15), 303 (6), 273 (11), 255 (21), 171 (22), 159 (37), 145 (46), 143 (39), 105 (65), 91 (100), 79 (62); ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.69 (3H, *s*, H-18),

0.82 (3H, *s*, H-29), 0.83 (3H, *d*, *J* = 4 Hz, H-26), 0.86 (3H, *s*, H-27), 0.93 (3H, *d*, *J* = 6.5, H-21), 1.02 (3H, *s*, H-19), 3.53 (*m*, H-3), 5.04 (1H, *dd*, *J*=15.5, 8.5 Hz, H-23), 5.18 (1H, *dd*, *J*= 15.5, 8.5 Hz, H-22), 5.36 (1H, *br s*, H-6)[4,5].

Liriodenine (3)

The compound was obtained as yellow needle-shaped crystals with R_f value of 0.50 (50% ethyl aceatate:chloroform). IR (KBr) ν_{max} cm^{-1}: 1737. UVλ_{max} nm (log ε), MeOH: 260, 280, 338 nm. EIMS *m/z* (relative intensity %): 275 ([M]$^+$, 100), 247 (21), 219 (11), 188 (20), 161 (9).^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.90 (1H, *d*, *J* = 5.5 Hz, H-5), 8.67 (1H, *d*, *J* = 8.0 Hz, H-11), 8.60 (1H, *d*, *J* = 8.0 Hz, H-8), 7.76 (1H, *d*, *J* = 7.5 Hz, H-10), 7.59 (1H, *t*, *J* = 15.5 Hz, H-9), 7.20 (1H, *s*, H-3) 6.38 (2H, *s*, -OCH_2O-),^{13}C-NMR (125 MHz, $CHCl_3$): 180.0 (C-7), 152.5 (C-2), 147.3 (C-1), 146.4 (C-3a), 145.3 (C-5), 135.1 (C-6a), 134.0 (C-10), 132.8 (C-11a), 131.1 (C-7a), 129.1(C-9), 128.4 (C-8), 127.4 (C-11), 124.1 (C-4), 123.3 (C-1b), 103.3 (C-3), 102.0 (O-CH_2-O)[4].

Lysicamine (4)

The compound was obtaind as yellow needle-shaped crystals with R_f value of 0.50, (0.5% methanol:chloroform). IR (KBr) ν_{max} cm^{-1}: 1652. UV λ_{max} nm (log ε), MeOH: 238 , 268. EIMS 70 eV, *m/z* (rel. int.): 291 ([M$^+$], 100), 266 (62), 248 (86), 233 (22), 177 (30), 149 (32). ^{1}H-NMR ($CDCl_3$, 500 MHz): 9.19 (1H, *dd*, *J*=5.5, 0.7 Hz, H-11), 8.92 (1H, *d*, *J*=5.0 Hz, H-5), 8.61 (1H, *dd*, *J*=5.6, 0.7 Hz, H-8), 7.84 (1H, *d*, *J*=5.0 Hz, H-4), 7.78 (1H, *ddd*, *J*=8.4, 8.4, 1.4 Hz, H-10), 7.60 (1H, *ddd*, *J*=8.4, 8.4, 1.4 Hz, H-9), 7.24 (1H, *s*, H-3), 4.10 (3H, *s*, 1-OCH_3), 4.02 (3H, *s*, 2-OCH_3). ^{13}C-NMR ($CDCl_3$, 125 MHz): 182.9 (C-7), 157.5 (C-1), 152.8 (C-2), 145.4 (C-6a), 144.9 (C-5), 135.5 (C-3a), 134.4 (C-11a), 134.2 (C-10), 132.2 (C-7a), 129.0 (C-8), 128.6 (C-9), 128.4 (C-11), 123.8 (C-4), 122.3 (C-11c), 120.0 (C-11b), 106.6 (C-3), 60.2 (2-OCH_3), 56.3 (1-OCH_3)[4,9].

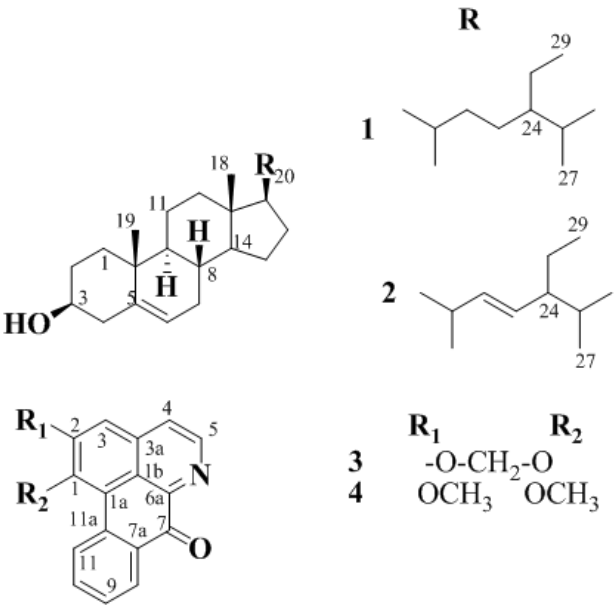

Fig. 1: Structures of isolated compounds

RESULTS AND DISCUSSION

The crude ethyl acetate extract from the leaves of *Cananga odorata* was fractionated using a column chromatography on silica gel to afford several fractions. Purifications were carried out on these fractions by repeated column chromatography, yielding four pure compounds. The compounds were identified by spectroscopic methods, i.e. NMR, MS, UV and IR, and compared with literature data.

β-sitosterol (**1**) and stigmasterol (**2**) are two steroidal compounds reported to occur in many plants in the world. Liriodenine (**3**) is ubiquitous in most Annonaceous plants especially in *Cananga* species. The other compound has been identified as lysicamine (**4**). The alkaloids belong to the oxoaporphine skeleton whose structures are known but their high field NMR (500 MHz) spectral data are reported here for the first time.

Compounds (**3**) and (**4**) exhibited intense yellow fluorescent coloration in solution, indicating the characteristic of an oxoaporphine chromophore. Compound (**3**) gave UV spectrum that displayed the typical maxima at 260, 280, 338 nm, while compound (**4**) absorbed at 238 and 268 nm, suggesting the presence of highly conjugated aromatic system[10,11]. The IR spectrum of compounds (**3**) and (**4**) showed the presence of carbonyl groups based on the strong broad band absorption at 1737 and 1658 cm^{-1}, respectively.

The EIMS of compounds (**3**) and (**4**) showed molecular ions at *m/z* 275 and *m/z* 291, corresponding to the formulae of $C_{17}H_9NO_3$ and $C_{18}H_{13}NO_3$, respectively. Based on the ^{1}H and ^{13}C-NMR spectra, compounds (**3**) and (**4**) were very similar to each other except for the presence of a methylenedioxy group in compund (**3**) and two methoxy moieties in compound (**4**).

In the ^{1}H-NMR spectrum of (**3**), the two singlets at δ 7.20 and 6.38 were assigned to H-3 and the methylene protons of a methylenedioxy group (O-CH_2-O), respectively, and a doublet at δ 8.90 ppm (*J*=5.5 Hz) showed the presence of H-5. In the aromatic region, the three doublets at δ 8.60 (*J*=8.0 Hz), 7.76 (*J*=7.5 Hz) and 8.67 (*J*=8.0 Hz) ppm were assigned to H-8, H-10 and H-11 protons, respectively, while the triplet at δ 7.59 ppm (1H, *t*, *J*=15.5 Hz, H-9) was due to H-9. The ^{13}C-NMR spectrum of (**3**) indicated the presence of one methylene, seven methines and eight quartenary carbons. These spectroscopic properties were similar to those of the known compound liriodenine[4,12].

The ^{1}H spectrum of (**4**) revealed a total of seven aromatic protons and two aromatic methoxyl groups at δ 4.10 (3H, s) and 4.02 (3H, s) ppm. A typical AB quartet of the two doublets at δ 8.92 and 7.84 ppm that had a coupling constant *J*=5.0 Hz were assigned to H-5 and H-4 of oxoaporphine skeleton. In the HMBC spectrum also showed appropriate three-bond correlation between H-4 (δ 7.84) and the carbon at 106.6 (C-3) and 122.3 (C-11c) ppm, while the H-5 (δ 8.92) showed a three-bond correlation to the carbon at 135.5 (C-3a) and 145.4 (C-6a) ppm. Another four signals of aromatic proton centered at δ 9.19 (*dd*, *J*=5.6, 0.7 Hz, H-11), 8.61 (*dd*, *J*=5.6, 0.7 Hz, H-8), 7.78

(*ddd*, *J*=8.4, 8.4, 1.4 Hz, H-10) and 7.60 (*ddd*, *J*=8.4, 8.4, 1.4 Hz, H-9) ppm were assigned as an ABMX system characteristic of 1,2-disubstituted benzene nucleus of ring D. The remaining signals of (**4**) are also indicated for one aromatic proton, resonating as a singlet at δ 7.24 ppm (H-3) and two sets of three proton singlets (3H) for an aromatic methoxyl at δ 4.10 and 4.02 ppm. The presence of this singlet aromatic proton (δ 7.24) showed ^{2}J correlation with an oxyquartenary carbon at δ 152.8 ppm (C-2) and ^{3}J correlation with two quartenary carbon at δ 122.3 (C-11c) and δ 157.5 (C-1) ppm, which then defined the position of two methoxyl groups at C-2 and C-1, respectively. Meanwhile, a connection between C-6a (δ 145.4 ppm) and C-11b (δ 120.0 ppm) was consistent with deshielding effect of the carbonyl group at C-7 (δ 182.9 ppm) on the doublet proton at δ 8.93 ppm (H-5) and one of methoxyl proton at δ 4.10 ppm (1-OCH_3). Therefore, compound (**4**) is an oxoaporphine-type of alkaloid with two methoxyl groups which are located in the A ring at C-1 and C-2, respectively. Considering all these spectral data the structure lysicamine (**4**) was assigned to this compound.

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