Chemical Constituents of the Insecticidal Active Extract of *Tinospora crispa*

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ABSTRACT

Tinospora crispa Miers (Menispermaceae) is a climbing vine with stems rich in warts. The plant is called Akar Seruntum or Patawali in Malaysia and is widely used for treating skin complaints, malaria, bacterial abscess, high blood pressure and diabetes. In the present study, the stems of T. crispa were collected from the locality and succesively extracted with petroleum ether, followed by chloroform and ethanol. The insecticidal active extract (ethanol extract) was subjected to column chromatography of silica gel eluted with a gradient mobile phase containing hexane, chloroform and ethanol. Among the chemical constituents isolated are n-tetracosyl trans-ferulate and n-octacosyl alcohol, along with three known aporphine alkaloids; N-formylnornuciferine, N-acetylnornuciferine and lysicamine. All compounds were identified by comparing their spectroscopic data (UV, IR, ¹H NMR, MS) with data from corresponding values in the literature. Isolation of n-tetracosyl trans-ferulate and n-octacosyl alcohol is reported the first time for T. crispa.

Keywords: n-octacosyl alcohol, n-tetracosyl trans-ferulate, Tinospora crispa, aporphine alkaloid

Introduction

Tinospora crispa, Miers (Figure 1) is one of the essential plants used in traditional medicine throughout Southeast Asian countries. In fact, the genus (*Tinospora*) is widely used in most areas in Asia and Africa [1]. The plant belongs to the family of Menispermaceae, which consists of about 70 to 80 genera, mostly found in tropical or almost tropical climate. *T. crispa* is found in Asia starting from Southwest of China to Southeast Asia such as Vietnam, Thailand, Malaysia, Indonesia, Philippines and India [2].

It is a sun-loving plant but it can tolerate a certain degree of shade. This plant grows in prime rainforest at a height that is not exceeding 1000 m. In Malaysia, *T.crispa* is usually planted at home to be applied as gate. The young stem is very succulent and it is green in colour. It gradually changes to dark grayish-brown as it matures. A mature shoot has a diameter of about 1.0 – 1.5 cm. It is fleshy and the surface is warty, with long filiform aerial roots hanging down from the branches. The stem climbs around other plants support to reach for the sunlight [3].

Previous studies on *Tinospora* species have revealed the presence of several clerodane diterpenes, clerodane diterpene glucosides, steroids, lignans, flavanoids and alkaloids [4]. Chemical investigation of the stems of *T. crispa* have indicated the presence of quaternary alkaloids including barberine (0.0067-0.0750%), palmatine (0.0014%), jatrrorrhizine (0.004%), tembetarine (0.019%) and choline (0.29%) [2]. Pachalay *et al.* reported that the extract also contain aporphine alkaloids; tinocrispicine, N-formyl-anonaine and N-acetylnornuciferine [5]. Protoberberine alkaloids berberine and palmatine exhibit a wide range of pharmacological effects including anti-inflammation, antibacterial, anti-protozoal and analgesic activities [6].

Figure 1: The leaves and stems of *Tinospora crispa*

Preliminary screenings of biopesticidal properties of *T. crispa* showed that the ethanol extract of *T. crispa* possessed potent insecticidal properties against flea beetles and small mottled willow moth [7]. The present study was therefore undertaken to gain further information on the chemical constituents of the ethanol extract as a potential biopesticide.

Material and Methods

General Experimental Procedures

Melting points were recorded on a Gallenkamp (England) melting point apparatus and were uncorrected. UV and IR spectra were obtained on Hitachi U-2000 spectrophotometer and Nexus FT-IR, respectively. ^{1}H-NMR and ^{13}C-NMR were recorded on Bruker 400 MHz and 300 MHz respectively, using $CDCl_3$ as solvent. GC-MS spectra were obtained using Agilent 5793N/6980 GC-MS system. TLC analysis of the compound was performed on pre-coated silica gel plates (F254, 0.2 mm, Merck) and used routinely for monitoring chromatographic fractions. TLC spots were detected by spraying

with Dragendorff's reagent. Dry-column flash chromatography with silica gel (Art No. 7730, Merck) was used to fractionate the crude extract into fractions. The fractions were rechromatographed on gravity silica gel (Art No. 9385, Merck) column for further isolation process. Semi-preparative column (Whatman Partisil-10 ODS-3, 9.4 × 250 mm) was used for HPLC analysis. The HPLC system consisted of a Waters 510 (MILLIPORE) delivery pump connected to a Rheodyne 7125 (USA) containing 2.0 mL sample loop. The peaks for the fraction were recorded using a Waters 486 UV/VIS detector monitored at 270 nm and the signals from the detector were analyzed with a HITACHI D-2500 chromato-integrator.

Reagents

Acetonitrile (LiChrosolv grade) was purchased from Merck, Germany. Methanol (HPLC grade) was purchased from BDH Supplies, England. Freshly prepared distilled water was used for HPLC analysis. $CDCl_3$ for NMR analysis and Dragendorff's reagent were purchased from Sigma-Aldrich Co., USA.

Plant Materials

The stem of *T. crispa* were collected from the locality of Parit Buntar, Perak, Malaysia and identified by comparison with example of existing specimens in the herbanium, School of Pharmaceutical Science, USM (serial number 011). Fresh plant sample (25 kg) were cleaned and cut into small pieces (± 1 cm) and dried in the laboratory of Chemistry, UiTM Cawangan Pulau Pinang under shaded area.

Extraction and Isolation

The air-dried *T. crispa* stems (4.4 kg) were ground into powder and then macerated for 18 hours with petroleum ether (3 × 12.5 L) in a hot water bath (60-70ºC). The petroleum ether solution was filtered and concentrated using rotary evaporator to give crude petroleum ether extract (62.84 g). The marc obtained after petroleum ether extraction, was further macerated for 18 hours with chloroform (3 × 12.5 L) and followed by ethanol (18 hours, 3 × 12.5 L). The solution was filtered and concentrated in vacuum to give the crude chloroform extract as a dark solid (138.25 g) and crude ethanol extract (457.55 g). The crude ethanol extract (10.0 g) was subjected to a

dry-column flash chromatography on silica gel (70 g) [8]. The column was eluted with hexane, followed by a gradient mobile phase containing a mixture of hexane, chloroform and ethanol; and finally with methanol which afforded 22 fractions of 100 mL each. The fractions collected were then combined into four fractions (F1-F4) based on the similarity of the TLC profiles. Fraction F1 which was a colourless solution, was concentrated to afford white solid, *n*-tetracosyl *trans*-ferulate (**1**, 9 mg). Fraction F2, also a colourless solution was concentrated to give another white solid, n-octacosyl alcohol (**2**, 15 mg). Fraction F3 (1.2 g) was subjected to silica gel column chromatography (100 g) and eluted with gradient mobile phase consisting a mixture of chloroform-ethanol to give 20 subfractions. Subfractions F_{12}-F_{13} (0.1 g) was rechromatographed on silica gel (20 g) column chromatography, eluted with petroleum ether-ethyl acetate-methanol (35:60:5) to give five combined fractions. Fraction 3 which was a colourless solution, gave F3B1 (50.2 mg) upon evaporation of sovent. F3B1 (25 mg) was further separated by semi-preparative reverse phase HPLC (acetonitrile-water 40:60, flow rate = 4.0 ml/min) yielding N-formylnornuciferine (3) (Rt 43.07 minute, 18.9 mg) and N-acetylnornuciferine (**4**) (Rt 48.11 minute, 4.4 mg). The samples collected were concentrated in vacuo at 50 ºC. Fraction 4, which was a yellowish solution, was concentrated to afford lysicamine (**5**, 2.5 mg).

Physical and Spectral Data

n-tetracosyl *trans*-ferulate (**1**)
Colourless needles, mp. 58-60ºC, IR (KBr) ν_{max} cm^{-1}: 3419 (OH), 2915, 2844 (-CH), 1715 (C=O ester), 1600, 1518 (C=C) and 1039 (-CO). EIMS *m/z*: 530 (M$^+$, 100%), 515 ((M-CH$_3$)$^+$, 50.3%). ^{1}H-NMR (400 MHz, CDCl$_3$) δ ppm: 0.81 (3H, t, *J=6.7 Hz*, CH$_3$), 1.18 (many H, br, s, (CH$_2$)$_n$), 1.60 (2H, t, *J*=6.7 Hz, OCH$_2$-CH$_2$), 3.86 (3H, s, OCH$_3$), 4.10 (2H, t, *J*=6.7 Hz, OCH$_2$), 5.77 (1H, s, OH), 6.22 (1H, d, *J*=16.0 Hz, H-2), 6.85(1H, d, *J*=8.1 Hz, H-5'), 6.96 (1H, s, H-2'), 6.97 (1H, d, *J*=8.1 Hz, H-6'), 7.54 (1H, d, *J*=16.0 Hz, H-3); ^{13}C-NMR (300 MHz, CDCl$_3$): 14.5 (CH$_3$), 23.0, 26.1, 29.2, 29.9, 30.0 ((CH$_2$)$_n$), 27.9 (OCH$_2$-CH$_2$), 56.3 (OCH$_3$), 65.0 (OCH$_2$), 109.7 (C-2'), 115.1 (C-5'), 116.1 (C-2), 123.4 (C-6'), 129.0 (C-1'), 146.0 (C-3), 148.5 (C-4'), 151.0 (C-3'), 168.0 (C-1).
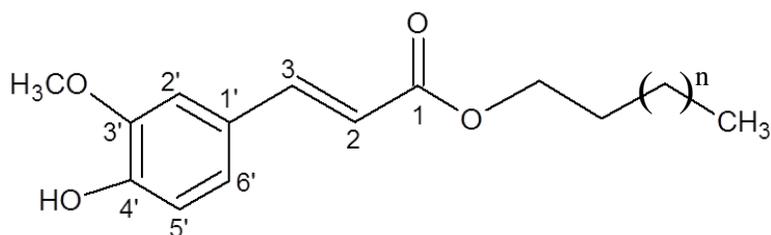
n-tetracosyl *trans*-ferulate (**1**)   n=21

n-octacosyl alcohol (**2**)
White flakes from chloroform, mp. 76-78°C; IR (KBr) cm^{-1}: 3315 (OH), 2918, 2848, 1473, 1462 (-CH) and 1063 (C-OH); EIMS *m/z* : 392 (M-H$_2$O)$^+$, 336; ^{1}H NMR (400 MHz, CDCl3) δ ppm: 0.81 (3H, t, *J*= 6.5 Hz), 1.19 (*br, s*), 1.50 (2H, m), 3.57 (2H, t, *J*=6.6 Hz); ^{13}C NMR (300 MHz, CDCl$_3$) δ ppm : 14.12, 22.71, 25.76, 29.38, 29.46, 29.63, 31.95, 32.85 and 63.13.

$$CH_3\text{-}(CH_2)_n\text{-}CH_2\text{-OH}$$ n-octacosyl alcohol (**2**)   n=26
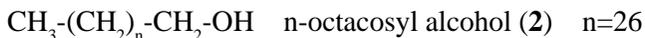
N-formylnornuciferine (**3**)
Colourless needles, mp. 141-143°C, IR (KBr) ν_{max} cm^{-1}: 1666 (C=O), UV λ_{max} (EtOH): 235, 270, 248(valley). EIMS *m/z*: 309 (M$^+$), 264, 251, 237, 178, 165. ^{1}H-NMR (400 MHz, CDCl$_3$) δ ppm: 2.75-2.95 (7H, *m*, H-4, H-7 eq (E, Z); H-7ax, Z), 3.14 -3.25 (2H, *m*, H-5ax, H-7ax, E), 3.40 (1H, *ddd*, *J*=12.0, 12.0, 3.0 Hz, H-5ax, Z), 3.69 (6H, *s*, C$_1$-OCH$_3$, E, Z), 3.85 (1H, *m*, H-5eq, Z), 3.93 (6H, s, C$_2$-OCH$_3$, *E, Z*), 4.41-4.53 (2H, *m*, H-6a, H-5eq, E), 4.95 (1H, *dd, J*=13.6, 4.0 Hz, H-6a, Z), 6.68 (1H, s, H-3, *Z*), 6.71 (1H, s, H-3, *E*), 7.28-7.35 (6H, *m*, H-8, H-9, H-10, *E, Z*), 8.28 (2H, s, CHO, *E, Z*), 8.42 (1H, d, *J*=11.8 Hz, H-11, *Z*), 8.44 (1H, *d, J*=14.0 Hz, H-11, *E*). ^{13}C-NMR (300 MHz, CDCl$_3$): Table 1.

N-acetylnornuciferine (**4**)
Colourless needles, mp. 229-231°C, IR (KBr) ν_{max} cm^{-1}: 1633 (C=O), UV λ_{max} (EtOH): 231, 272, 246(valley). EIMS *m/z*: 323 (M$^+$), 264, 251, 237, 178, 165. ^{1}H-NMR (400 MHz, CDCl$_3$) δ ppm: 2.19 (3H, *s*, COCH$_3$, E), 2.25 (3H, s, COCH$_3$, *Z*), 2.65-2.95 (6H, m, H-4, H-7eq, *E, Z*), 2.78 (1H, d, J=14.3 Hz, H-7ax, Z), 3.06-3.31 (2H, m, H-5ax, H-7ax E), 3.33 (1H, t,

J=12.0 Hz, H-5ax, Z), 3.69 (6H, s, C_1-OCH$_3$, E, Z), 3.93 (6H, s, C_2-OCH$_3$, E, Z), 4.03 (1H, d, J=13.0 Hz, H-5eq, Z), 4.58 (1H, d, J=15.0 Hz, H-6a, E), 5.00 (1H, d, J=10.0 Hz, H-5eq, E), 5.12 (1H, d, J=14.3 Hz, H-6a, Z), 6.65 (1H, s, H-3, Z), 6.72 (1H, s, H-3, E), 7.29-7.38 (6H, m, H-8, H-9, H-10, E, Z), 8.44 (1H, d, J=7.8 Hz, H-11, Z), 8.48 (1H, dd, J=11.0, 3.4 Hz, H-11, E). ^{13}C-NMR (300 MHz, CDCl$_3$) : Table 1.
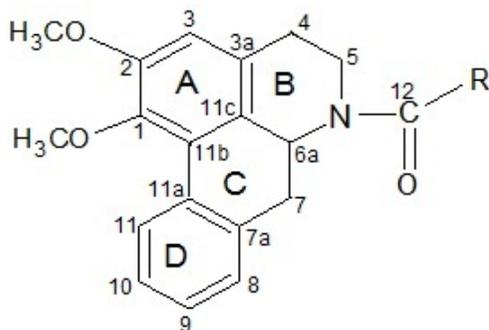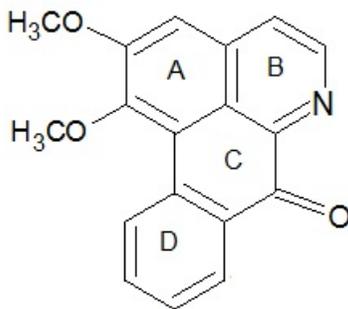

Z isomer	**3**$_Z$	R = H
	4$_Z$	R = CH$_3$
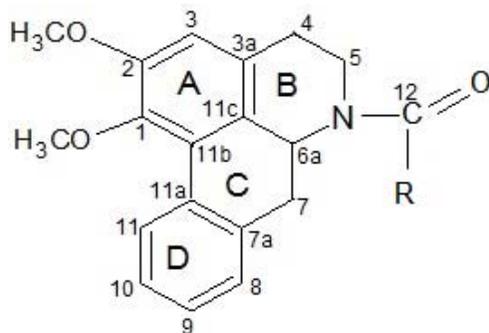
E isomer	**3**$_E$	R = H
	4$_E$	R = CH$_3$

Lysicamine (**5**)

Yellow powder (2.5 mg), mp. 183-185 °C, IR (KBr) v_{max} cm^{-1}: 1677 (C=O), UV λ_{max} (EtOH): 252, 268, 344, 404 nm. EIMS *m/z*: 291 (M$^+$), 248, 233, 177. ^{1}H-NMR (400 MHz, CDCl$_3$) δ ppm: 4.03 (3H, *s*, C$_1$-OCH$_3$), 4.11 (3H, *s*, C$_2$-OCH$_3$), 7.25 (1H, *s*, H-3), 7.60 (1H, *t, J*=8.0 Hz, H-9), 7.78 (1H, *d, J*=8.0 Hz, H-10), 7.82 (1H, *d, J*=5.0 Hz, H-4), 8.59 (1H, *d, J*=8.0 Hz, H-8), 8.93 (1H, *d, J*=5.0 Hz, H-5), 9.19 (1H, *d, J*=8.0 Hz, H-11).

Lysicamine (**5**)

Results and Discussion

Isolation of *T. crispa* extract using extensive chromatographic techniques resulted in the isolation of an alkyl *trans- ferulate*, a long chain saturated alcohol, two N-acylaporphine and one oxoaporphine alkaloids. Their structures were established by spectral methods and compared to the published literatures [5], [9-10].

Compound **1** and **2** were roughly suggested by EIMS and proton NMR revealed to be *n*-tetracosyl *trans*-ferulate and *n*-octacosyl alcohol, respectively. The mass spectrum of **1** is characterized by an abundant molecular ion that is also the base peak at *m/z* 530. The coupling constant between H-2 and H-3 (*J*=16.0 Hz) clearly suggested the *trans-geometry* of compound **1** [11]. The IR spectrum of **2** showed absorption bands for hydroxyl group (3315 cm^{-1}), methyl group (2918 cm^{-1}) and long aliphatic chain (782, 719 cm^{-1}). The ^{1}H-NMR spectrum showed two one-proton doublet at δ 3.57 ppm assigned as oxygenated methylene protons. The other

methylene protons appeared as a broad signal at 1.19 ppm. A three-proton multiplet at 0.81 ppm was assigned as the terminal primary methyl protons. A single proton of hydroxyl group appeared at 7.20 ppm. The ^{13}C-NMR spectrum of **2** exhibited important signals for oxygenated methylene carbon at δ 63.13 ppm and primary methyl carbons at δ 14.12 ppm. All the methylene carbons resonated between δ 32.85 – 22.71 ppm.

N-formylnornuciferine (**3**) was obtained as colourless needles with a melting point of 141–143ºC (lit. 140ºC) [5]. The UV spectrum showed strong absorption at λ_{max} (EtOH) 235, 270 and 248 (valley) nm suggested an aporphine alkaloid skeleton with substituents at C-1 and C-2. The IR spectrum exhibited a strong absorption band at 1666 cm^{-1} indicating the presence of a carbonyl group. The mass spectrum showed a strong molecular ion peak at *m/z* 309, which corresponds to the molecular formula $C_{19}H_{19}NO_3$. N-formylnornuciferine was found to be a mixture of *Z* (**3***$_Z$*) and *E* (**3***$_E$*) isomers as was reported before [5, 12]. The ratio of *Z: E* isomer was 1.7:1.0, based on the intensity of proton H-3 which resonated at δ 6.68 and 6.71 ppm respectively. The ^{1}H-NMR spectrum exhibited two singlets at 3.69 and 3.93 ppm, corresponding to two methoxyl groups at C-1 and C-2 respectively. A singlet at 8.28 ppm was assigned as the aldehyde proton at C-12 and the multiplet at 7.28 – 7.35 ppm was assigned as the three aryl protons, H-8, H-9 and H-10. Another aryl proton (H-11), resonated as a doublet at 8.42 ppm (*J*=11.8 Hz, Z isomer) and 8.44 ppm (*J*=14.0 Hz, *E* isomer). A doublet-doublet at 4.95 ppm (*J*=13.6, 4.0 Hz) was attributed to H-6a (*Z* isomer) and a multiplet at 4.41-4.53 ppm were assigned as H-6a and H-5eq (*E* isomer). Proton H-5eq (Z isomer) resonated as a multiplet centered at 3.85 ppm. Proton H-5ax and H-7ax of *E* isomer resonated as a multiplet at 3.14-3.25 ppm. Proton H-5ax (*Z* isomer) resonated at 3.40 ppm (*ddd, J*=12.0, 12.0, 3.0 Hz). The multiplet peaks centered at 2.85 ppm was assigned as the remaining protons at H-4 (*E, Z* isomers), H-7eq (E, Z isomers) and H-7ax (Z isomer). The ^{13}C-NMR chemical shift of both isomers of N-formylnornuciferine (**3***$_Z$*, Z isomers; **3***$_E$*, E isomer) is summarized in Table1.

N-acetylnornuciferine (**4**) was isolated as colourless needles with a melting point of 229-231ºC (lit. 230ºC) [5] and had a molecular ion peak at *m/z* 323, consistent with $C_{20}H_{21}NO_3$. The IR spectrum showed the presence of a carbonyl group by a strong absorption at 1633 cm^{-1}. The UV spectrum of **4** resembles that of **3** with absorption at λ_{max} (EtOH) 231, 272 and 246 (valley) nm. N-acetylnornuciferine was also found to be a mixture of *Z* and

E isomers as was reported before [5]. The ratio of *Z: E* isomer was 1.3:1.0, based on the intensity of proton H-3 which resonated at 6.65 and 6.72 ppm respectively. The ^{1}H-NMR spectrum was also similar to **3**, except there were significant two 3-H singlets at 2.19 and 2.25 ppm, which were due to the presence of the methyl group bonded to the carbonyl group in *E* and *Z* isomer, respectively. The two methoxyl groups at C-1 and C-2 appeared as singlets at 3.69 and 3.93 ppm respectively. The aromatic signal at 8.48 ppm (*dd, J*=11.0, 3.4 Hz) was assigned as H-11 (*E* isomer) and another signal at 8.44 ppm (d, 7.8 Hz) was due to H-11 of *Z* isomer. A multiplet at 7.29-7.38 ppm was attributed to the three protons, H-8, H-9 and H-10 in ring D of both isomers. The methylene proton, H-5eq was resonated as a doublet at 4.03 ppm (*J*=13.0 Hz, *Z* isomer) and 5.00 ppm (*J*=10.0 Hz, *E* isomer). Signal for H-6a appeared as a doublet at 4.58 ppm (*J*=15.0 Hz, *E* isomer) and 5.12 ppm (14.3 Hz, *Z* isomer). A triplet at 3.33 ppm (*J*=12.0 Hz) was due to H-5ax of *Z* isomer while a multiplet at 3.06-3.31 ppm was attributed to H-5ax and H-7ax of *E* isomer. Another multiplet centered at 2.85 ppm was due to H-7eq and both of H-4 for *E* and *Z* isomer. A doublet at 2.78 ppm (*J*=14.3 Hz) was assigned as H-7ax (*Z* isomer). The above assignments were consistent with literature values [10]. The ^{13}C-NMR chemical shift of both isomers of N-acetylnornuciferine (**4**$_Z$, *Z* isomers; **4**$_E$, *E* isomer) is summarized in Table1.

Table 1: ^{13}C-NMR data for **3** and **4** (CDCl$_3$, δ (ppm), 300MHz)

C-atom	**3**$_Z$	**3**$_E$	**4**$_Z$	**4**$_E$
1	144.2	144.1	145.8	145.8
2	152.5	152.5	152.5	153.0
3	112.0	112.2	111.7	112.1
3a	125.5	125.5	126.6	125.2
4	31.4	30.0	31.2	30.1
5	42.4	36.5	42.4	36.8
6a	49.8	54.0	50.7	54.0
7	34.5	38.3	34.4	37.0
7a	136.5	136.0	137.0	136.5
8	129.0*	128.5*	128.8*	128.2*

Table 1: contd.

9	128.2*	127.9*	127.9*	127.9*
10	127.5*	127.5*	127.4*	127.6*
11	128.8*	129.1*	128.3*	129.0*
11a	131.5	131.5	132.0	132.0
11b	128.2	127.5	127.4	126.5
11c	129.0	129.8	129.0	129.1
12	162.5	162.3	164.5	164.5
13	-	-	23.0	22.0
1-OCH$_3$	60.4	60.5	60.4	60.4
2-OCH$_3$	56.4	56.4	56.4	56.4

* These assignments may be interchanged.

Lysicamine (**5**) was obtained as yellow solid with a melting point of 183-185°C (lit. 185-187°C) [11]. The molecular formula is $C_{18}H_{13}NO_3$ as determined by its molecular ion peak, *m/z* of 291. The UV spectrum of **5** showed a complex pattern characteristic of a highly conjugated system, with absorption bands at λ_{max} (EtOH) 404, 268, 344 and 252 nm. The IR spectrum of **5** shows conjugated carbonyl absorption at 1677 cm^{-1}. The ^{1}HNMR spectrum showed the presence of four aryl proton signals at 8.59 ppm (*d, J*=8.0 Hz, H-8), 7.60 ppm (t, *J*=8.0 Hz, H-9), 7.78 ppm (*d, J*=8.0 Hz, H-10) and 9.19 ppm (*d, J*=8.0 Hz, H-11), characteristic signals for oxoaporphines [10]. Another three aryl proton signals which were attributed to H-3, H-4 and H-5, appeared at 7.25 (s), 7.82 (*d, J*=5.0 Hz) and 8.93 (*d, J*=5.0 Hz), respectively. Two significant 3-H singlets at 4.03 ppm and 4.11 ppm were due to two methoxyl groups at C-1 and C-2, respectively. Due to the limitation of the sample amount, we failed to get its ^{13}C-NMR spectrum. The mass fragmentation, which demonstrated 99% agreement with the library values for lysicamine, proved the identification.

(-)-N-formylnornuciferine was reported to show in vitro cardiotonic activity. It exhibited a significant increase in the force of contraction of an isolated rat heart with no significant change in the heart rate [12-13]. However, none of the compounds isolated have been studied for their insecticidal activity. Thus, the bioactive components could be further explored in relevant experimental models for its potential benefit.

Acknowledgement

References

[1]   S. K. Pathak, D. C. Jain and R. P. Sharma, 1995. Chemistry and Biological Activities of the Genera *Tinospora*, a review. *Int. J. Pharm.* Vol. 33, No. 4, pp. 277-287.

[2]   N. G. Bisset and J. Nwaiwu, 1983. Quaternary Alkaloids of Tinospora Species. *Planta Medica*, Vol. 48, No. 4, pp. 275-279.

[3]   W. W. E. Hassan, 2007. *Healing Herbs of Malaysia*. Malaysia: FELDA. pp. 90-161.

[4]   V. Wazir, R. Maurya and R. S. Kapil, 1995. Cordioside, A Clerodane Furano Diterpene Glucoside from *Tinospora cordifolia. Phytochemistry*, Vol. 38, No. 2, pp. 447-449.

[5]   P. Pachalay, A. Z. Adnan and G. Will, 1992. NMR-Assignments of N-Acylaporphine Alkaloids from *Tinospora crispa*. *Planta Medica*, Vol. 58, pp. 184-187.

[6]   N. R. Farnsworth and N. Bunyaphatsara, 1992. Thai Medicinal Plants, Prachachon Co., Thailand, pp. 244-245.

[7]   I. Norain., A. S. Syazwani., B. Nor Aziyah, F. Siti Nur Amirah Diana, B. T. Lim and W. K. Wan Zarina, 2013. The effect of *Tinospora crispa* extract against *Spodoptera exigua on Spinacia oleracea*. Malaysian Journal of Fundamental and Applied Sciences Vol. 9, No.2, pp. 110-114.

[8] J. T. Sharp, J. Gosney and A. G. Rowley, 1989. Practical Organic Chemistry, A student   Handbook of techniques, Chapman & Hall, London, pp. 160-163.

[9] F. R. Chang, C. Y. Chen, T. J. Hsieh, C. P. Cho and C. Wu, 2000. Chemical constituents from *Annona glabra* III, *J. Chin. Chem. Soc.,* Vol. 47, No. 4B, pp. 913-920.

[10] T. J. Hsieh, F. R. Chang and Y. C. Wu, 1999. The Constituents of *Cananga odorata. J. Chin. Chem. Soc.,* Vol. 46, No. 4, pp. 607-611.

[11] S. J. Chang, T. H. Lin and C. C. Chen, 2001. Constituents from the Stems of Dendrobium *Clavatum Var. Aurantiacum. J. Chin. Med.,* Vol. 12, No. 3, pp. 211-218.

[12] I. Komsan, K. Ngampong, D. Prasan, M. Rungnapa and K. Boonsong, 2009. Synthesis of N-formylnornuciferine with Cardiotonic Activity. *Kasetsart J. (Nat. Sci.)*, Vol. 43, pp. 738 – 744.

[13] A. Sunthitikawinsakul, 2005. Part I. Isolation of Active Constituents with Antimycobacterial, Antifungal and Anti-HIV-1 Activities from *Clausena excavata* Burm.f (*Rutaceae*). Part II. Isolation of Active Constituents with Cardiotonic and Anti- HIV-1 Activities from *Tinospora crispa* Miers (Menispermaceae). Ph.D. Thesis, Kasetsart University, Bangkok.