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Alkaloids from *Fissistigma fulgens* Merr. (Annonaceae)

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ABSTRACT A study on the alkaloids of *Fissistigma fulgens* Merr. was performed. Five alkaloids have been isolated from this species, i.e liriodenine, anonaine, argentinine, discretamine and kikemanine. Discretamine and kikemanine belong to the protoberberine type of alkaloids.

ABSTRAK Kajian telah dijalankan terhadap kandungan alkaloid dari *Fissistigma fulgens* Merr. Lima alkaloid telah dapat dipisahkan daripada sepsis ini, iaitu liriodenina, anonaina, argentinina, diskritinina dan kikemanina. Diskritimina dan kikemanina merupakan alkaloid dari jenis protoberberina.

(Alkaloid, protoberberine, Annonaceae, NMR, *Fissistigma*)

INTRODUCTION

In continuing our investigation on the alkaloids of the Annonaceaous plant species, we have studies the constituents of the trunk bark of *Fissisitigma fulgens* Merr. The genus *Fissisitigma* (family Annonacaea, subfamily, Annonaideae, tribe Xylopieae) consists more than fifty species native to Africa, East India, South-East Asia and North-East Australia [1]. So far *Fissisitigma oldhamii* and *F. glaucescens* have been studied for their alkaloid contents [2]. The present paper deals with the structural elucidation of alkaloids isolated from the dichloromethane extract of *F. fulgens* Merr.

EXPERIMENTAL

Melting points were taken on a Leitz Wetzler microscope hot stage and are uncorrected. UV spectra were obtained in methanol on a Varian Superscan 3 UV-Vis Spectrometer. IR spectra were recorded on Beckmann Acculab IR spectrometer. ^{1}H-NMR spectra were recorded in $CDCl_3$ on a JEOL-JNM-FX100 with TMS as the internal standard, and chemical shifts are reported in ppm. Mass spectra were obtained on AEI MS3074 Mass Spectrometer. Column Chromatography was carried out using Merck kieselgel 60 (230-400 mesh) and Merck kieselgel 60_{254} was used for preparative thin layer chromatography.

Dried and milled stem-barks of *Fissisitigma fulgens* (1.0kg) were defatted with petroleum-ether (40-60°C), then the barks were moistened with 20% NH_4OH and followed by the extraction with dichloromethane. The dichloromethane extracts were evaporated to the half of the volume and then extracted with 5% aqueous HCl. The HCl extracts were basified with ammonia to the pH 11, then reextracted with dichloromethane. Dichloromethane extracts were washed with distilled water and then evaporated to dryness to give a crude alkaloid (0.06%). The crude alkaloid was subjected to column chromatography over silica gel with dichloromethane: methanol (99:1, 95:5, 90:10) as solvent systems. The structural elucidation of the individual alkaloid was done using spectroscopic techniques such as UV, IR, MS and ^{1}H-and ^{13}C-NMR.

RESULTS AND DISCUSSION

Fissisitigma fulgens Merr. a large climer, was collected at Gua Musang, Kelantan. The dried stembark of *Fissisitigma fulgens* Merr. were extracted for their alkaloid contents (0.06%) by the conventional method.

Alkaloid **1** was isolated as yellow needles with m.p. 277-279°C (lit. m.p. 280-282°C, dec.)[3]. The UV spectrum revealed maxima absorptions at 247, 269 and 302 nm, and in the presence of HCl 0.1 N the maxima absorptions were shifted to longer wavelengths at 256, 280 and 334 nm, respectively typical of that for oxoaporphine skeleton. Its IR spectrum showed absorption at

which gave possible molecular formula of $C_{17}H_9NO_3$. ^{1}H-NMR spectrum showed an unsubstituted ring D. The pair of doublets (AB system, J=5.4 Hz) at 8.93 and 7.82 ppm significant of H-5 and H-4, respectively. A proton singlet was observed at 7.25 ppm attributable to H-3. Moreover, two sets of multiplets at 7.62-7.80 and 8.63-8.72 ppm corresponding to four protons comfirmed that ring D was not substituted. The latter pair was assigned to H-8 and H-11 because they are usually found more downfield than the other aromatic protons. A two proton singlet at 6.42 ppm indicated a methylenedioxy group at C-1,2. Alkaloid **1** was identified as liriodenine based on the above findings and other spectroscopic data [3].

Alkaloid **2** was isolated as white crystal with melting points at 122°C (decomposed). Its mass spectrum showed a molecular ion peak at m/e 265 which gave possible molecular formula of $C_{17}H_{15}NO_2$. A peak at m/z 236 $[M-29]^+$ was also observed due to the loss of methylene imine and significant $[M-1]^+$ peak was also present. The UV spectrum showed the maxima absorptions at 234, 272 and 312 indicated that this compounds belongs to the noraporphine alkaloid. No hyperchromic and bathochromic changes were observed upon addition with alkali, hence suggested that there was no hydroxyl substituent present. The infrared spectrum showed no strong absorption at the carbony and hydroxyl region. However, two peaks were observed at 1042 cm^{-1} as result of the C-O stretching vibrations of methoxyl or methylenedioxy group and at 946 cm^{-1} which is characteristic of the methylenedioxy group.

The ^{1}H-NMR spectrum revealed a pair of doublets at 5.92 and 6.06 ppm with J = 1.5Hz which indicated the presence of a methylenedioxy group at C-1,2. In addition, a singlet was observed at 6.56 ppm, a rather highfield value for an aromatic proton, which belonged to most probably to H-3 that experienced the inductive effect by the neighbouring methylenedioxy group. On the contrary, a lowfield shift, assigned to H-11 was observed at 8.06 ppm, which was due to the deshielding effect of the facing aromatic ring A. A set of multiplet centered at 7.2 ppm was attributable to the other three aromatic protons. Therefore ring D was free from any substituent. Finally, comparison of the observed data and the literature values [4,5] of a known compound left no doubt that alkaloid **2** was anonaine.

Alkaloid **3** was obtained as colorless crystal from chloroform with m.p 235°C. The UV spectrum showed the maximum absorption at 232, 252, 309 and 314 nm and in alkali medium it experienced a bathochromic shift indicated that the hydroxyl group was present in the aromatic ring. In infrared spectrum, an hydroxyl group absorbed at 3450 cm^{-1} and another absorption attributed to the aliphatic C-O vibrations stretching of methoxyl at 1035 cm^{-1} was also observed. Its mass spectrum gave a molecular ion peak at m/e 295 which gave possible molecular formula of $C_{19}H_{21}NO_2$. A base peak was observed at m/e 58 and a peak at m/z 237 $[M-58]^+$ was also visible. These fragmentations are diagnostical for a phenanthrene having two methyl groups attached to the nitrogen. Other significant fragmentation peaks observed were m/z 222, 294, 193 and 165.

Its ^{1}H-NMR spectrum showed the presence of a methoxyl group at 3.83 ppm as a three proton singlet and two N-methyl groups were observed at 2.42 ppm. H-3 proton appeared at 7.24 ppm as a singlet indicating that the C-1 and C-2 positions were substituted with hydroxyl or methoxyl group. Two batches of multiplet were observed, each at 3.23 and 2.7 ppm which were typical of phenanthrene C-11 and C-12 protons, respectively. A very down field shift was observed as a doublet at 9.47 ppm attributable to H-10. The other five aromatic protons gave a series of multiplets at 7.5-7.9 ppm. All the data obtained suggested that alkaloid **3** was argentinine [6].

Alkaloid **4** was isolated as a brownish amorphous solid. In the UV region, it absorbed at 207 and 282 nm, with a minimum at 251 nm and a shoulder at 230 nm typical of that for a tetrahydroprotoberberine skeleton [7]. The IR spectra showed a peak at 3450 cm^{-1} typical of an intramolecular hydrogen bonded hydroxyl group. In addition, a peak was observed at 1350 cm^{-1} which is due to the in plane O-H bend. The mass spectrum revealed a molecular ion peak at m/e 327 which gave a possible molecular formula of $C_{19}H_{21}NO_4$. $[M-149]^+$ and $[M-178]^+$ peaks were also observed in the mass spectrum. The former peak was indicated for the presence of hydroxyl group in ring D, while the latter was formed by an expulsion of a proton which is typical for the 9-methoxy, 10-hydroxy substitution type.

From the ^{1}H-NMR spectrum, two singlets attributed to two methoxyl groups appeared at 3.82 and 3.92 ppm. A half quartet was observed at 4.18 ppm (J = 16Hz) due to the resonance of the sp^3 C-8 equatorial proton. The other half of the axial

proton was hidden under the signals of methoxyl groups. Furthermore, a singlet corresponding to two aromatic protons, attributable to H-1 and H-4 was observed at 6.68 ppm. H-11 and H-12 exhibited an AB quartet system centered at 6.82 ppm with a coupling constant of 6 Hz. From the observed data and comparison with the literature values [8], it was confirmed that alkaloid **4** was discretamine.

Alkaloid **5** was isolated in a brownish amorphous state. The basic skeleton of the alkaloid **5** was deduced from its characteristic UV spectrum and supported by the ^{1}H-NMR and mass spectrum data. The UV spectrum revealed maxima at 209 and 282 nm, a minimum at 257 and a shoulder at 230 nm typical for the tetrahydroprotoberberine skeleton. The IR spectrum showed absorption at 3550 nm^{-1} indicating the presence of the hydroxyl group. Other peaks observed were at 1350 and 1040 cm^{-1}. The former was due to the in plane O-H bend while the latter was caused by the C-O stretching vibrations of the methoxyl group. The mass spectrum showed a molecular ion peak at m/e 341 which permitted the possibility of the molecular formula to be $C_{20}H_{23}NO_4$. The mass spectra also exhibited additional two peaks at m/z 192 and 149, which proved that the ring A carried two methoxyl groups and the hydroxyl group belonged to ring D. Morever, the $[M\text{-}OCH_3]^+$ peak at 310 was 16% in intensity relative to the molecular ion peak, which indicated that the ring D has a C-9 methoxy, C-10 hydroxy substitution pattern.

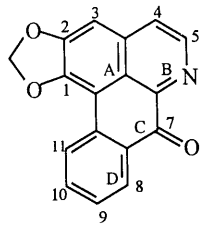
The ^{1}H-NMR gave further prove that C-9 was substituted by revealing a half AB quartet, a signal revealed by the C-8 equatorial proton, at 4.3 ppm with coupling constant of 16 Hz. Two singlets corresponding to nine methoxyl hydrogens were observed at 3.70 and 3.74 ppm. Furthermore, a singlet attributable to H-1 and H-4 was present at 6.85 ppm. A distorted quartet of an AB system centered at 6.80 ppm with J = 6 Hz was also exhibited and it was assigned to H-11 and H-12. Finally, after comparison the observed spectral data with the literature values [9], it is confirmed that compound **5** was kikemanine.

For further structural study the ^{13}C-NMR of discretamine and kikemanine were performed and peak assignments were based on comparison with tetrahydropalmitine as shown in Table 1.

Table 1 ^{13}C chemical shifts of tetrahydropalmitine, discretamine and kikemanine

Carbon	Tetrahydropalmitine	Discretamine	Kikemanine
1	108.9	114.9	109.0
1a	129.9	128.2	129.6
2	147.6	146.1	147.5
3	147.6	147.3	147.5
4	111.5	109.3	111.6
4a	127.8	128.4	129.6
5	29.1	28.5	28.7
6	51.5	51.3	53.7
8	54.0	51.3	51.3
8a	126.9	125.8	126.6
9	150.2	144.7	146.8
10	145.2	143.3	115.0
11	111.1	114.9	124.2
12	123.7	123.6	128.1
12a	128.7	125.8	37.1
13	36.4	35.5	60.0
14	59.3	59.1	56.1
C-2 OMe	55.8	55.9	55.8
C-3 OMe	55.8	-	59.3
C-9 OMe	60.1	55.9	-
C-10 OMe	56.1	-	-

Spectra recorded in $CDCl_3$, in ppm with TMS as internal reference.



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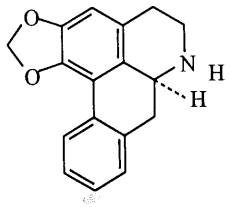

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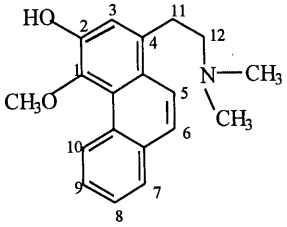

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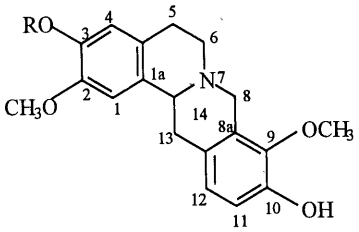
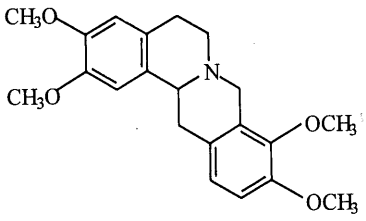


4 R = H

5 R = CH_3



Tetrahydropalmitine

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