PERTANIKA 14(3), 355-358 (1991)

# COMMUNICATION VI

## Phaeanthine and Limacine from *Phaeanthus crassipetalus* Becc.

**Key words:** ***Phaeanthus crassipetalus*, Annonaceae, alkaloid, limacine, phaeanthine, antibacterial activity.**

**ABSTRAK**

Phaeanthus crassipetalus *Becc. (Fam. Annonaceae) telah diselidiki. Dua alkaloid major telah diasing dan dicirikan berdasarkan maklumat spektroskopi. Alkaloid tersebut adalah feantina (I) dan limasina (II). Alkaloid tersebut menunjukkan sifat antibakteria terhadap bakteria Gram positif dan Gram negatif.*

**ABSTRACT**

Phaeanthus crassipetalus *Becc. (Fam: Annonaceae) has been studied. Two major alkaloids have been isolated and characterised based on their spectral information. The alkaloids, phaeanthine (I) and limacine (II), show antibacterial properties against Gram positive and Gram negative bacteria.*

### INTRODUCTION

*Phaeanthus crassipetalus* Becc. represents one of the three *Phaeanthus* spp. found in Borneo. The other two species are *P. ebracteolatus* and *P. ophthalmicus.* Only two species of *Phaeanthus* have been investigated chemically, *P. ebracteolatus* and *P. macropodus* (Leboeuf *et al.* 1982). Limacine and phaeanthine were isolated from a species of *Phaeanthus*, possibly *P. macropodus* (Johns *et al.* 1968) whilst phaeanthine and phaenthaarine have been isolated from *P. ebracteolatus* (Van-Beek *et al.* 1983). Some *Phaeanthus* spp. such as *P. ebracteolatus* have been used in traditional medicine as an antispasmodic as well as to cure ulcers and minor wounds (Perry 1980).

*Phaeanthus crassipetalus*, locally known as 'mempisang', can be found in several places in Sabah. This species has been chosen for further investigation because of its high alkaloid content (Fasihuddin and Kamaruddin 1989) and its use by the local community to reduce blood pressure.

### MATERIALS AND METHODS

*General Experimental Procedures*

IR spectra were measured as KBr discs using a Pye Unicam Model SB 3-200 spectrometer. $^{1}$H-NMR and $^{13}$C-NMR were recorded in $CDCl_3$ using NMR spectrophotometer model Bruker WP'T (90 MHz for $^{1}$H and 20.1 MHz for $^{13}$C(TMS as an internal standard). MS were recorded on a JEOL model JMS-DX 303 mass spectrometer linked to a JMA-DA 5000 Mass Data System. Specific rotations were measured in $CHCl_3$ using a Polarimeter Optical Activity Model AA-10.

*Plant Material*

*Phaeanthus crassipetalus* was collected from Pulau Tiga, Sabah. A voucher specimen was deposited at UKMS Herbarium, reference number KMS 1517. Leaves and stem bark were separated, dried at room temperature and ground to a powder.

*Extraction*

About 455g of leaves and 542 g of stem bark were extracted separately. Both samples were defatted with n-hexane for a week and extracted with $CHCl_3$-$NH_4OH$ for a week and filtered. The extract was concentrated under reduced pressure. The concentrated extract was transferred into a separating funnel and shaken with several portions of 2% $H_2SO_4$ until no alkaloid was detected in the organic phase. The non-alkaloid impurities in the aqueous acid solution were removed by shaking under acidic conditions with portions of $CHCl_3$ and the $CHCl_3$ extract discarded. The aqueous acidic solution in a separating funnel was covered with $CHCl_3$ and 10% ammonia was slowly added to make the solution alkaline. Extraction was then carried out by shaking with several portions of

$CHCl_3$ until no alkaloid was detected in the aqueous phase. Removal of water from the organic extract was achieved by stirring into it anhydrous sodium sulphate. Finally the $CHCl_3$ was evaporated to dryness to yield crude alkaloids: 0.72% on leaf and 0.78% on stem bark.

*Thin Layer Chromatography (TLC)*

For the separation of the alkaloids, the following TLC systems were used S1:$CHCl_3$-cyclohexane-$Et_2NH$ (10:8:3); S2 toluene-$Et_2NH$ (7:2:1); S3 $CHCl_3$-MeOH-conc. $NH_4OH$ (14:5:1); S4 MeOH-$H_2O$-conc. $NH_4OH$ (8:1:1). All solvent systems were used in combination with pre-coated plates (Si gel $Si_{60}$ $F_{254}$, Merck) in saturated chromatography chambers. Alkaloids were detected using UV light (254 nm), iodoplatinate reagent or with 0.2 M $FeCl_3$ in 30% $HClO_4$ followed by heating.

*Column Chromatography*

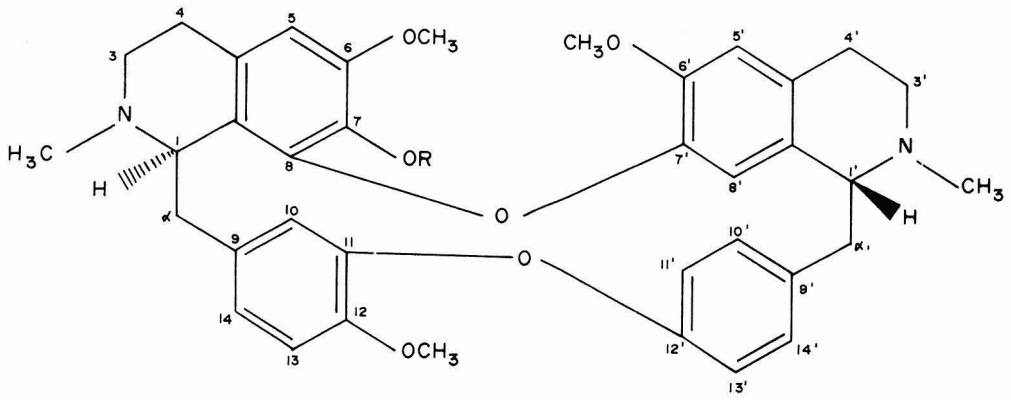
Crude alkaloids were dissolved in $CHCl_3$ and separated by means of adsorption chromatography on ready made Si gel 60 columns, Merck (Size C), using $CHCl_3$, -cyclohexane-$Et_2NH$ (10:8:3) as a mobile phase. 10mL fractions were collected and monitored by TLC. Fractions containing the same component were combined and evaporated to dryness at room temperature. This process was repeated until pure alkaloids were obtained. Limacine was obtained in a yield of 92 mg and phaeanthine in a yield of 32 mg.

*Antibacterial Screening*

This was carried out according to the method described by Verpoorte *et al.* (1982).

*Characterisation of Alkaloids*

**Limacine** Colourless crystal (crystallized from benzene), m.p. 173-174° C (decomp.), mixed m.p. with an authentic sample undepressed (Reported m.p.173-174°C, Siwon *et al.* 1981) UV$\lambda_{max}$ 284 nm (MeOH), $[\alpha]_D$-240° (c = 0.20 in $CHCl_3$). MS m/z (% abundance) 609 (22%), 608 (44,$M^+$), 607 (24) 471 (1), 382 (23), 381 (70), 367 (26) 192 (60), 191 (100), 174 (13) and 168 (14). $^1$H-NMR (80 MHz, $CDCl_3$). δ (p.p.m) 2.30 (s, 2-*N*-methyl) 2.60 (s,2'-*N*-methyl), 3.30 (s' 6'-*O*-methyl) 3.74 (s, 6-*O*-methyl), 3.90 (s, 12-*O*-methyl) and 6.05-7.40 (10H, aromatic). $^{13}$C-NMR (20.1 MHz, $CDCl_3$), 22.0 (C-4), 25.2(C-4), 38.0 (C-α,), 42.0 ((C-α), 42.4 (N-$CH_3$), ($C_3$), 42.6 (N-$CH_3$), 44.3 (C-3), 45.4 (C-3'), 56.1 (3 × O-$CH_3$), 61.4 (C-I), 63.7 (C-1'), 104.8 (C-5), 112.0 (C-13), 113.0 (C-5'), 116.2 (C-10), 120.4 (C-8'), 121.8 (C-13'), 122.8 (C-11'), 123.0 (C-8a & C-14), 127.8




(i) R = $CH_3$ PHAEANTHINE

(ii) R = H LIMACINE

(C-8a') 128.3 (C-4a''), 128.3 (C-4a), 129.9 (C-10'), 132.4(C-14'), 134.7 (C-9), 134.9 (C-9'), 135.9 (C-7), 142.0 (C-7'), 143.7 (C-11), 146.0 (C-8), 147.0 (C-6'), 148.8 (C-12), 149.3 (C-6) and 153.6 (C-12').

**Phaeanthine** Colourless crystals, m.p. 220-221°C, mixed-m.p. with an authentic sample undepressed (Reported m.p. 220-221°C, Johns *et al.* 1968) $[\alpha]_D$ -280° (c = 0.20 in $CHCL_3$). MS m/z (% abundance) 622 ($M^+$, 2%), 396(7), 395(34), 381(27), 364(5), 349(6), 198(100), 175(28), 174(93). $^1$H-NMR (80 Mhz, $CDCI_3$): δ (p.p.m) 2.32 (s, 2-*N*-methyl), 2.61 (s, 2'-*N*-methyl), 3.19 (s, 6'-*O*-methyl), 3.38(s,7-*O*-methyl), 3.72 (s, 6-*O*-methyl), 3.92 (s, 12-*O*-methyl) and 6.05-7.44 (10H, aromatic). $^{13}$C-NMR (20.1 MHz, $CDCI_3$)-22.3 (C-4), 25.2 (C-4'), 38.4 (C-α), 42.0 (C-α), 42.5 (N-$NH_3$), 42.6(N'$CH_3$), 44.5(C-3), 45.6 (C-3'), 56.2(2×$OCH_3$), 56.3-$OCH_3$), 60.4(C-7-$OCH_3$), 61.8(C-1), 64.2(C-1'), 106.2 (C-5), 112.1(C-13), 113.0(C-5'), 116.7(C-10), 120.4(C-8'), 122.1 (C-11'), 122.2(C-13'), 123.2(C-8a), 123.4(C-14), 128.2(C-4a' and C-8a'), 128.3 (C-4a), 130.4 (C-10'), 132.8(C-14'), 135.0 (C-9), 135.3(C-9'), 138.5(C-7'), 144.1(C-7'), 147.5(C-11), 148.6(C-8), 148.9 (C-6'), 149.8 (C-12), 151.8 (C-6) and 154.2 (C-12').

## RESULTS AND DISCUSSION

The main alkaloids from the stem bark and leaves were identified as phaeanthine (I) and limacine (II). The other alkaloids are still under investigation. This is the first report of these alkaloids from *Phaeanthus crassipetalus.*

The mass spectrum of phaeanthine exhibited a molecular ion, $M^+$ at m/z 622, which is consistent with the formula $C_{38}H_{42}N_2O_6$ and for limacine at m/z 608 which is consistent with the formula $C_{37}H_{40}N_2O_6$. The $^1$H-NMR spectrum (80 MHz) showed the presence of 4 methoxyl groups (δ3.19, 3.38, 3.72 and 3.92 ppm) and 2*N*-methyl groups (δ2.32 and 2.61 ppm) and 10 aromatic protons at 6.05-7.44 ppm and additional signal in the region 2-4 ppm which integrated for a further 14 hydrogen atoms for phaeanthine whilst the spectra of limacine showed only 3 methoxyl groups (δ3.30, 3.74 and 3.90 ppm). The melting point of limacine was in good agreement with the previously published results (Siwon *et al.* 1981).

The $^{13}$C-NMR spectra agreed well with published spectra of limacine and phaeanthine. The optical rotation of phaeanthine $[\alpha]_D$-280° indicated that the substance was phaeanthine and not the enantiomeric tetrandrine (Reported specific rotation $[\alpha]_D$–280°, Johns *et al.* 1968). Limacine was methylated with diazomethane in methanol-diethyl ether solution to give phaeanthine, melting point 220-222°C. The infrared spectrum of limacine showed the presence of a hydroxyl group at 3400 $cm^{-1}$ which disappeared when the sample was methylated with diazomethane. The fragmentation patterns of limacine and phaeanthine agreed well with their structure.

Antibacterial properties were tested against Gram negative and Gram positive bacteria using the agar diffusion test. Both limacine and phaeanthine showed a certain degree of antibacterial activity. Phaeanthine was active against *Streptococcus pneumoniae* (minimum inhibiting concentration 68μg/mL), *Streptococcus faecalis* (75 μg/mL, *Bacillus subtilis* (70 μg/mL), *Staphylococcus aureus* (80 μg/mL) and *Escherichia coli* (100 μg/mL), while limacine was active against *Streptococcus pneumoniae* (85 μg/mL), *Streptococcos faecalis* (100 μg/mL and *Escherichia coli* (125 μg/mL).

## ACKNOWLEDGEMENTS

We thank MPKSN for IRPA Research Grant No. 4-07-03-11 and Sabah Parks for the plant material.

FASIHUDDIN, B.A, SHANTY, V and ATAN [1], M.S

*Department of Chemistry*
*Faculty of Science and Natural Resources*
*Universiti Kebangsaan Malaysia,*
*Sabah Campus, L.B. No. 62*
*88996 Kota Kinabalu, Sabah.*
[1]*Department of Chemistry*
*Faculty of Science and Evironmental Studies*
*Universiti Pertanian Malaysia,*
*43400 UPM, Serdang, Selangor Darul Ehsan.*
*Malaysia.*

## REFERENCES

FASIHUDDIN, B.A. and M.S. KAMARUDDIN. 1989. Penyaringan Fitokimia Tumbuh-tumbuhan di Sabah. *In Proceedings Malaysian Traditional Medicine,* Kuala Lumpur, ed. E. Soepadmo, S.H. Goh, W.H. Wong, Laily Din and C.H. Chuah. p. 147.

JOHNS, S.R., J.A. LAMBERTON and A.A. SIOUMIS. 1968. Alkaloid of a *Phaeanthus* species from New Guinea. Isolation of Phaeanthine and Limacine. *Aust. J. Chem.* **21**: 1387.

LEBOEUF, M., A. CAVE, P.K. BHAUMIK, B. MUKHERJEE

and R. MUKHERJEE. 1982. Phytochemistry of the Annonaceae. *Phytochemistry* **21(12)**: 2783.

PERRY, L.M. 1980. *Medicinal Plants of East and Southeast Asia.* Massachusetts: Massachusetts Institute of Technology Press.

SIWON J., R. VERPOORTE, T. VAN-BEEK, H. MEERBURG and A.B. SVENDSEN. 1981. Alkaloids from *Pycnarrhena longifolia. Phytochemistry* **20**: 323-325.

VAN-BEEK, T.A., R. VERPOORTE and A.B. SVENDSEN, 1983. Revised Structure of Phaeantharine. *Journal of Natural Product* **46(2)**:226.

VERPOORTE, R., A. TJIN, A. TSOI, H. VAN-DOORNE and A.B. SVENDSEN. 1982. Screening of Antimicrobial Activity of some Plants Belonging to the Apocynaceae and Loganiaceae. *J. Ethnopharmacology* **5**: 221.

*(Received 26 November, 1990)*