ISSN:0128-7680
Pertanika J. Sci. & Technol. 4(1): 17-22 (1996)

Constituents of the Rhizomes of *Alpinia rafflesiana*

Hasnah Mohd. Sirat,*[1] Dian Masri,[1] Ahmad A. Rahman,[2] H. Itokawa[3] and H. Morita[3]

[1]*Dept. of Chemistry, Faculty of Science*
Universiti Teknologi Malaysia
KB 791, 80990 Johor Bahru, Johor, Malaysia

[2]*Dept. of Biology,*
Faculty of Science and Environmental Studies
Universiti Pertanian Malaysia
43400 Serdang, Selangor, Malaysia

[3]*Tokyo College of Pharmacy,*
1432-1 Horinouchi, Hachioji,
Tokyo 192-03, Japan

Received 6 August 1994

ABSTRAK

Juzuk kimia rizom *Alpinia rafflesiana* telah dikaji. Empat komponen telah diasingkan melalui teknik kromatografi. Strukturnya telah dikenal pasti melalui kaedah spektroskop. Juzuk utama dikenal pasti sebagai kalkon flavokawin B, manakala juzuk sampingan dikenali sebagai sinamit metil, diarilheptanoid 1,7-difenil-5-hidroksi-6-heptena-3-on dan 5,6-dihidrokawin.

ABSTRACT

The chemical constituents of the rhizomes of *Alpinia rafflesiana* were studied and four components were isolated by chromatographic techniques and their structures identified by spectroscopic methods. The major constituent has been identified as chalcone flavokawin B, while the minor constituents have been assigned as methyl cinnamate, diarylheptanoid 1,7-diphenyl-5-hydroxy-6-hepten-3-one and 5,6-dehydrokawain.

Key words: *Alpinia rafflesiana*, Zingiberaceae, phenolics, diarylheptanoid

INTRODUCTION

Alpinia rafflesiana (Zingiberaceae) is one of the 23 species of *Alpinia* found in Peninsular Malaysia (Holttum 1950). Several species of *Alpinia* are used for flavouring agents, while several others are used as ingredients in traditional

* Author to whom correspondence should be addressed.

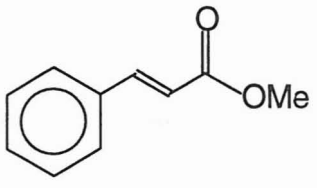
medicine formulations (Burkill 1966). Several papers have reported the chemical constituents of the seeds and rhizomes of *Alpinia* species. These include sesquiterpenes from *A. paponica* (Itokawa *et al.* 1980b), diterpenes from *A. speciosa* (Itokawa *et al.* 1980a) and *A. galanga* (Morita and Itokawa 1988), phenolics from *A. speciosa* (Itokawa *et al.* 1981b), *A. galanga* (Barik *et al.* 1987), and *A. formosana* (Itokawa *et al.* 1988), and diarylheptanoids from *A. oxyphylla* (Itokawa *et al.* 1981a), *A. officinarum* (Itokawa *et al.* 1981c, 1985), and *A. katsumudai* (Kuroyanagi *et al.* 1983). In the present paper we wish to report the isolation and structural elucidation of the rhizome constituents of *A. rafflesiana*. The constituents of this plant have, to our knowledge, never been reported before.

METHODS AND MATERIALS

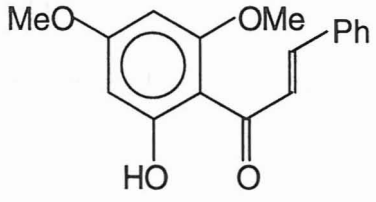
The rhizomes of *A. rafflesiana* were collected from cultivated plants in Universiti Pertanian Malaysia, Serdang. A voucher specimen was deposited in the Herbarium of the Dept. of Biology, UPM. TLC was performed on Merck pre-coated plates of silica gel F_{254}. Column chromatography was performed on silica gel (Merck 70-230 and 230-400 mesh). IR spectra were recorded on a Perkin Elmer 727B spectrophotometer, MS were recorded on a VG instrument, and ^{1}H-NMR and ^{13}C-NMR were recorded on a Bruker AM 400 spectrometer in $CDCl_3$ with TMS as an internal standard.

Extraction, Isolation and Identification of Constituents

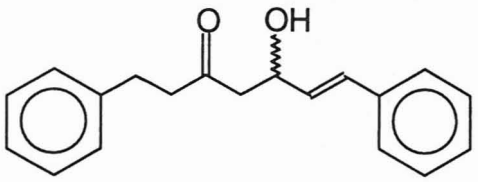
Air-dried powdered rhizomes of *A. rafflesiana* (140 g) were extracted with chloroform in a Soxhlet apparatus for 24 h and the excess solvent was distilled off. Concentration under reduced pressure gave the crude product (7.3 g). The crude extract was fractionated using vacuum liquid chromatography (VLC) using petroleum ether and ether mixtures stepwise to give three fractions (A-C). Fraction A was subjected to silica gel column chromatography (CC) using hexane as an eluent to give **1** (100 mg). Fraction B was further purified by CC on SiO_2 using petroleum ether-ether (4:1), followed by recrystallization from petroleum ether-ether to yield **2** (280 mg). Fraction C was subjected to multiple CC using petroleum ether - ethyl acetate (4:1) followed by recrystallization to give compounds **3** (80 mg) and **4** (210 mg).



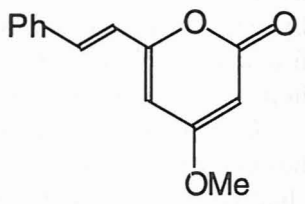
1



2



3



4

*Methyl cinnamate (**1**)*
Colourless oil; IR ν_{max} cm^{-1}: 3350, 2950, 1630, 1590, 1450, 1350, ^1H NMR: δ 3.7(3H, s, OMe), 6.02(1H, d, J = 16 Hz, styryl proton), 7.4 (5H, m, phenyl protons) and 7.8 (1H, d, J = 16Hz, styryl protons).

*Flavokawin B (**2**)*
Yellow needles, m.p. 94-94°C, (Itokawa *et al.* 1981b, 91.5-92.0°C). IR ν_{max} cm^{-1}: 2950, 1630 1590; ^1H NMR: δ 3.83 and 3.91 (each 3H, s, OMe), 5.97 (1H, d, J = 2.4 Hz, H-5'), 6.11 (1H, d, J = 2.4 Hz, H-3'), 7.34-7.61(5H,m, phenyl protons), 7.80 (1H, d, J = 15.6 Hz, H-8), 7.90(1H,d, J = 15.6 Hz, H-9), and 14.24 (1H, s, OH). EIMS: m/z (rel. int.) 284 $C_{17}H_{16}O_2$ [M^+](78), 207(100).

*1,7-diphenyl-5-hydroxy-6-hepten-3-one (**3**)*
Pale yellow crystals, m.p. 60-61°C (Kuroyanagi *et al.* 1983, 59.5-60.5°C); IR ν_{max} cm^{-1}: 3450, 1720,1610,1500,1460 and 1370; ^1H NMR: δ 2.72 (2H, br. d, H-4), 2.80 and 2.95 (each 2H,t, J = 5.8 Hz, $PhCH_2CH_2CO$-), 4.75 (1H,q, J = 5.8 Hz, H-5), 6.20(1H, dd, J = 16.1 and 5.8 Hz, H-6) and 6.65 (1H,d, J = 16.1 Hz, H-7), and 7.15-7.40 (10H, m, phenyl protons); EIMS: m/z (rel.int) 280, $C_{19}H_{20}O_2$ [M^+](17), 262(9), 175(18), 148 (26), 133(52), 105(80), 91(100), 77(27).

*5,6-dehydrokawain (**4**)*
Pale yellow crystals, m.p. 139-140°C (Itokawa *et al.* 1981b, 136.5-137.5°C); IR ν_{max} cm^{-1}: 3040, 1730, 1610, 1560, 1460, ^1H NMR(C_6D_6): δ 3.75(3H,s,OMe), 5.40(1H,d, J = 2.5 Hz, H-3), 5.85 (1H,d, J = 2.5 Hz, H-5), 6.40(1H,d, J = 16 Hz,H-7), 7.30(5H,m, phenyl protons), and 7.40 (1H,d, J = 16 Hz, H-8). EIMS: m/z (rel. int.) 288 $C_{14}H_{12}O_3$ [M^+](100), 200(52), 157(55), 77(50), 69(42).

RESULTS AND DISCUSSION

The $CHCl_3$ extract of the rhizomes was subjected to silica gel vacuum liquid chromatography to give three fractions (A-C). Repeated column chromatography of fraction A using silica gel resulted in the isolation of

methyl cinnamate (**1**). Purification of fraction B using silica gel CC followed by recrystallization afforded chalcone flavokawin B (**2**). Repeated CC of fraction C followed by recrystallization gave 1,7-diphenyl-5-hydroxy-6-hepten-3-one (**3**) and 5,6-dehydrokawain (**4**).

Compound (**1**) was readily identified as methyl *trans*-cinnamate, which has frequently been isolated from Zingiberaceous plants. This ester showed characteristic methoxyl group at δ 3.9 (s), *trans* styryl protons at δ 6.02 and 7.40 (d, $\mathcal{J}$ = 16.0 Hz) and phenyl group at δ 7.4 (m) in the ^{1}H-NMR spectrum.

Compound (**2**) revealed a strong UV absorption maximum at 343 nm characteristic of chalcone. The ^{1}H NMR spectrum agreed almost completely with that of flavokawin B, which had been previously isolated from *Piper methysticum* (Sauer and Haensel 1967) and *A. speciosa* (Itokawa *et al.* 1981b). Notable was the presence of two methoxyl signals at δ 3.83 and 3.91 and the two aromatic peaks at δ 5.97 and 6.11 for H-3' and H-5' respectively. The structure was supported by the ^{13}C NMR data and DEPT, which showed the presence of two methyl carbons, nine methine carbons and five quarternary carbons.

Compound (**3**) showed strong hydroxyl and carbonyl absorptions at 3450 and 1720 cm^{-1} respectively. The ^{1}H NMR spectrum of (**3**) revealed styryl protons signals at δ 6.20 (dd, $\mathcal{J}$ = 16.1 and 5.8 Hz) and 6.65 (d, $\mathcal{J}$ = 16.1 Hz) indicating a *trans* orientation, and resonances corresponding to two phenyl groups at δ 7.15-7.40. A signal at δ 4.75 was assigned to a proton adjacent to a hydroxyl group and a broad singlet at δ 3.07 was assigned to the hydroxyl proton. A set of triplets at δ 2.80 and 2.95 was attributable to four methylene protons, indicating the presence of the structure of $PhCH_2CH_2CO$, and a peak at δ 2.72, d, $\mathcal{J}$ = 5.8 Hz was assigned to the methylene protons at C-4. These data were in agreement with those for the previously isolated diarylheptanoid from *A. katsumudai* (Kuroyanagi *et at.* 1983). ^{13}C NMR and DEPT showed the presence of three methylene carbons, thirteen methine carbons, and three quarternary carbons which were also in agreement with those published previously.

Compound (**4**) was identified as 5,6-dehydrokawain by comparison of its physical and spectral properties with those of the compound previously isolated from *A. speciosa* (Itokawa *et al.* 1981b). The compound had a molecular formula $C_{14}H_{12}O_3$ (228, M^+) as shown in the MS. The ^{1}H NMR spectrum showed the presence of a methoxyl peak at δ 3.78, and the olefinic signals of an $\alpha\beta$ – unsaturated carbonyl group at δ 6.48 and 7.46 with $\mathcal{J}$ = 15.6 Hz. A set of small doublets observed at δ 5.45 and 5.85 with coupling constants of 2.4 Hz was assigned to H-3 and H-5 respectively (Itokawa *et al.* 1981b).

The 13C NMR signals of compounds (**2** – **4**) were assigned by substitution chemical shift and also by comparison with these for the previously isolated compounds (Table 1).

TABLE 1
^{13}C NMR data of compounds 2, 3 and 4

C	2	3	4
1	135.7	29.4	–
2	128.4	45.2	158.6
3	128.9	209.9	88.9
4	130.0	49.4	171.2
5	128.9	68.6	101.4
6	128.4	130.3	164.0
7	127.6	130.5	118.6
8	142.3	–	135.7
9	192.7	–	135.1
10	–	–	127.4
11	–	–	128.9
12	–	–	129.4
13	–	–	128.9
14	–	–	127.4
1'	106.4	140.7	–
2'	166.3	128.3	–
3'	91.3	128.6	–
4'	168.4	126.3	–
5'	93.7	128.6	–
6'	162.6	128.3	–
1''	–	136.6	–
2''	–	126.6	–
3''	–	128.6	–
4''	–	127.8	–
5''	–	128.6	–
6''	–	126.6	–
4–OMe	–	–	55.9
2'–OMe	55.6	–	–
3'–OMe	55.6	–	–

CONCLUSION

Three phenolics and one diarylheptanoid have been isolated from the rhizomes fo *A. rafflesiana*. As these phenolics have also been obtained from the rhizomes of *A. speciosa*, it seems likely that *A. rafflesiana* and *A. speciosa* are closely related plants in the genus of *Alpinia*.

ACKNOWLEDGEMENTS

The authors wish to thank Mrs. Mek Zum Mohamed of UTM for recording the NMR spectra, Dr. Graeme B. Russell, DSIR, New Zealand for his help with high resolution MS and the Malaysian government for financial support.

REFERENCES

BARIK, B.R., A.B. KUNDU and A.K. DEY. 1987. Two phenolic constituents from *Alpinia galanga* rhizomes. *Phytochemistry* **26**: 2126.

BURKILL, I.H. 1966. *A Dictionary of the Economic Products of the Malay Peninsula*, Kuala Lumpur: Ministry of Agriculture and Cooperatives.

HOLTTUM, R.E. 1950. The Zingiberaceae of the Malay Peninsula. *Gard. Bull. Sing.* **13**: 1.

ITOKAWA, H., R. AIYAMA and A. IKUTA. 1981a. A pungent diarylheptanoid from *Alpinia oxyphylla*. *Chem. Pharm. Bull.* **29**: 2283.

ITOKAWA, H., M. MORITA and S. MIHASHI. 1980a. Labdane and bisnorlabdane type diterpenes from *Alpinia speciosa* K. Schum. *Chem. Pharm. Bull.* **28**: 3452.

ITOKAWA, H., M. MORITA and S. MIHASHI. 1981b. Phenolic compounds from the rhizomes of *Alpinia speciosa*. *Phytochemistry* **20**: 2503.

ITOKAWA, H., M. MORITA and S. MIHASHI. 1981b. Two diarylheptanoids from *Alpinia officinarum Hance*. *Chem. Pharm. Bull.* **29**: 2383.

ITOKAWA, H., H. MORITA, I. MIDORIKAWA, R. AIYAMA and M. MORITA. 1985. Diarylheptanoids from the rhizomes of *Alpinia officinarum* Hance. *Chem. Pharm. Bull.* **33**: 4889.

ITOKAWA, H., K. WATANABE, S. MIHASHI and Y. IITAKA. 1980b. Isolation of agarofuran-type sesquiterpenes from *Alpinia japonica* (Thunb.) Miq. *Chem. Pharm. Bull.* **28**: 681.

ITOKAWA, H., S. YOSHIMOTO and H. MORITA. 1988. Diterpenes from the rhizomes of *Alpinia farmosana*. *Phytochemistry* **27**: 435.

KUROYANAGI, M., T. NORO, S. FUKUSHIMA, R. AIYAMA, A. IKUTA, H. ITOKAWA and M. MORITA. 1983. Studies on the constituents of the seeds of *Alpinia katsumudai* Hayata. *Chem. Pharm. Bull.* **31**: 1544.

SAUER, H. and R. HAENSEL. 1967. Components of *Piper* species XVI. Kava lactones and flavonoids from an endemic *Piper* species of New Guinea. *Planta Med.* **15**: 443.