Available online at www.sciencedirect.com
SCIENCE DIRECT®
PERGAMON
Phytochemistry 62 (2003) 1121–1124
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Meliternatin: a feeding deterrent and larvicidal polyoxygenated flavone from *Melicope subunifoliolata*

Shuit Hung Ho[a,*], Jing Wang[a], K.Y. Sim[b], Gwendoline C. L. Ee[c], Zamrie Imiyabir[d], K.F. Yap[e], Khozirah Shaari[e], Swee Hock Goh[e]

[a]*Department of Biological Sciences, National University of Singapore, Singapore 117543, Singapore*
[b]*Department of Chemistry, National University of Singapore, Singapore 117543, Singapore*
[c]*Department of Chemistry, Universiti Putra Malaysia, Malaysia*
[d]*Forest Research Centre, Sepilok, Sandakan, Sabah, Malaysia*
[e]*Forest Research Institute of Malaysia, Kuala Lumpur, Malaysia*

Received 28 May 2002; received in revised form 1 November 2002

Abstract

We screened more than 60 Malaysian plants against two species of insects and found that *Melicope subunifoliolata* (Stapf) T.G. Hartley (Rutaceae) showed strong feeding deterrent activity against *Sitophilus zeamais* Motsch. (Curculionidae) and very good larvicidal activity against *Aedes aegypti* L. (Diptera). One anti-insect compound, meliternatin (3,5-dimethoxy-3′,4′,6,7-bismethylendioxyflavone) (**6**) and six other minor polyoxygenated flavones were isolated from *M. subunifoliolata*.

Keywords: *Melicope subunifoliolata*; Rutaceae; Feeding deterrence; Larvicidal activity; *Sitophilus zeamais*; *Aedes aegypti*; Flavonoid; Meliternatin

1. Introduction

The use of synthetic pesticides has many drawbacks, including toxicity to non-target organisms, development of pesticide resistance and environmental pollution. Renewed interest has been shown in the development of alternative strategies, including the use of suitable types of natural insecticides derived from a re-evaluation of age-old traditional botanical pest control agents (Heyde et al., 1984). In our studies to discover botanical insecticides and their modes of action, we have screened more than 60 Malaysian plants based on such factors as availability and species related to those with ethnobotanical uses for medicine and pesticides. We found that the crude methanolic extract of *Melicope subunifoliolata* (Stapf) T.G. Hartley (Rutaceae) showed strong feeding deterrent activity against the storage pest, *Sitophilus zeamais* Motsch. (Curculionidae) as well as larvicidal activity against *Aedes aegypti* L. (Diptera). Active fractions from the leaf extracts provided one major and six other minor polymethoxyflavonoids. We now report the isolation and structural elucidation of these flavonoids, and the feeding deterrent and larvicidal activities of one of the flavonoids, meliternatin, the major compound isolated.

2. Results and discussion

The methanolic extract of *M. subunifoliolata* which showed strong feeding deterrent activity against *S. zeamais* and larvicidal activity against *Ae. aegypti*, was separated into various fractions by column chromatography on silica gel with an *n*-hexane–EtOAc gradient solvent system. The feeding deterrent and larvicidal activities of the fractions were evaluated and fractions exhibiting high activity were separated further to obtain pure compounds. The compounds characterized by NMR and MS were 5-hydroxy - 3,6,7,8 - tetramethoxy - 3′,4′ - methylenedioxyflavone (**1**, 0.01%) (Higa and Scheuer, 1974), 5-hydroxy-3,7,8-trimethoxy-3′,4′-methylenedioxyflavone (**2**, 0.005%) (Higa and Scheuer, 1974), 3,5,6,7,8-pentamethoxy-3′,4′-methylenedioxyflavone (**3**, 0.01%) (Jong and Wu, 1989),

* Corresponding author. Tel.: +65-6874-2698; fax: +65-6779-2486.
E-mail address: dbshosh@nus.edu.sg (S.H. Ho).

0031-9422/03/$ - see front matter © 2003 Published by Elsevier Science Ltd.
PII: S0031-9422(02)00632-5

3,5,6,7-tetramethoxy-3′,4′-methylenedioxyflavone (**4**, 0.005%) (Jong and Wu, 1989) 3,5,7-trimethoxy-3′,4′-methylenedioxyflavone (**5**, 0.005%) (Higa et al., 1987), 3,3′,4′,5,7-pentamethoxyflavone (**7**, 0.01%) (Higa et al., 1990) and meliternatin, 3,5-dimethoxy-3′,4′,6,7-bis-methylendioxyflavone (**6**, 0.16%) (Higa et al., 1987).

Compound **6** was found to have feeding deterrent action and larvicidal activity toward *S. zeamais* and *Ae. Aegypti*, respectively. The nutritional and feeding deterrence indices of insects exposed to meliternatin (**6**) are shown in Table 1. Compound **6** significantly ($P<0.05$) reduced the growth rate (RGR), food consumption rate (RCR) and efficiency of food conversion (ECI) of *S. zeamais* adults at concentrations of 30 ppm and above in a concentration-dependent manner. The feeding deterrence index (FDI) at 30–300 ppm increased from 16.6 to 81.4%. The EC_{50} (the concentration needed to inhibit insect feeding by 50% relative to controls) for *S. zeamais* is 125 ppm, as determined by probit analysis (Finney, 1971).

Meliternatin (**6**) showed very good larvicidal activity against *Ae. aegypti* with an LC_{50} value of 0.47 μg/ml (Table 2). For comparison, LC_{50} values are shown for three other larvicidal principles from insecticidal plants in Sarawak, Malaysia (Table 2).

Table 1
Nutritional and feeding deterrence indices of *Sitophilus zeamais* adults on flour disks treated with meliternatin (**6**)[a]

Concentrations (ppm)	RGR (mean±S.D.) (μg/mg/day)	RCR (mean±S.D.) (μg/mg/day)	ECI (mean±S.D.)	FDI (%)
0	28±2.3 a	185±5.8 a	15±1.2 a	
30	16±0.1 b	127±2.8 b	13±0.6 ab	16.6
60	12±1.1 c	117±2.2 c	10±0.9 b	25.8
100	4.8±0.03 d	116±0.8 c	4±0.3 c	40.4
200	−31±1.2 e	73±2.5 d	–	59.5
300	−31±1.0 f	34±2.1 e	–	81.4

[a] Means in the same column followed by the same letters do not differ significantly ($P>0.05$) in ANOVA and Tukey's tests.

3. Experimental

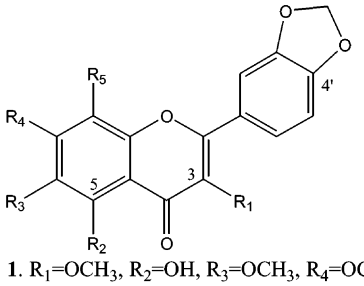
^{1}H NMR spectra were run at 500 MHz (Bruker AMX 500) in $CDCl_3$ using TMS as internal standard. EIMS were obtained at 70 eV direct inlet system of the VG Micromass 7035 instrument. Chromatographic separations were carried out on Merck 9385 silica gel. TLC spots were detected under UV (254 nm) and the plates heated to 100 °C after spraying with 5% H_2SO_4.

3.1. Plant material

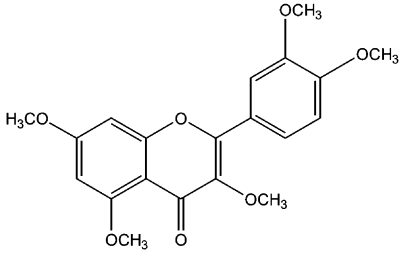
The leaves of *M. subunifoliolata* were collected in September 1998 in K. Kinabalu, Malaysia and identified by L. Madani. Voucher specimens (SAN145390) were deposited at the hebarium of the Forest Research Centre, Sepilok, Sandakan, Sabah, Malaysia.

3.2. Extraction and fractionation

Air dried, milled leaves (1.25 kg) were extracted repeatedly with MeOH and after removal of solvent in vacuo provided 62 g of crude extract. The crude dried extract was found to exhibit feeding deterrent activity against *S. zeamais* and larvicidal activity against *Ae. aegypti*. Column chromatography of the extract was undertaken using an *n*-hexane and *n*-hexane–EtOAc mixture of increasing polarity and afforded compounds **1–7** successively.



1. R_1=OCH_3, R_2=OH, R_3=OCH_3, R_4=OCH_3, R_5=OCH_3
2. R_1=OCH_3, R_2=OH, R_3=H, R_4=OCH_3, R_5=OCH_3
3. R_1=OCH_3, R_2=OCH_3, R_3=OCH_3, R_4=OCH_3, R_5=OCH_3
4. R_1=OCH_3, R_2=OCH_3, R_3=OCH_3, R_4=OCH_3, R_5=H
5. R_1=OCH_3, R_2=OCH_3, R_3=H, R_4=OCH_3, R_5=H
6. R_1=OCH_3, R_2=OCH_3, R_3, R_4=-OCH_2O-, R_5=H



7

Table 2
Larvicidal effect on *Aedes aegypti* of meliternatin compared with three other botanical insecticides

Compound	LC_{50} (μg/ml)[a] (95% C.L.)[b]	LC_{90} (μg/ml)[a] (95% C.L.)[b]	Slope ±S.E.[c]
Meliternatin	0.47 (0.37–0.59)	1.45 (1.03–2.62)	2.60±0.40
Naringenin[d]	3.7 (3.1–4.5)	15.1 (11.0–24.5)	2.10±0.24
Annonacin[d]	9.5 (7.5–12.2)	22.3 (16.4–41.0)	3.48±0.69
Goniothalamin[d]	15.0 (12.0–18.0)	57.7 (44.5–85.1)	5.89±0.81

[a] LC = lethal concentration.
[b] 95% CL = confidence interval at 95% confidence level.
[c] S.E. = standard error.
[d] Ee (1996).

3.3. Feeding deterrence test

S. zeamais was obtained from laboratory cultures maintained in incubators at 30±1 °C and 70–80% relative humidity. *S. zeamais* was reared on whole wheat at 12–13% moisture content. Adults of this species used in the experiments were about 2 weeks old. A flour disk bioassay was used to direct the isolation of active compounds from *M. subunifoliolata* according to the method of Xie et al. (1996) with some modifications. Wheat flour (1 g) was ultrasonically stirred in 5 ml of distilled water and 50 μl ethanol containing an active fraction or compound was added. Aliquots of 200 μl of this stirred suspension were placed on the bottom of a polystyrene Petri dish to form disks. The same amounts of ethanol, wheat flour and water were applied to produce the control flour disks. The disks were left in the fume-hood overnight to air dry. The disks were then transferred to an incubator to equilibrate at 30±1 °C and 70–80% relative humidity for 48 h. Each flour disk weighed between 36 and 39 mg. The moisture content of the disk was determined to be 13.5±0.1% using the Kett's Grain moisture tester (Model PB-1D_2, Japan). The disk was placed in glass vials for weighing. Ten group-weighed, unsexed insects were then added to each vial prior to further weighing. All the insects were starved for 24 h before use. The experimental set-up was left in the incubator for 3 days, after which the weights of the live insects and flour disks were taken. The following calculations were made for the study of nutritional indices (Manuwoto and Scriber, 1982; Farrar et al., 1989):

Relative growth rate

$$\mathrm{RGR} = (A - B)/(B \times 3)$$

Relative consumption rate

$$\mathrm{RCR} = D/(B \times 3)$$

Efficiency of conversion of ingested food

$$\mathrm{ECI}\ (\%) = (\mathrm{RGR}/\mathrm{RCR}) \times 100$$

Feeding deterrence index

$$\mathrm{FDI}\ (\%) = (C - T)/C \times 100$$

where A = weight of live insects on the 3rd day/number of live insects on the 3rd day, B = initial weight of insects/original number of insects, D = biomass ingested food/number of live insects on the 3rd day, C = the weight of control disks consumed and T = the weight of treated disks consumed, as the control and treated disks were placed in separate vials in no-choice tests. The EC_{50} (the concentration that inhibits insect feeding by 50% relative to control) was determined by probit analysis using the PriProbit Program (Sakuma, 1998) and the means were compared by using ANOVA and Tukey's Tests.

3.4. Mosquito larvicidal assay

Investigations on the larvicidal activity of meliternatin on *Ae. aegypti* were carried out using the method recommended by WHO (World Health Organization, 1980). A standard stock solution of 5000 μg/ml was prepared by dissolving 100 mg extract or compound in 20 ml of absolute ethanol. A test solution was made by pipeting a sample of the stock solution into 25 ml of chlorine-free tap water in a glass container. The concentrations of the test solutions were 50, 100 and 150 ppm. A control was prepared by using 1.5 ml ethanol in 50 ml of chlorine-free water. The test sample was made up to 50 ml with chlorine-free water. Ten late third-instar mosquito larvae were introduced into each glass container by a dropper. A little larval food (roasted cow's liver) was added. Mortality of the mosquito larvae was evaluated after 24 h. A series of at least 5 concentrations in duplicates was needed to obtain LC_{50} and LC_{90} values. Results were analyzed using Probit Analysis.

Acknowledgements

This work was supported by an A*STAR grant No. R-154-000-016-305 under the Singapore-Ontario Joint Research Program. The authors thank L. Madani for help in identifying the plant material.

References

Ee, G.C.L., 1996. Larvicidal Principles from Some Insecticidal Plants of Sarawak. PhD thesis, Department of Chemistry, University of Malaya, Malaysia.

Farrar, R.R., Babour, J.D., Kennedy, G.G., 1989. Quantifying food consumption and growth in insects. Ann. Entom. Soc. Am. 82, 593–598.

Finney, D.J., 1971. Probit Analysis, 3rd ed. Cambridge University Press, Cambridge.

Heyde, J.V.D., Saxena, R.C., Schmutterer, H., 1984. Neem oil and neem extracts as potential insecticides for control of Hemipterous rice pests. Nat. Pestic. Neem Tree Other Trop. Plants 161, 377–390.

Higa, T., Scheuer, P.J., 1974. Hawaiian plant studies. XVI. Coumarins and flavones from *Pelea barbigera* (Rutaceae). J. Chem. Soc., Perkin Trans. 1, 1350–1352.

Higa, M., Miyagi, Y., Yogi, S., Hokama, K., 1987. Flavonoid constituents of *Melicope triphylla* Merr. Yakugaku Zasshi 107, 954–958.

Higa, M., Ohshiro, T., Ogihara, K., Yogi, S., 1990. Flavonoid constituents of *Melicope triphylla* Merr. II. Yakugaku Zasshi 110, 822–827.

Jong, T.T., Wu, T.S., 1989. Highly oxygenated flavonoids from *Melicope triphylla*. Phytochemistry 28, 245–246.

Manuwoto, S., Scriber, J.M., 1982. Consumption and utilization of three maize genotypes by the southern armyworm. J. Econ. Entom. 75, 163–167.

Sakuma, M., 1998. Probit analysis of preference data. Appl. Entomol. and Zool. 33, 339–347.

World Health Organization, 1980. Instructions for determining the susceptibility or resistance of mosquito larvae to insecticide (WHO/VBC/8.807).

Xie, Y.S., Bodnaryk, R.P., Fields, P.G., 1996. A rapid and simple flour-disk bioassay for testing substances active against stored-product insects. Can. Entomol. 128, 865–875.