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Arborine, a Larval Growth Inhibitor from *Glycosmis pentaphylla*

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ABSTRAK

Arborina (1), suatu alkaloid kuinazolon telah diasingkan daripada daun *Glycosmis pentaphylla* (Rutaceae) dan didapati memberikan kesan perencat pembesaran ke atas larva *Drosophila melanogaster*.

ABSTRACT

Arborine (1), a quinazolone alkaloid was isolated from the leaves of *Glycosmis pentaphylla* (Rutaceae) and was found to have a growth inhibitory effect on the larvae of *Drosophila melanogaster*.

Keywords: *Glycosmis pentaphylla*, Rutaceae, arborine, larval growth inhibitor, *Drosophila melanogaster*

INTRODUCTION

In the course of our search for insect development inhibitors in plants, we screened the EtOH extracts of dried leaves of *Glycosmis pentaphylla* Correa by the *Drosophila test* (Nakajima and Kawazu 1977). We found that the EtOH extracts of *G. pentaphylla* inhibited the larval growth of fruit-fly (*Drosophila melanogaster*). *G. pentaphylla*, of the Rutaceae family, is a well-known plant in South East Asia. It is used in traditional preparations to treat facial inflammation, itchiness, intestinal problems and coughs (Perry 1980). In this paper we report the isolation of the active compound, arborine **(1)** and its development inhibition activity against the fruit-fly.

MATERIALS AND METHODS

General

UV spectrum was recorded on a Hitachi 200-20 spectrophotometer. IR spectrum was recorded on a Perkin Elmer 727B spectrophotometer. NMR spectra were recorded on a Bruker 300 MHz machine operating at 300 MHz(^{1}H) and 75 MHz(^{13}C). Samples were dissolved in $CDCl_3$ containing TMS as an internal standard. TLC were carried out on Merck pre-coated silica gel F_{254} plates while column chromatography was performed using TLC grade silica gel without binder.

Plant Material

Leaves of *G. pentaphylla* were collected from Kelantan in early 1990. A voucher specimen was deposited at the Herbarium, Department of Botany, Universiti Kebangsaan Malaysia, Bangi, Selangor.

Extraction and Isolation

The powdered air-dried leaves of *G. pentaphylla* (667.3 g) were defatted with petroleum ether and then extracted with ethanol (4L × 3) at room temperature for 24 h. After removing the solvent, the residue was extracted with HCl (1 N) which was then neutralised (NaOH) and extracted with $CHCl_3$. The evaporation of the $CHCl_3$ layer gave a thick oily residue (3.2 g). Treatment with ethyl acetate - methanol mixture gave a crystalline solid which was then recrystallised from the same solvent (2 : 8) to give **(1)**.

Arborine **(1)** was isolated as white needles (0.6 g, 0.1%), m.p 160-161°C (Chakravarti *et al.*, 1961); UV λ_{max} (EtOH): 245, 260, 277, 284 and 306 nm; IR ν_{max} (KBr): 3560, 3370, 1650, 1610, 1530 and 1490 cm^{-1}; ^{1}H-NMR δ ($CDCl_3$): 3.60 (s, 3H, N-CH_3), 4.26 (s, 2H, -CH_2Ph), 7.27(dd, J = 8.6, 1.3 Hz, 1H, H-8), 7.29(s, 5H, Ph), 7.44(t, J = 7.8 Hz, 1H, H-6), 7.68(ddd, J = 8.6, 7.8, 1.3 Hz, 1H, H-7), 8.35(dd, J = 7.8, 1.3 Hz, 1H, H-5); ^{13}C-NMR ppm : (Table 1); MS m/z (%): 250 (47), 249 (100), 149(10), 133 (21), 132(14), 125 (15), 117(13), 106(9), 105(22), 92(15), 91(28) and 90 (15).

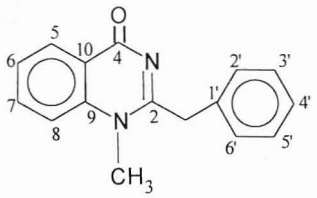
Bioassay

The larval growth inhibitory activity of *D. melanogaster* was assayed by the *Drosophila* test (Kawazu *et al.* 1977). Ten newly-hatched 1st-instar larvae were introduced into the diet (2 g) [a mixture of dry yeast powder (20 g), honey (40 g), agar (3 g), *n*-butyl *p*-hydroxybenzoate (0.3 g) and water (250 g)] containing the test material in a 30 ml sample vial (ϕ 25 mm × 55 mm) and left for two weeks at room temperature. The development state of the larvae was observed, and the number of survivors at successive stages of development recorded every other day and compared with that of a control. The assay was carried out in five duplicates and at gradient concentrations.

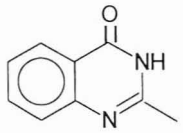
RESULTS AND DISCUSSION

The alkaloid arborine **1** was first isolated from the leaves of *Glycosmis arborea* and its structure was further confirmed as 2-benzyl-1-methylquinazol-4-one based on UV, IR and ^{1}H-NMR data (Chakravarti *et al.*, 1961). Compound **1** is optically inactive with a molecular formula $C_{16}H_{14}N_2O$, and gives intense peaks at m/z 249 ($M^+ - 1$), 133 and 91 in the MS spectrum. We would like here to update the spectral data of **1** since the last reported data (Chakravarti *et al.* 1961) were incomplete.

The ^{1}H-NMR spectrum of **1** showed the presence of two singlets at δ3.60 and 4.26 due to the N-CH_3 and benzylic methylene protons, respectively. A singlet at δ 7.29 was assigned to the five protons of the isolated phenyl nucleus. A doublet of doublet at δ 7.27 which was partly masked by the phenyl nucleus signal and a triplet centred at δ 7.44 (J = 7.8 Hz) were assigned to the protons at C-8 and C-6, respectively. Proton resonances of H-7 occurred as a doublet of doublet of doublet at δ 7.68 (J = 8.6, 7.8 and 1.3 Hz). Another doublet of doublet at δ 8.35 (J = 7.8 and 1.3 Hz) was assigned to the deshielded aromatic proton in the *peri*-position to the carbonyl group of a quinazolone system. The ^{13}C-NMR spectrum showed the presence of 16 carbons with 4 quartenary carbons at δ 169.0, 162.1, 141.5 and 120.0. The spectrum also showed the presence of one methylene carbon which was confirmed by DEPT experiment. Complete assignments for the ^{13}C-NMR chemical shifts of arborine were made by comparison with the data of similar compounds **(2)**, **(3)** and **(4)** for both the heterocylic and the isolated phenyl part of the molecule (Table 1) (Dreyer and Brenner 1980).



(1)



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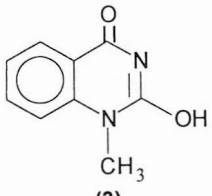

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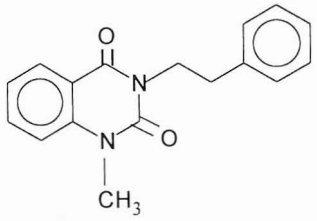

(4)

TABLE 1
^{13}C-NMR chemical shift data for **(1)**, **(2)**, **(3)** and **(4)** in ppm

Carbon no.	**(2)**[a]	**(3)**[a]	**(4)**[a]	**(1)**
2	155.2	141.6	140.4	162.2
4	162.2	161.7	161.4	169.0
5	126.9	127.2	128.9	128.5
6	126.1	122.3	122.8	125.9
7	134.4	135.1	134.9	133.7
8	126.1	114.5	113.3	114.4
9	149.5	150.2	150.7	141.5
10	121.2	115.5	155.4	120.0
N-CH_3		29.4	30.6	34.8
CH_2				43.4
1'			138.6	134.5
2'			128.9	128.2[b]
3'			128.4	129.1[b]
4'			126.4	127.4
5'			128.4	129.1[b]
6'			128.9	128.2[b]

[a] From reference 2
[b] Assignment may be interchanged

The growth inhibition activity of arborine is shown in Table 2. The 1st-instar larvae were introduced into the diet containing arborine. Arborine completely retards the larval growth, resulting in very small and abnormally shaped larvae and pupae. The minimum concentration for the inhibitory effect on the 1st instar larvae of *D. melanogaster* was 0.8 mg/2 g diet (Table 2). However, at lower concentrations, arborine has no effect on the larval growth. From these results, we conclude that arborine has potential as a larval growth inhibitor.

TABLE 2
Larval growth inhibitory activity of arborine **(1)** against *Drosophila melanogaster*[a]

Days Dose/Diet (mg/2g)	2	3	4	6	8	10	12
4.0	5Ls	5Ls	4Ls	2Ls	0	0	0
3.0	7Ls	6Ls	5Ls	3Ls	2Ls	0	0
2.5	8L	7Ls	5Ls	3Ls	1Ls	0	0
0.8	8L	5L	5L	4Ps	2Ps	2Ps	0
0.6	8L	8L	8L	2P6L	3A5P	6A2P	8A
0.2	8L	8L	8L	4P4L	4A4L	7A1P	8A
Control	10L	10L	10L	10P	10A	10A	10A

[a]L = larvae P = pupae A = adult s = small

Apart from quinazolone, other alkaloids such as foruquinoline, acridone and carbazole are known to be distributed in *Glycosmis* species (Sarkar and Chakraborty 1977; Sarkar Chakraborty 1978). We will further test the alkaloids from this genus to evaluate their effectiveness to control insect pest.

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