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POTENTIAL AGROCHEMICALS FROM LEAVES OF *WEDELIA BIFLORA*

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Key Word Index—*Wedelia biflora*; Compositae; *Rhizoctonia solani*; *Pythium ultimum*; *Anthonomous grandis*; 3,3′-di-*O*-methylquercetin; 2,7-dihydroxy-3(3′-methoxy-4′-hydroxy)-5-methoxyisoflavone; 3′,7-di-*O*-methylquercetin.

Abstract—Four compounds, veratrylidenehydrazide, 3,3′-di-*O*-methylquercetin, 2,7-dihydroxy-3(3′-methoxy-4′-hydroxy)-5-methoxyisoflavone and 3′,7-di-*O*-methylquercetin were isolated from the methylene chloride extract of dried leaves of *Wedelia biflora*. The structures were elucidated from spectroscopic evidence. The third compound possessed antifungal and boll weevil antifeedant activity while the fourth showed antifungal activity.

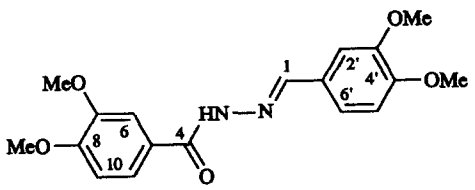
INTRODUCTION

The Thai plant, *Wedelia biflora*, is used in folk medicine for headaches and fevers [1]. Guerrero showed that the leaves, in decoction, are vulnerary and antiscabious [1]. According to Ridley, the leaves are used for dressing ulcers, while the juice of the leaves is given internally with cow's milk as a tonic [1] after childbirth. Only a few species of *Wedelia* have been investigated [2–4]. Five compounds were isolated from extracts of stems of *W. biflora* [5], 16-ene-kaur-19-oic acid, 24-ethylcoprostanone, stigma-7-en-3-ol, stigmasterol, grandifloric acid and *ent*-kauradienoic acid.

RESULTS AND DISCUSSION

In our study, the methylene chloride extract of leaves from *W. biflora* was shown to posses antifeedant properties against the cotton boll weevil, (*Anthonomus grandis* Boh.) using the Hedin method [6] and antifungal activity against *Pythium ultimum* and *Rhizoctonia solani* using the 'paper disc' method [7]. Utilization of the antifungal bioassay as a guide led to the isolation of veratrylidenehydrazide (1), 3,3′-di-*O*-methylquercetin (2), 2,7-dihydroxy-3(3′-methoxy-4′-hydroxy)-5-methoxyisoflavone (3), and 3′,7-di-*O*-methylquercetin (4). These compounds have not been reported previously from this plant. Also, this is the first report of **1** from natural sources.

Veratrylidenehydrazide (**1**) was isolated as an orange powder, mp 140°. A molecular formula of $C_{18}H_{20}N_2O_5$ was established by HR mass spectrometry ($[M]^+$ *m/z* obsd 334.13581). This formula indicated 10 degrees of unsaturation. The presence of aromaticity in the molecule



1

was suggested by an IR band at 1600 and 1480 cm^{-1}. A band at 3400 cm^{-1} indicated the presence of a N–H stretch, while a band at 1620 cm^{-1} provided evidence for a carbonyl group. The aromatic nature of **1** was confirmed by its ^1H NMR spectrum, which showed resonances at δ7.82 (2H, *d*, $J=2$ Hz, H-9 and H-5′). There were four methoxy resonances at δ3.90, 3.88, 3.86 and 3.84, signals for a H–C=N proton at δ6.30 and a N–H proton at δ1.58. The IR and mass spectral data were identical to those published for veratrylidenehydrazide in the literature [8]. To our knowledge, **1** has not been previously isolated from a plant. But it has been synthesized [4]; however, complete ^1H NMR data of **1** has not been previously reported.

Compound **2** was identified as 3,3′-di-*O*-methylquercetin by mmp, co-TLC, IR, NMR and mass spectral comparison with an authentic sample [9–11]. Compound **2** showed antifeedant activity against the boll weevil of 50% (Table 1). Compound **3** was identical to 2,7-dihydroxy-3(3′-methoxy-4′-hydroxy)-5-methoxy-isoflavone upon comparison by mmp, co-TLC, NMR and mass spectral comparison with an authentic sample [12]. Compound **3** showed activity against the fungus *R. solani* (78%) (Table 2).

Upon comparison with standard ^1H NMR and physical properties reported in the literature, **4** was identified

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Table 1. Boll weevil bioassay of compounds from *W. biflora* leaves

Compound	Dosage	% T/C	% Inhibition
2	3.5	50	50.0
3	2.0	29	71.0

% T/C = [No. of punctures of test paper (T) /no. of punctures of control paper (C)] × 100.

Table 2. Antifungal activity of compounds from *W. biflora* leaves

Compound	P	R
3	10%	78%
4	—	100%

Dosage = 1 mb ml^{-1}. P = *P. ultimum*. R = *R. solani*.

% T/C = [Inhibition zone radius (mm) caused by sample/inhibition zone radius (mm) caused by control]100.

Control = Dithane M-45.

as the flavone, 3′,7-di-*O*-methylquercetin [13]. Compound **4** exhibited 100% inhibition against the fungus *R. solani* (Table 2) and 71% inhibition against the feeding of cotton boll weevils (Table 1).

In summary, of the four compounds, three possess antifungal and/or boll weevil antifeedant activity. While these compounds have previously been isolated from other sources or synthesized, this is the first report of the investigation of their bioactivity against boll weevils and fungi (Tables 1 and 2). The activity of 3′,7-di-*O*-methylquercetin against the fungus *R. solani* is equivalent to that of the commercially available fungicide Dithane M-45. Also, to our knowledge, this is the first report of veratrylidenehydrazide (**1**) as a natural product and confirmation of the structure via NMR studies.

EXPERIMENTAL

General. IR spectra were recorded as KBr pellets. ^1H NMR were recorded at 200 MHz in $CDCl_3$ with TMS as int. std. GC analysis were performed on a DB-5 column. MS were recorded in the EI mode. CC was performed with silica gel 60–200 mesh ID (Grade 62) Fisher, TLC with silica gel GF_{254} (Type 60) Merck.

Antifungal bioassay was performed by using the preliminary 'paper disc' method [7].

Boll weevil bioassay. Performed using the Hedin method [6].

Plant material. Wedelia biflora Linn. DC was collected in March in Samut Sakron, Thailand. Voucher number 46402 is deposited in the forest Herbarium (BFK) of the Royal Forest Department, Flora of Thailand.

Extraction and isolation. Dried powdered leaves (2 kg) were extracted with CH_2Cl_2 (10 l) in an extractor for 7 days at room temp. The CH_2Cl_2 soln was removed from the extractor and evapd *in vacuo* to yield 30 g of a crude ext. This extract (24 g) was dissolved in $CHCl_3$ and added to 10 g of silica gel (Woelm 63–200 mesh). The $CHCl_3$ solvent was removed *in vacuo* to yield a dry sample which was chromatographed on a open column of silica gel. The column was eluted with a hexane–CH_2Cl_2–MeOH system. Four compounds were isolated, veratrylidenehydrazide, 3,3′-di-*O*-methylquercetin, 2,7-dihydroxy-3(3′-methoxy-4′-hydroxy)-5-methoxyisoflavone and 3′,7-di-*O*-methylquercetin.

Veratrylidenehydrazide (**1**) Orange powder (60 mg) mp 140°. HRMS $[M]^+$ at *m/z* 334.13581, $C_{18}H_{20}N_2O_5$ requires 334.1370. UV λ_{max}^{EtOH} 320, (log ε 4.50), 368 (log ε 4.56), 376 (log ε 4.57) nm. IR ν_{max}^{KBr} 3400, 2900, 1620, 1600, and 1480. ^1H NMR (200 MHz, $CDCl_3$): δ 1.58 (1H, *s*), 3.84 (3H, *s*, OMe), 3.86 (3H, *s*, OMe), 3.88 (3H, *s*, OMe), 3.90 (3H, *s*, OMe), 6.30 (1H, *s*), 6.90 (2H, *d*, *J* = 6.2 Hz, H-9 and H-5′), 7.60 (2H, *d*, *J* = 2 Hz, H-10 and H-6′), and 7.82 (2H, *s*, H-6 and H-2′). MS *m/z* $[M]^+$ 334, 329, 301, 285, 255, 210, 195, 165. Anal. Calcd for $C_{18}H_{20}N_2O_5$: C, 62.78; H, 5.85; Found: C, 61.13, H, 5.79.

3,3′-*Di-O-methylquercetin* [2]. Yellow crystals (100 mg) mp 256°. HRMS $[M]^+$ at *m/z* 330.293, $C_{17}H_{14}O_7$ requires 330; 293. UV λ_{max}^{EtOH} 372, 354, 300, 252 nm. IR ν_{max}^{KBr} 3520, 3240, 1650, 1620, 1600, 1480. ^1H NMR (200 MHz, $CDCl_3$): δ 3.95 (3H, *s*, OMe), 4.05 (3H, *s*, OMe), 6.40 (1H, *d*, H-6, Ar), 6.50 (1H, *d*, H-8, Ar), 7.00 (1H, *d*, H-5′, Ar), 7.80 (2H, *d*, H-2′ and H-6′, Ar). MS *m/z* $[M]^+$ 330, 315, 287, 259, 135, 79. Anal Calcd for $C_{17}H_{14}O_7$: C, 61.82; H 4.27, mol wt 330.293. Found: C, 62.21; H, 3.72.

Acetylation of compound **2**. 3,3′-Di-*O*-methylquercetin (2 mg) was treated with Ac_2O in pyridine at room temp. for 16 hr. The mixt. was then poured into crushed ice with stirring. After all the ice had melted, the mixt. was extracted with CH_2Cl_2. Evapn of solvent after drying with Na_2SO_4 gave the triacetate of **2** as yellow crystals, mp 192–193°.

2,7-*Dihydroxy*-3(3′-*methoxy*-4′-*hydroxy*)-5-*methoxy-isoflavone* (3). Yellow needles (140 mg), mp 232°. HRMS $[M]^+$ at *m/z* 330.00, $C_{17}H_{14}O_7$ requires 330, 293. UV λ_{max}^{EtOH} 320, 300, 298, nm. IR ν_{max}^{KBr} 3540, 3100, 1640, 1600, 1540. ^1H NMR (200 MHz, $CDCl_3$): δ 3.8 (3H, *s*, OMe), 3.9 (3H, *s*, OMe), 6.2 (2H, *d*, H-6 and H-8, Ar), 6.98 (2H, *d*, H-3′ and H-5′, Ar), 7.02 (1H, *d*, H-6′, Ar). MS *m/z* $[M]^+$ 330, 315, 287, 259, 203, 149, 77.

3′,7-*Di-O-methylquercetin* (4). Yellow needles (110 mg). mp 240°. HRMS $[M]^+$ at *m/z* 330.070, $C_{17}H_{14}O_7$ requires 330.293. UV λ_{max}^{EtOH} 396, 380. IR ν_{max}^{KBr} 3460–3440, 3380, 1650, 1600, 1580, 1500. ^1H NMR (200 MHz, $CDCl_3$): δ 3.9 (3H, *s*, OMe), 4.00 (3H, *s*, OCH_3), 6.40 (1H, *d*, H-8, Ar), 6.90 (1H, *s*, H-5′), 7.49 (1H, *d*, H-6′), 7.81 (1H, *d*, H-2′, Ar), 11.70 (1H, *s*, OH), 12.02 (1H, *s*, OH). MS *m/z* $[M]^+$ 330, 329, 315, 299, 273, 193, 167, 151, 137, 107, 95, 77.

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