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TERMITE ANTIFEEDANT ACTIVITY IN *AFRAMOMUM MELEGUETA**

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Key Word Index—*Aframomum melegueta*; zingiberaceae; seeds; *Reticulitermes speratus*; Isoptera; antifeedants; aryldecanones; gingerdione; gingerol; shogaol; paradol.

Abstract—*n*-Hexane and methanolic seed extracts of *Aframomum melegueta* were studied for termite antifeedant activity against workers of *Reticulitermes speratus*. Strong antifeedant activity was observed when the termite workers were tested in a choice filter paper disk bioassay containing 1% crude extracts. Bioassay-directed fractionation led to the isolation of gingerdione, 5-oxo-1-(4-hydroxy-3-methoxyphenyl)decan-3-one, [6]-paradol, 1-(4-hydroxy-3-methoxyphenyl)decan-3-one, [6]-gingerol, 5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)decan-3-one and [6]-shogaol, 1-(4-hydroxy-3-methoxyphenyl)dec-5-en-3-one, as the antifeedant compounds. [6]-Gingerol and [6]-shogaol exhibited the strongest antifeedant activity at 1000 ppm, corresponding to 8 $\mu g\,cm^{-2}$.

INTRODUCTION

In continuation of our search for natural pesticides in plants, we have focused part of our efforts on the discovery of naturally occurring termite feeding deterrent compounds from tropical plants. The pungent principles of *Aframomum melegueta* have been extensively studied [2–4]. These compounds have been used traditionally as preservatives in herbal formulations and in the preservation of stored grains [5]. They have also been patented in an antihelminthic composition [6], while antimicrobial, molluscicidal and antischistosomal activities have also been reported [7, 8]. To our knowledge, there is little known about the insect antifeedant activity of these compounds. The strong antifeedant activity of a *A. melegueta* seed extract against the subterranean termite, *Reticulitermes speratus*, in a choice filter paper disk bioassay and the active compounds responsible for this activity are discussed in this report. The structures of the active compounds were confirmed by spectroscopic methods and comparison with authentic samples. Gingerdione is reported in *A. melegueta* for the first time.

RESULTS AND DISCUSSION

The combined hexane and methanolic extracts of seeds of *Aframomum melegueta* showed strong antifeedant activity against workers of *Reticulitermes speratus*, when tested initially at 1% in a choice bioassay. Initial separation of the crude extract by TLC revealed the presence of four major compounds and eight minor ones. Bioassay directed fractionation of the crude extracts and subsequent purification of the active fractions by silica gel column chromatography led to the isolation of compounds **1**–**4**. The structures of these compounds were confirmed by ^1H and ^{13}C NMR, ^1H–^1H COSY, DEPT, ^{13}C–^1H COSY, low and high resolution mass spectrometry and by comparison of the data with those already reported in the literature [2–4, 9–11]. The variation in the chemical constituents of *A. melegueta* has been noted previously [2], and gingerdione (**2**) was isolated as a major constituent from plant material collected from SW Nigeria, a climatic zone between forest and the true savannah.

Individual compounds were tested later for termite antifeedant activity. Table 1 shows their Antifeedant Index values for a range of concentrations. Compound **4**, with a hydroxyl group at C-5, and **1** were the most active at 1000 ppm (8.0 $\mu g\,cm^{-2}$), while **3**, with a *trans*-double bond between C-5 and C-6, which is in conjugation with the carbonyl group, was only effective as a feeding deterrent at 5000 ppm (39.8 $\mu g\,cm^{-2}$), a five-fold decrease when compared with **1** or **4**. When a synthetic analogue (**5**), with two double bonds on both sides of the carbonyl group and two 1,3-benzodioxole groups at C-1 and C-6 was tested for antifeedant activity at 10000 ppm, its activity ($I = 17.98$) was less than that for compounds **1**–**4**, being about equal to that of **3** at 5000 ppm. This observation suggests that introduction of a double bond either at C-1 or C-5, if in conjugation with the carbonyl group, reduces antifeedant activity. Gingerdione (**2**), possessing an extra carbonyl group at C-5, was the least active of the four compounds tested. These results also imply that the

*Part 2 in the series 'Termite antifeedant activity in tropical plants'. For Part 1 see ref. [1].

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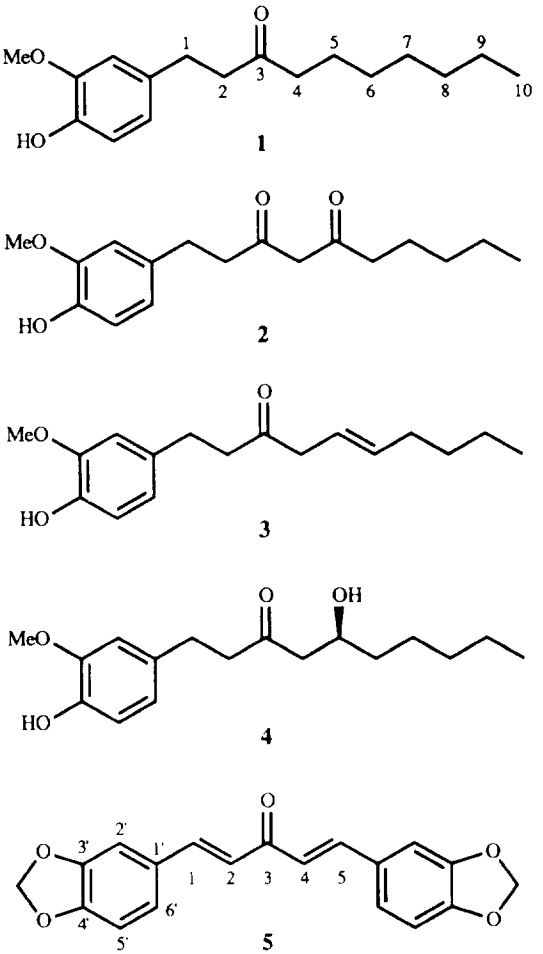
Table 1. Antifeedant Index values for compounds 1–5

Dose (ppm)	1	2	3	4	5
10000	0.9*		5.8	3.4*	18.0*
7500	10.2*	8.4*	17.5*	5.6	
5000	22.8*	41.9	15.2*	8.2*	
2500	1.3*	35.4	34.5	3.0*	
1000	17.6	27.3	45.8	19.6*	
Control	33.9				

*Significant differences between treatment and control; Mann–Whitney U-test, $P < 0.05$.



carbonyl group at C-3 with or without a hydroxyl group at C-5 is important for antifeedant activity observed against workers of *R. speratus*, but the introduction of a hydroxyl group at C-5 enhanced the activity.

Compounds **1** (0.87%), **2** (1.71%), **3** (0.81%) and **4** (1.25%) all demonstrated complete feeding inhibition at their natural concentrations as seen in their low antifeedant index values (Table 1); this suggests a protective role for these compounds against seed predators in their natural habitat. The potent termite antifeedant activity demonstrated by **1** and **4** could provide new leads in the search for environmentally acceptable, alternative forms of termite control [12].

EXPERIMENTAL

Plant material. Dried fruits of *A. melegueta* were collected in Ogbomosho Local Government Area, Nigeria, and identified by Dr M. O. Akanbi of the Forestry Research Institute of Nigeria, Ibadan.

Extraction and isolation. Seeds (32 g) from 85 g of fruits were ground into a fine powder and extracted first with n-hexane (100 ml) for 24 hr at room temp. and then with MeOH (100 ml). The combined extract, after removal of solvent, yielded a pungent pale yellow oil (1.85 g, 5.8% of seed wt). The crude extract (100 mg) was initially sepd by prep. TLC (EtOAc–n-hexane, 1:4) to give four major compounds (**1–4**) which were individually purified on a short silica gel column using the same solvent system. Their structures were established by spectroscopic analysis and comparison with data already published in lit.

1-(4-*hydroxy*-3-*methoxyphenyl*) *Decan*-3-*one* (**1**). Yield 8.7 mg, 0.87%. Found *m/z* $[M]^+$, 278.1870, $C_{17}H_{26}O_3$. Calc. for 278.1872. Lit. [2].

5-*Oxo*-1-(4-*hydroxy*-3-*methoxyphenyl*) *decan*-3-*one* (**2**). Yield 17.7 mg and 1.77%. Found *m/z* $[M]^+$, 292.1814, $C_{17}H_{24}O_3$. Calc. for 292.1816. Lit. [10, 11].

1-(4-*hydroxy*-3-*methoxyphenyl*) *Dec*-5-*en*-3-*one* (**3**). Yield 8.1 mg, 0.81%. Found *m/z* $[M]^+$, 276.1739, $C_{17}H_{24}O_3$, calcd for 276.1740. Lit. [2].

5-*Hydroxy*-1-(4-*hydroxy*-3-*methoxyphenyl*) *decan*-3-*one* (**4**). Yield 12.5 mg, 1.25%. Found *m/z* $[M]^+$, 294.1858, $C_{17}H_{26}O_4$, calcd for 294.1860. Lit. [2, 9].

1,5-*Bis*-(3,4-*methylenedioxyphenyl*)*pent*-1,4-*dien*-3-*one* (**5**). A mixt. of 3,4-methylenedioxybenzaldehyde (3.75 g), 10% aq. NaOH (5 ml) and Me_2CO (2.5 ml) in 1,2-dimethoxyethane (50 ml) was stirred at room temp. for 12 hr. The reaction mixt. was diluted with H_2O and extracted with EtOAc. The EtOAc layer was washed with H_2O, brine and dried. Evapn of solvent gave a quantitative yield of **5**, which was recrystallized from EtOAc–*n*-hexane as yellow flakes, mp 198–200°. IR ν_{max}^{KBr} cm^{-1}: 1640 (C=O), 1620, 1580, 1490 (Ar). ^1H NMR ($CDCl_3$): δ6.0 (2H, *s*, 2 × OCH_2O), 6.79 (4H, *m*, H-2′, H-2″, H-6′, H-6″), 6.86 (2H, *d*, J = 15 Hz, H-2, H-4), 7.11 (2H, *d*, J = 7.9 Hz, H-5′, H-5″), 7.65 (2H, *d*, J = 15 Hz, H-1, H-3). ^{13}C NMR ($CDCl_3$): δ 101.6 (*t*, OCH_2O), 108.6 (*d*, C-2′, C-2″, C-5′, C-5″), 125.1 (*d*, C-2, C-4), 128.8 (*d*, C-6′, C-6″), 142.6 (*d*, C-1, C-5), 148.4 (*s*, C-3′, C-3″), 149.8 (*s*, C-4′, C-4″), 188.5 (*s*, C-3). EI-MS *m/z* (rel. int.) : 322 $[M]^+$, (100), 294 (5), 264 (7), 200 (44), 175 (22), 145 (29), 135 (59), 117 (27), 89 (61), 63 (26). EI-HR-MS: Found *m/z* $[M]^+$, 322.0840, $C_{19}H_{14}O_5$, calcd for 322.0845.

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