Pergamon

0031-9422(95)00153-7

Phytochemistry, Vol. 40, No. 4, pp. 1105–1112, 1995

0031-9422/95 $9.50 + 0.00

TERMITE ANTIFEEDANT ACTIVITY IN *XYLOPIA AETHIOPICA**

LABUNMI LAJIDE†, ‡PIERRE ESCOUBAS§ and JUNYA MIZUTANI

Research Development Corporation of Japan, (JRDC), Eniwa RBP, Megumino Kita 3-1-1, Eniwa-Shi, Hokkaido 061-13, Japan

(*Received in revised form* 4 *January* 1995)

Key Word Index—*Xylopia aethiopica*; Annonaceae; seeds; fruits; *Reticulitermes speratus*; Isoptera; antifeedants; ent-kaurane diterpenes; phenolic amides; lignanamides.

Abstract—A hexane extract of *Xylopia aethiopica* fruits and an aqueous methanol extract of the seeds were studied for termite antifeedant activity against workers of the subterranean termite, *Reticulitermes speratus*. The crude extract, at 1%, exhibited strong antifeedant activity in a choice filter paper disk bioassay. Bioassay-directed fractionation led to the isolation and identification of six ent-kaurane diterpenes in the hexane extract. Feeding deterrent activity varied significantly with the structures when the compounds were tested at concentrations ranging from 5000 ppm (40 μg cm^{-2}) to 100 ppm (0.824 μg cm^{-2}). (−)-Kaur-16-en-19-oic acid had the strongest termite antifeedants activity among the ent-kauranes isolated. Two phenolic amides and four lignanamides were also isolated from the aqueous methanolic extract of seeds. *E*-3-(4-hydroxy-3-methoxyphenyl)-*N*-2-[4-Hydroxyphenylethyl]2-Propenamide was more active than *E*-3-(3,4-dihydroxyphenyl)-*N*-2-[4-Hydroxyphenylethyl]2-propenamide, whilst grossamide and the new lignanamide, demethylgrossamide, as well as (−)-cannabisins B and D exhibited potent feeding deterrent activity at 5000 ppm. The identity of these compounds was established by spectroscopic analysis and synthesis. Two synthetic amides, *E*-3-(3,4-methylenedioxyphenyl)-*N*-2-[4-Hydroxyphenylethyl] propenamide and *E*-3-(3,4-methylenedioxyphenyl)-*N*-2-[octadecyl]-2-propenamide were also tested for antifeedant activity.

INTRODUCTION

Environmental and health problems related to the use of synthetic pesticides have made more urgent the need for less toxic, environmentally friendly insect control substances. As part of this effort, we have conducted an investigation of naturally occurring substances that possess insect antifeedant or growth retarding activity. One of our insect targets was the subterranean termite, *Reticulitermes speratus*, chosen as representative of wood-destroying insects of economic importance. Several alternatives to commonly used synthetic pesticides have been suggested, including cultural methods, biological control, selection of resistant crops, physical barriers and the use of natural insecticides or repellents [1]. In the latter case, relatively few studies have been conducted with the aim of finding efficient compounds with a potential for control [2–4], although the structures of many termite antifeedant allelochemicals have been described [5].

We present here results of a study of termite feeding-deterrent activity in fruits of *Xylopia aethiopica* and the isolation of the active constituents. The genus *Xylopia* has been extensively studied chemically and compounds already isolated include ent-kaurane diterpenes from fruits and bark [6–13], and alkaloids [14–16]. In Nigeria, dried fruits of *X. aethiopica* are used as a spice and in the treatment of oedema [17]. It has been reported that the wood of *X. aethiopica* is exceptionally hard and is believed to withstand attack from termites and other insects destructive to wooden structures [18]. A crude hexane extract of the fruits showed strong antifeedant activity against workers of *R. speratus* in a choice filter paper disk bioassay and subsequent bioassay-directed fractionation led to the isolation of ent-kaurane diterpenes, phenolic amides and lignanamides from an aqueous methanolic extract of the seeds as active constituents.

RESULTS AND DISCUSSION

The crude hexane extract of fruits of *X. aethiopica* was fractionated initially by gradient elution using silica gel column chromatography (n-hexane–EtOAc). Fractions were monitored by TLC and compounds with similar R_f values were pooled to give 16 fractions. Subsequent

* Part 1 in the series 'Termite antifeedant activity from tropical plants'.

† Author to whom correspondence should be addressed.

‡ Present address: Department of Chemistry, Federal University of Technology, P.M.B. 704, Akure, Nigeria.

§ Present address: Suntory Institute for Bioorganic Research, Wakayamadai, Shimamoto-cho, Mishima-gun, Osaka 618, Japan.

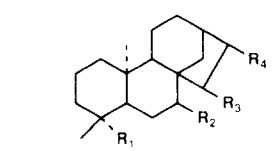
choice bioassay of the fractions at 1% indicated that activity was present in all of the fractions. The active fractions were concentrated, redissolved in n-hexane–chloroform (9:1) and allowed to recrystallize slowly at room temperature. Individual crystals were filtered and identified by physicochemical methods: ^1H and ^{13}C NMR, H–H COSY, DEPT, C–H COSY, low and high resolution mass spectra, and by comparing these data with those already reported in the literature [6–13]. The compounds identified were 7β-acetoxy (−)-kaur-16-en-19-oic acid (**1**), (−)-kaur-16-en-19-oic acid (**2**), (−)-kauran-16α-ol (**3**), 15β-acetoxy-(−)-kaur-16-en-19-oic acid (**4**), 15-oxo-(−)-kaur-16-en-19-oic acid (**5**) and (−)-kauran-16α-19-diol (**6**). When individual compounds were tested for antifeedant activity against *R. speratus*, all but four were potent at 5000 ppm. The antifeedant index values for the six compounds are listed in Table 1.

Table 1. Antifeedant index values for compounds **1–6**

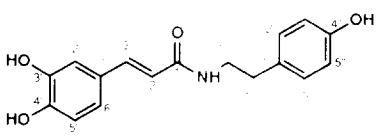
Dose (ppm)	1	2	3	4	5	6
5000	0*	0*	0*	47.1	0*	18.0
2500	0*	7.78	14.4*	32.9	14.1	22.4
1000	20.9	5.4*	30.3	37.4	32.2	46.5
750	29.8	12.2	20.7	51.6	25.2*	74.2
500	33.3	26.5	52.6	68.4	27.4	61.4
250	56.8	10.8	65.2	53.5	45.4	46.7
100	47.3	39.5	57.9	—	47.1	61.5
Control	46.7					

*Significant differences between treatment and control, Mann-Whitney U-test, $P^{69} < 0.05$.

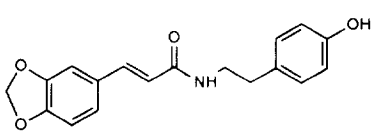
These results suggest that activity varies significantly with structure. With the exception of **4** and **6**, all the compounds demonstrated very strong feeding inhibition at 5000 ppm (40 μg cm^{-2}). Compound **2**, which displayed the strongest activity was used, as a basis for a structure–activity comparison among the compounds. It appeared that modification of (−)-kaur-16-en-19-oic acid either by reduction of the carboxylic acid at C-19 to a methyl or hydroxymethyl group, as in **3** and **6**, reduction and oxidation of the exomethylene group at C-16, as in **3** and **6**, or oxidation at C-15 and substitution with an acetoxy group, as in **1**, **4**, and **5**, all resulted in lower activity. However, **1–3** and **5** showed little variation in potency. The most drastic activity loss was observed for **4**, which was essentially inactive, and **6**, in which the activity was reduced by *ca* one order of magnitude.



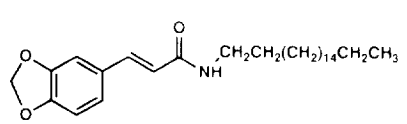
	R_1	R_2	R_3	R_4
1	CO_2H	β-OAc	H	H_2
2	CO_2H	H	H	H_2
3	CH_3	H	H	α-CH_3; β-OH
4	CO_2H	β-OAc	H	H_2
5	CO_2H	H	=O	H_2
6	CH_2OH	H	H	α-CH_3; β-OH



8



9



10

The crude aqueous methanolic seed extract of *X. aethiopica* also gave a very strong feeding deterrent activity at 10 000 ppm using a similar bioassay procedure. The crude extract was separated into 10 fractions by preparative TLC developed (× 3) with n-hexane–ethylacetate–chloroform–methanol (10:5:5:2). These fractions were then tested for antifeedant activity. Activity was found in all of them and further purification led to the isolation and identification of active compounds using a combination of ^1H, ^{13}C NMR, ^1H–^1H, COSY, ^{13}C-DEPT, ^{13}C–^1H COSY and low and high mass spectroscopic analyses. *E*-3-(4-Hydroxy-3-methoxyphenyl)-*N*-2-[4-hydroxyphenyl ethyl]-2-propenamide (**7**) and *E*-3-(3,4-dihydroxyphenyl)-*N*-2-[4-hydroxyphenylethyl]-2-propenamide (**8**) were isolated from the less polar fraction as the active compounds. The ^1H, ^{13}C NMR (Table 2) and mass spectra of **7** and **8** were identical to those previously reported in the literature [19–23] and their structures were confirmed by synthesis when tyramine was condensed with either ferulic acid or caffeic acid in the presence of diethyl phosphorocyanidate (DEPC). Grossamide, (**11**), a lignanamide previously isolated from *Capsicum annum* var. *grossum* [21] and from *Cannabis sativa* [24], and a new lignanamide, demethylgrossamide (**12**), (−)-cannabisin B (**13**) and (−)-cannabisin D (**14**) [25] were also isolated from the more polar fraction of the seed extract. The structures of these compounds were established by spectroscopic methods and comparison with data previously reported [21, 23–25].

The new lignanamide (**12**) because of the paucity of material was only identified by ^1H and ^{13}C NMR and

Table 2. ^{13}C NMR spectral data of compounds **7**–**10** (67 MHz), in CD_3OD

C	7	8	9		1 ($CDCl_3$)
1	166.6 *s*	166.5 *s*	166.9 *s*		166.0 *s*
2	122.5 *d*	121.6 *d*	120.9 *d*		118.9 *d*
3	140.5 *d*	140.9 *d*	139.8 *d*		140.5 *d*
1′	130.9 *s*	131.6 *s*	130.9 *s*		129.3 *s*
2′	111.2 *d*	114.2 *d*	106.8 *d*		106.3 *d*
3′	148.5 *s*	146.2 *s*	149.2 *s*		148.2 *s*
4′	149.1 *s*	147.9 *s*	149.7 *s*		148.8 *s*
5′	119.2 *d*	114.8 *d*	109.1 *d*		108.5 *d*
6′	130.6 *d*	130.9 *d*	124.2 *d*		123.7 *d*
1″	130.6 *s*	130.6 *s*	130.6 *s*		39.8 *t*
2″	130.7 *d*	130.7 *d*	130.6 *d*		31.8 *t*
3″	115.9 *d*	116.4 *d*	116.0 *d*		29.7 *t*
4″	156.4 *s*	156.6 *s*	156.6 *s*	4″–16″	26.9 *t*
5″	115.9 *d*	116.4 *d*	116.0 *d*	17″	22.7 *t*
6″	130.7 *d*	130.7 *d*	130.6 *d*	18″	14.1 *q*
α	41.4 *t*	41.9 *t*	41.9 *t*		—
β	35.6 *t*	35.6 *t*	35.6 *t*		—
OMe	56.1 *q*	—			
OCH_2O	—	—	102.4 *t*		101.4 *t*

Multiplicities were assigned using DEPT and $^{13}C-^1H$ COSY experiments.

mass spectroscopic methods and comparison with **11**. The ^1H and ^{13}C NMR spectra (Table 3) were very similar to those of **11**, but only one methoxy signal was observed for **12**. DIFNOE measurement on H-2 or H-3 of **11** or **12** confirmed the *trans*-configuration between the C-7 aryl and C-8 amide groups [23]. The FD-mass spectrum gave *m/z* 611.2392 ($[M + H]^+$, $C_{35}H_{35}N_2O_8$) and *m/z* 633 ($[M + Na]^+$).

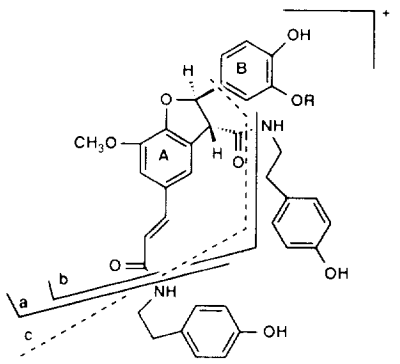
Furthermore, the fragmentation ions at *m/z* 352 and 229 from **11**, and *m/z* 338 and 229 from **12**, confirmed the position of the methoxy group in ring A of **12** (Scheme 1). The FAB-mass spectrum of **13** gave *m/z* 597.2235 ($[M + H]^+$, $C_{34}H_{33}N_2O_8$) while that of **14**, gave *m/z* 625.2550, ($[M + H]^+$, $C_{36}H_{37}N_2O_8$). The IR spectra for both compounds had absorption bands, for hydroxyl (3365 cm^{-1}) and amide (1650 cm^{-1}).

The ^1H NMR spectrum of **14** showed the presence of two tyramine moieties, six aromatic and/or olefinic protons, two methoxy signals and two methine protons signals which were coupled with each other. Similar data was observed for **13**, but with no methoxy signals. The ^1H and ^{13}C NMR data of **13** and **14** coincided with those reported for the racemic dihydronaphthalene lignans, cannabisins B–D [25]. The only difference was that in our case, **13** gave a negative specific rotation of $-38°$, while **14** gave a specific rotation of $-46°$. The coupling constants between H-1 and H-2 ($J_{1,2}$ = 3.9 Hz) of **13** and **14** possibly indicate a *cis*-configuration. As a result of the H-1, H-2 coupling constants, four possible optical isomers **a**–**d** (Fig. 1) were considered in order to determine the stereochemistry at C-1 and C-2. Structure **a** was ruled out because it shows a dihedral angle of *ca* 90° between H-1 and H-2 and the coupling constant should be close to zero. Structure **b** with a quasi-equatorial aryl substituent was also ruled out despite the fact that it is thermodynamically more stable having a coupling constant of the order of $J_{1,2}$ = 10–12 Hz. Structures **c** and **d** possess a dihedral angle of *ca* 55° between H-1 and H-2 and should, therefore, provide observable couplings of

Table 3. ^{13}C NMR spectral data for compounds **11** and **12** (67 MHz)

C	11			12	
2	88.5 *d*			89.4 *d*	
3	58.6 *d*	3a	172.8 *s*	58.6 *d*	171.7 *s*
4	113.0 *d*	4a	131.0 *s*	111.9 *d*	131.3 *s*
5	131.2 *s*			130.0 *s*	
6	110.8 *d*			111.1 *d*	
7	145.9 *s*	7a	148.0 *s*	146.9 *s*	148.2 *s*
1′	141.7 *d*			141.9 *d*	
2′	119.4 *d*			119.7 *d*	
3′	168.9 *s*			168.1 *s*	
1″	132.5 *s*	1‴	130.4 *s*	132.9 *s*	129.6 *s*
2″	116.2 *d*	2‴	130.8 *d*	116.4 *d*	131.1 *d*
3″	149.2 *s*	3‴	116.3 *d*	148.9 *s*	117.3 *d*
4″	151.1 *s*	4‴	156.9 *s*	151.0 *s*	157.3 *s*
5″	118.1 *d*	5‴	116.3 *d*	119.3 *d*	117.3 *d*
6″	120.0 *d*	6‴	130.8 *d*	120.4 *d*	131.1 *d*
α	42.2 *t*	α′	42.5 *t*	42.1 *t*	42.6 *t*
β	35.3 *t*	β′	35.5 *t*	35.2 *t*	35.9 *t*
1‴	130.4 *s*	4‴	156.8 *s*	129.6 *s*	157.2 *s*
2‴	130.8 *d*	5‴	116.2 *d*	130.9 *d*	116.6 *d*
3‴	116.2 *d*	6‴	130.7 *d*	116.6 *d*	130.9 *d*
–OMe	56.7 *q*; 56.4 *q*;			56.7 *q*	

Assignments made by ^{13}C-DEPT and $^{13}C - ^1H$ COSY measurements



m/z		11 R = CH_3	12: R = H
[M + H]$^+$		625	611
a	[M - tyr]$^+$	487	473
b	[M - 2 x tyr]$^+$	350	338
c	[b - ring B]$^+$	229	229

Scheme 1. Mass fragmentation pattern for compounds **11** and **12**

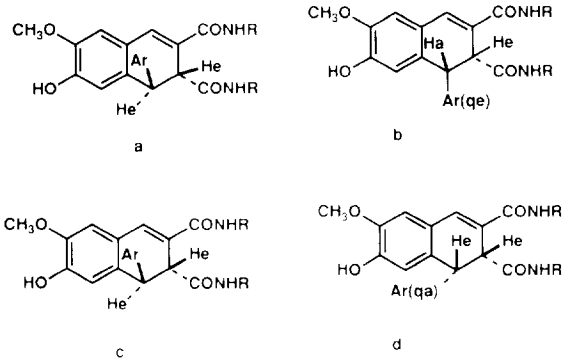


Fig. 1. Possible optical isomers of compound **14**.

$J_{1,2}$ = 3–4 Hz. To confirm which of the structures **c** and **d** fit our data for (−)-cannabisin D, DIFNOE measurements (Table 4) were carried out, in which irradiation of the H-1 signal resulted in the enhancement of not only H-2, but also the H-8, H-6′ and H-2′ signals. Similar irradiation of H-2 signals caused enhancement of the H-1, H-2′ and H-6′ signals, while irradiation of the H-6′ signals resulted in enhancement of the H-1 and H-2 signals. These observations confirmed the *cis*-configuration between the C-1 aryl group and the C-2 amide group similar to DIFNOE measurements of *Podophylum* lignans [26]. Thus, the most likely structure for (−)-cannabisins B and D is **d**. This observation is consistent with spectroscopic data of similar naturally occurring tetrahydronaphthalene lignans, such as polygamain [27] and podophyllotoxin [28] or synthetic dihydronaphthalene lignans [29], in contrast to the *trans*-arrangement between H-1 and H-2 [30].

The structures of **11**, **13** and **14** were further confirmed by synthesis (Scheme 2). Phenolic oxidative coupling of

Table 4. DIFNOE ^{1}H NMR spectral data for compound **14** (at 270 MHz, ppm TMS, in CD_3OD)

Irradiation (δ)	Enhanced peak (δ)
6.68 (H-2′)	4.32, 3.62
6.51 (H-8)	4.32
6.40 (H-6′)	4.32, 3.62
4.32 (H-1)	6.68, 6.51, 6.40, 3.62
3.62 (H-2)	6.68, 6.40, 4.32

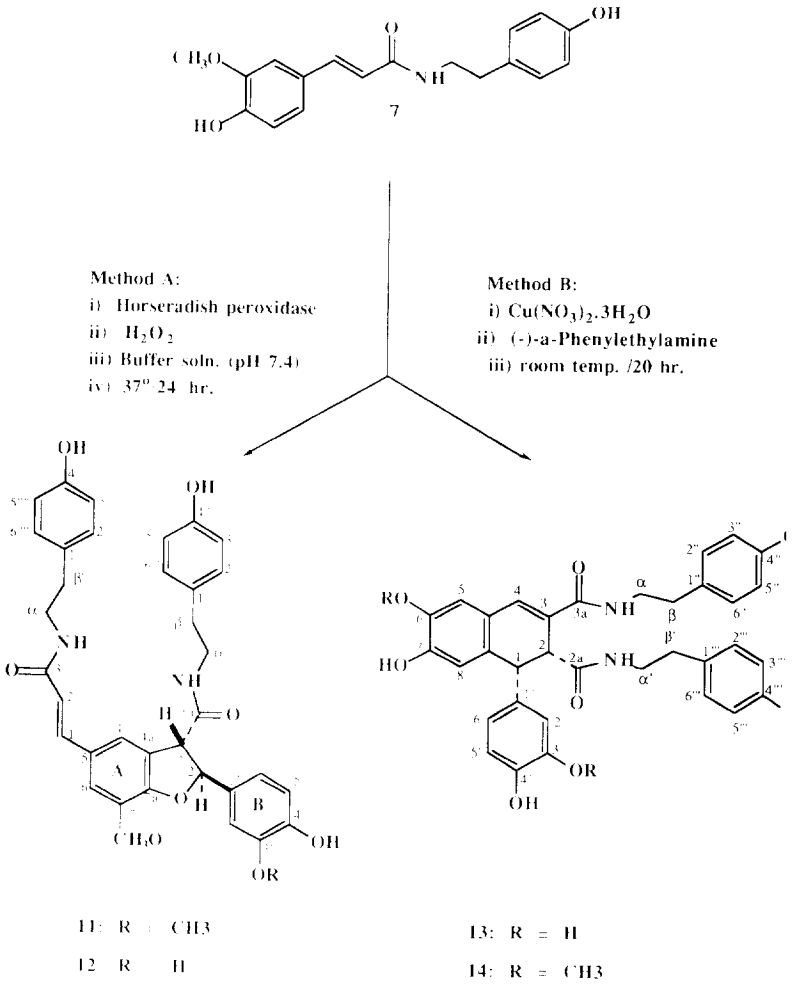
Table 5. Antifeedant index values for compounds **7–10**

Dose (ppm)	**7**	**8**	**9**	**10**
10,000	1.0	21.6	—	—
7500	8.5*	14.6*	—	—
5000	24.8	81.5	25.9*	10.9*
2500	23.3	78.6	—	—
1000	17.6	67.1	—	—
750	38.7	59.4	—	—
Control 75.8*				

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

7 mediated either by H_2O_2–horseradish peroxidase [16] or chiral Cu(II)-α (−)phenylethylamine complex [31] led to the synthesis of **11** and **14**. In both synthetic methods, grossamide was obtained as racemate, while **14** was obtained predominantly as the (−)-antipode. Although, it is yet to be proven experimentally, the kinetics of the cyclization process of the *trans*-intermediate, may require a *cis*-product, in a manner similar to the biosynthetic pathway proposed for *Podophyllum* lignans [32]. Finally, demethylation of **14** using anhydrous lithium iodide in dry DMF afforded **13**, which was identical in all respects to the authentic sample.

In an attempt to explore the commercial viability of these amides as termite feeding deterrents, two synthetic cinnamoyl derivatives, **9** and **10**, were synthesized by reacting 3,4-methylenedioxycinnamoyl chloride with the appropriate amine. The pure compounds **7–10** were tested for antifeedant activity against *R. speratus* in a similar manner and their antifeedant index values are given in Table 5. The synthetic amides, **9** and **10** were only tested at 5000 ppm and the lignanamides **11**, **13** and **14** were tested only at 10 000 ppm because the termite colony was already depleted after the structures were confirmed. Compound **7** was a very effective feeding deterrent, whereas **8** showed somewhat weaker activity. The synthetic compound **10** was the most potent, when compared to **7** and **8** at 5000 ppm. This seems to justify further exploration of this chemical series for its antitermite potential. The lignanamides **11–14** were all effec-



Scheme 2. Synthesis of compounds **11**, **13** and **14**.

tive feeding deterrents at 5000 ppm (with index values of 1.91, 29.49, 7.10 and 12.93, respectively). Compound **11** was particularly active and variations of activity were also observed according to modification of the structure between **11** and **12**, as well as **13** and **14**.

There are many literature reports on the biological activities of lignans [33] but few on phenolic amides. Compounds **7**, **8** and tyramine (**15**) the hydrolysis product of the amides, have been reported as human platelet aggregation inhibitors [34]. Tyramine also has been implicated in the inhibition of sucking actions of leaf hoppers on rice plants [35]. However, this is the first report of termite antifeedant activity of the phenolic lignanamides **7**, **8**, **11**–**14** and the ent-kaurane diterpenes, **1**–**6**.

The occurrence of diterpenes in fruits and bark and the presence of phenolic amides and lignanamides in seeds of *X. aethiopica* may suggest a defensive role for these compounds against wood destructive insects and, in this case, may protect *X. aethiopica* seeds against termites or other seed-consuming insects before the next growth cycle begins.

Although the level of activity varies according to the structure, a synergistic effect in admixture cannot be excluded. In addition, the wide range of chemical structures possessing biological activities, which have been described here seems to suggest that the defence mechanisms may be broader, providing the plant with protection against other insects or pathogens as well.

Compounds **7**, **8**, **11**, **13** and **14** have been previously isolated from the Solanaceae [19], Piperaceae [20], Liliaceae [34], Lauraceae [22], Cannabidaceae [24], Rutaceae [36] and Menispermaceae [37], but to our knowledge, this is the first report on the isolation of such amides from the Annonaceae.

EXPERIMENTAL

General. Mps: uncorr. ^1H and ^{13}C NMR spectra (ppm, *J* Hz) were obtained at 270 MHz (^1H NMR) and 67 MHz (^{13}C NMR) with TMS as int. standard. CC: silica gel 60 (70–230 mesh, Merck). TLC: precoated Kieselgel 60F_{254} plates (Merck). Spots were visualized under UV light 254 nm irradiation and by spraying with 10% H_2SO_4 followed by heating. Organic solvents were dryed with Na_2SO_4.

Plant material. Dried fruits of *X. aethiopica* were collected in the Ogbomoso Local Government area, Nigeria, and were identified by Dr M. O. Akanbi of the Forestry Research Institute of Nigeria, Ibadan.

Extraction and isolation. Fruits (85 g) were ground and extracted with hexane at room temp. for 72 hr. The residue was filtered and the extract concd *in vacuo* to give a pungent greenish-brown oil (16.5 g, 0.19%). Seeds, sepd from pods and ground (16 g) were defatted with hexane and then extracted with 10% aq. MeOH. The extract was partitioned between water and n-BuOH and the n-BuOH layer concd *in vacuo* to give a brown solid (1.12 g, 6.8%).

Separation and purification of hexane extract. The oily extract (2 g) was charged into a silica gel column and compounds were eluted starting initially with n-hexane–EtOAc, (19:1, 4:1 and finally 7:3); 10 ml frs were collected. A total of 600 frs containing similar compounds were collected and pooled to give 16 frs of 15 ml each. These frs were allowed to evaporate slowly at room temp. Individual crystals from each fr. were filtered and analysed by ^{1}H and ^{13}C NMR, ^{1}H–^{1}H COSY, ^{13}C–^{1}H COSY, EI/HR-MS to give **1**–**6**, identical in all respects with the data previously reported for these compounds [6–13]. The homogeneity of the compounds was confirmed by their TLC (silica gel) behaviour in A: n-hexane–EtOAc–$CHCl_3$ (2:1:1), B: n-hexane–EtOAc (1:4) and C. EtOAC–C_6H_6 (1:9).

R_f	A	B	C
1	0.83	0.34	0.89
2	0.83	0.46	0.91
3	0.74	0.33	0.93
4	0.71	0.30	0.87
5	0.66	0.30	0.81
6	0.21	0.07	0.57

Separation and isolation of compounds from seed methanolic extract. Compounds were initially sepd using prep. TLC silica gel plates. Each plate was charged with 40 mg of crude extract and developed with n-hexane–EtOAc–$CHCl_3$–MeOH (10:5:5:2). Ten bands were scraped off the plates and extracted with MeOH. Termite antifeedant bioassay of these fractions revealed activity in all the frs. Crude extract (1 g) was then charged into a silica gel column and eluted with $CHCl_3$–MeOH (19:1), affording the active compounds **7**, **8**, **11**–**14**.

E-3-(4-*Hydroxy-3-methoxyphenyl*)-N-2-[4-*hydroxyphenylethyl*]-2-*Propenamide* (**7**). Yields 26 mg 0.16% dry wt. R_f 0.55. Found *m/z* [M]$^+$ 313.1325, calc. $C_{18}H_{19}NO_4$, 313.1333. Data identical with ref. [19].

E-3-(3,4-*dihydroxyphenyl*)-N-2-[4-*Hydroxyphenylethyl*]-2-*Propenamide* (**8**). Yield 106 mg, 0.72% dry wt. R_f 0.50. Found *m/z* [M]$^+$ 299.1134, calc. $C_{17}H_{17}NO_4$, 299.1129. Data identical with ref. [24].

Synthesis of compounds **7** *and* **8**. A mixt. of ferulic acid (1.94 g) and tyramine (1.4 g) in dry DMF in the presence of diethylphosphorocyanidate, (1 ml) was kept at 0° for 6 hr. The reaction mixt. was diluted with H_2O and extracted with EtOAc (80 ml), washed with dilute HCl (1 M), H_2O and dried. The residue obtained after the removal of solvent was chromatographed on a silica gel column and eluted with $CHCl_3$–MeOH (9:1) to give **7** (2.5 g, 80%), identical in all respects with an authentic sample [19]. Similar condensation of caffeic acid (1.82 g), tyramine (1.4 g), DEPC (1 ml) followed by usual wok-up gave **8** (2 g, 67%), identical in all respects with an authentic sample.

(±)-*Grossamide* (**11**). Yield 7 mg, 0.04% dry wt. R_f 0.45. Pale yellow needles from MeOH, mp 132–134°, lit. mp 133–135° [25].

(±)-2,3-*Dihydro*-2-(3,4-*dihydroxyphenyl*)-N-[2-(4-*hydroxyphenyl*)*ethyl*]-5E-[3-[2-(4-*hydroxyphenyl*)*ethyl*]*amino*-3-*oxo*-1-*propenyl*]-7-*methoxy*-3-*benzofurancarboxamide*, (*demethylgrossamide*) (**12**). Yield 18 mg, 0.01% dry wt. R_f 0.38. Amorphous powder. IR ν_{max} cm^{-1}: 3325 (OH), 1655 (C=O), 1600, 1505. UV λ_{max} nm: 320 (20 000), 304 (20 500), 286 (20 400). ^{1}H NMR (CD_3OD): δ 2.65 (2H, *t*, J = 7 Hz, H-β), 2.71 (2H, *t*, J = 7 Hz, H-β'), 3.41 (2H, *ddt*, J = 14, 6, 7 Hz, H-α), 3.51 (2H, *ddt*, J = 14, 6, 7, Hz, H-α'), 3.82 (3H, *s*, OMe), 4.09 (1H *d*, J = 5.8 Hz, H-3), 5.82 (1H, *d*, J = 5.8 Hz, H-2), 6.31 (1H, *d*, J = 15.15, 5 Hz, H-2'), 6.62 (2H, *d*, J = 8.5 Hz, H-3‴, H-5‴), 6.65 (2H, *d*, J = 8.5 Hz, H-3‴, H-5‴), 6.68 (1H, *d*, J = 8 Hz, H-5″), 6.71 (1H, *d*, J = 2 Hz, H-2″), 6.81 (1H, *d*, J = 2 Hz, H-6), 6.82 (1H, *dd*, J = 8, 2 Hz, H-6″), 6.87 (2H, *d*, J = 8.5 Hz, H-2‴, H-6‴), 6.98 (1H, *d*, J = 2 Hz, H-4), 6.99 (2H, *dd*, J = 8.5 Hz, H-2‴, H-6‴), 7.35 (1H, *d*, J = 15.5 Hz, H-1'). FD-MS *m/z* 611.2390 [M + H]$^+$, calcd $C_{35}H_{36}N_2O_8$, 611.2397. EIMS 70 eV *m/z* (rel. int.) 573 (10), [M − tyramine]$^+$, 473 (230), [M − 2 × tyramine]$^+$, 363 (17), 338 (10), 336 (8), 299 (19), 256 (30), 243 (11), 229 (50), 177 (11), 137 (16), 121 (91), 108 (100), 77 (31).

(−)-*Cannabisin B* (**13**). Amorphous powder, 3.5 mg, 0.02% dry wt. R_f 0.21, $[\alpha]_D$ − 38° (MeOH; *c* 0.25). FAB-MS *m/z* 597.2233, [M + H]$^+$ calc. $C_{34}H_{33}N_2O_8$, 597.2237. Data in good agreement with lit. [25].

(−)-*Cannabisin D* (**14**). Yield 4.5 mg, 0.03% dry wt. R_f 0.32. $[\alpha]_D$ − 46° (MeOH; *c* 0.25). Needles from MeOH, mp 164–166°, lit. mp 165–168° [25]. FAB-MS *m/z* 625.2556 [M + H]$^+$ calc. $C_{36}H_{37}N_2O_8$, 625.2550. ^{1}H and ^{13}C NMR spectra identical with ref. [25].

Synthesis of (±)-*grossamide* (**11**) and (−)-*cannabisin B* (**14**). Method A: to a mixt. of **7**, (0.8 g), dissolved in EtOH, (100 ml), buffer soln (pH 7.4) (500 ml), horseradish peroxidase (10 mg) was added dropwise, 0.06% H_2O_2 (350 ml) for 1 hr. The reaction mixt. was then stirred at 37° for 24 hr. NaCl (10 g) was then added followed by extraction with EtOAc (250 ml). The EtOAc layer was washed with H_2O, satd brine and dried. The residue obtained after solvent was removed was sepd on a silica gel column by vacuum liquid chromatography (VLC) using Me_2CO–$CHCl_3$ (1:9) to give starting material **7** (0.3 g), (±) **11** (175 mg, 18%) and (−)-cannabisin D (**14**) (95 mg, 10%) which were identical in all respects to authentic samples.

Method B: to a soln of α(−)-phenylethylamine (0.574 g) in degassed EtOH (15 ml) and satd with Ar, was added $Cu(NO_3)_2 \cdot 3H_2O$ (0.5 g) under Ar and the mixt. stirred at room temp. for 30 min. Compound **7** (0.8 g) in degassed EtOH (10 ml) was then added to the Cu(II)–PEA complex and stirring continued for 24 hr. 2 M HCl (50 ml) was then added and the mixt. extracted with EtOAc (100 ml). The organic layer was washed with H_2O, brine and then dried. The residue obtained after removal of the solvent was purified on a short silica gel column by VLC using Me_2CO–$CHCl_3$, (1:9) as eluent to give starting material (**7**) 400 mg, **11** (110 mg, 14%) and **14** (52 mg, 7%) identical in all respects to authentic samples.

Synthesis of compound **13**. Anhydrous LiI (50 mg) was added to a soln of **14** (30 mg) in dry HMPA (20 ml) and then boiled at 150° under Ar for 3 hr. The cooled reaction mixt. was poured into cold dilute HCl (1 M) and then extracted with EtOAc (40 ml). Usual work-up gave **13** (18 mg, 63%) identical in all respects to an authentic sample.

E-3-(3,4-*Methylenedioxyphenyl*)-N-2-[4-*hydroxyphenylethyl*]-2-*propenamide* (**9**). A mixt. of 3,4-methylenedioxycinnamoyl chloride (2.4 g) (prepd in quantitative yield by boiling under reflux, 3,4-methylenedioxycinanmic acid and thionyl chloride), tyramine hydrochloride (2 g) $NaHCO_3$ (5 g) in dry DME (50 ml) and a few drops of TEA was boiled under reflux for 1 hr. The reaction mixt. was poured into dilute HCl (1 M) and extracted Et_2O. The Et_2O layer was washed with H_2O, satd $NaHCO_3$ soln and then dried. The residue obtained after solvent was removed was chromatographed on a short silica gel column and eluted with ($CHCl_3$–MeOH, 9:1) to give **9**. Recrystallization from Me_2CO–$CHCl_3$ gave crystals, mp 168–170°. IR ν_{max} cm^{-1}; 3520 (OH); 1660 (C=O). ^1H NMR (CD_3OD): δ 2.74 (2H, *t*, *J* = 7.5 Hz, H-β), 3.5 (2H, *ddt*, *J* = 14, 6, 7.5 Hz, H-α), 6.04 (2H, *s*, OCH_2O), 6.52 (1H, *d*, *J* = 16 Hz, H-2), 6.75(2H, *d*, *J* = 8.5 Hz, H-3″, H-5″), 6.85 (1H, *d*, *J* = 8.5 Hz, H-5′), 7.02 (1H, *d*, *J* = 2, 8.5 Hz, H-6′), 7.10 (1H, *d*, *J* = 2 Hz, H-2′), 7.15 (2H, *d*, *J* = 8.5 Hz, H-2″, H-6″), 7.43 (1H, *t*, *J* = 6 Hz, NH), 7.46 (1H, *d*, *J* = 16 Hz, H-3), 8.28 (1H, *bs*, OH). EI-MS 70 eV *m/z* (rel. int.) 311.1167 $[M]^+$, (12), $C_{18}H_{17}NO_4$, calc. 311.1171, 192 (50), 191 (59), 175 (100), 145 (35), 120 (43), 107 (20), 89 (44), 77 (14), 63 (20).

E-3-(3,4-*Methylenedioxyphenyl*)-N-2-[*octadecyl*]-2-*Propenamide* (**10**). A mixt. of 3,4-methylenedioxycinnamoylchloride (2.4 g), octadecamine (2 g), TEA (2 ml) in dry DME (50 ml) was boiled under reflux for 12 hr. Work-up in the same manner as described above gave **10** in quantitative yield. Recrystallization from $CHCl_3$ gave flakes, mp 100–101°. IR ν_{max} cm^{-1}: 1640 (C=O). ^1H NMR ($CDCl_3$): δ 0.85 (3H, *t*, *J* = 6 Hz, H-18″), 1.19–1.38 (30H, *m*, H-3″–H-17″), 1.58 (2H, *m*, H-2″), 3.39 (2H, *ddt*, *J* = 14, 6, 7 Hz, H-1″), 6.14 (1H, *d*, *J* = 15 Hz, H-2), 6.77 (1H, *d*, *J* = 8.5 Hz, H-5′), 6.95 (1H, *dd*, *J* = 2, 8.5 Hz, H-6′), 7.01 (1H, *d*, *J* = 2 Hz, H-2′), 7.58 (1H, *d*, *J* = 15 Hz, H-3). EI-MS *m/z* (rel. int.) 443.3380 $[M]^+$ (24), $C_{28}H_{45}NO_3$ calc. 443.3388, 415 (5), 308 (5), 232 (13), 205 (18), 190 (36), 175 (100), 145 (26), 135 (33), 117 (13), 89 (21), 43 (59).

Insect bioassay. Logs containing *R. speratus* colonies were collected near Yokkaichi (Mie prefecture),. Shizuoka (Shizuoka prefecture) and Kagoshima (Kagoshima prefecture), Japan, logs were kept in the laboratory in plastic bags and termites removed as needed. Only workers older than the 3rd instar were used. Activity of the compounds isolated was tested in a choice feeding bioassay, using filter paper disks as a feeding substrate (Whatman no. 1, qualitative, 2.0 cm diameter, 0.18 mm thickness). For each expt, 30 termite workers were placed in a 55 mm diameter plastic Petri dish whose bottom was covered with a layer of gelified agar (10 $g\,l^{-1}$) covered with sand. In each dish, a control disk treated with solvent only and a disk treated with 25 μl of the sample were placed on Al foil; 3 replicates were made for each dose. The amount of compound per disk was as follows:

ppm:	10000	7500	5000	2500	1000	750	500	250	100
μg $disk^{-1}$:	250	187.5	125	62.5	25	18.75	12.5	6.25	2.50
μg cm^{-2}:	79.57	59.68	39.78	19.89	7.95	5.96	3.97	1.98	0.79

Termites were allowed to feed on the disks for 14 days, at 27°, 80% rel. hum. in the dark. Termites were removed and the remaining surface of the paper disks was measured, to evaluate feeding inhibition, as previously described [38]. Disk surfaces were numerized using a video camera connected to a computer, through a video interface. The process was controlled via scanning software. Data were stored using image editing software and individual disk surfaces measured using additional software. Square dots were chosen as the surface unit. The percentage consumed for each disk was calculated by reference to an average disk surface ($N = 6$). These values were transformed into an antifeedant index calculated as: $T/(C + T)*100$ where T = % of treated disks consumed and C = % of control disks consumed [39]. A value of less than 20 indicates significant feeding-deterrent activity, a value of 50 indicates equal consumption of treated and untreated disks and a value greater than 80 denotes strong feeding stimulation effect.

Acknowledgements—We are grateful to Miss Y. Matsuzawa for technical assistance, Drs M. Kim and M. Serit (Taiyo Kagaku Inc.), Prof. S. Takahashi and his group (Kyoto Wood Research Institute) and Dr A. Messer (Shizuoka Prefectural University) for assistance with termite collection and Mr K. Watanabe and Mrs E. Fukushi for MS measurements. This work was supported by The Research Development Corporation of Japan (JRDC) under the ERATO program.

REFERENCES

1. Logan, J. W. M., Cowie, R. H. and Wood, T. G. (1990) *Bull. Entomol. Res.* **80**, 309.
2. Carter, F. L. and Beal, R. H. (1982) *Int. J. Wood Pres.* **2**, 185.
3. Ishida, M., Serit, M., Nakata, K., Juneja, L. R., Kim, M. and Takahashi, S. (1992) *Biosci. Biotech. Biochem.* **56**, 1835.
4. Grace, J. K. and Yates, J. R. (1992) *Trop. Pest Mangnt.* **38**, 176.
5. Scheffrahn, R. H. (1991) *Sociobiology* **19**, 257.
6. Ekong, D. E. U. and Ogan, A. U. (1968) *J. Chem. Soc. (C)* 311.
7. Ekong, D. E. U., Olagbemi, E. O. and Odutola, F. A. (1969) *Phytochemistry* **8**, 1053.
8. Moraes, M. P. L. and Rogue, N. F. (1988) *Phytochemistry* **27**, 3205.
9. Hassan, C. M., Healey, T. M. and Waterman, P. G. (1982) *Phytochemistry* **21**, 177.
10. Hassan, C. M., Healey, T. M. and Waterman, P. G. (1982) *Phytochemistry* **21**, 1365.
11. Hassan, C. M., Healey, T. M. and Waterman, P. G. (1982) *Phytochemistry* **21**, 2134.
12. Hassan, C. M., Healey, T. M. and Waterman, P. G. (1985) *Phytochemistry* **24**, 192.
13. Faulkner, D. F., Lebby, U. and Waterman, P. G. (1985) *Planta Med.* 354.
14. Schmutz, J. (1959) *Helv. Chim. Acta* **42**, 385.
15. Richter, W. J. and Brochman, H. E. (1975) *Helv. Chim. Acta* **58**, 209.
16. Jossang, A., Lebceuf, M. and Cave, A. (1991) *J. Nat. Prod.* **54**, 466.
17. Sofowora, A., ed. (1979) in *African Medicinal Plants*, p. 54. University of Ife, Ile-Ife.
18. Dupriez, H. and De Leener, P. (1962) *African Gardens and Orchards: Growing Vegetables and Fruits*, p. 269. Oxford University Press, London.
19. Yoshihara, T., Takamatsu, S. and Sakamura, S. (1978) *Agric. Biol. Chem.* **42**, 623.
20. Nakatani, N., Inatani, R. and Fuwa, H. (1980) *Agric. Biol. Chem.* **44**, 2831.
21. Yoshihara, T., Yamaguchi, K. and Takamatsu, S. (1981) *Agric. Biol. Chem.* **45**, 2593.
22. Tanaka, H., Nakamura, T., Ichino, K. and Ito, K. (1989) *Phytochemistry* **28**, 2516.
23. Yoshihara, T., Yamaguchi, K. and Sakamura, S. (1983) *Agric. Biol. Chem.* **47**, 217.
24. Sakakibara, I., Katsuhara, T., Ikeya, Y., Hayashi, K. and Mitsuhashi, H. (1981) *Phytochemistry* **30**, 3013.
25. Sakakibara, I., Ikeya, Y., Hayashi, K. and Mitsuhashi, H. (1992) *Phytochemistry* **31**, 3219.
26. Broomhead, J. A. and Dewick, P. M. (1990) *Phytochemistry* **29**, 3839.
27. Provan, G. J. and Waterman, P. G. (1985) *Planta Med.* 271.
28. Fonseca, S. F., Ruveda, E. A. and McChesney, J. D. (1980) *Phytochemistry* **19**, 1527.
29. Takei, Y., Mori, K. and Matsui, M. (1973) *Agric. Biol. Chem.* **37**, 637.
30. Liu, J., Huang, M., Ayer, W. A. and Nakashima, T. (1984) *Phytochemistry* **23**, 1143.
31. Feringa, B. and Wynberg, H. (1978) *Bioorg. Chem.* **7**, 397.
32. Broomhead, A. J., Rahman, M. M. A., Dewick, P. M., Jackson, D. E. and Lucas, J. A. (1991) *Phytochemistry* **30**, 1492.
33. MacRae, W. D. and Towers, G. H. N. (1984) *Phytochemistry* **23**, 1207.
34. Okuyama, T., Shibata, S., Hoson, M., Kawada, T., Osada, H. and Noguchi, T. (1986) *Planta Med.* 171.
35. Kurata, S. and Sogawa, K. (1976) *Appl. Entom. Zool.* **11**, 89.
36. Rondest, J., Das, B. C. and Polonsky, J. (1968) *Bull. Soc. Chim. Fr.* 2411.
37. Fukuda, N., Yonemitsu, M. and Kimura, T. (1983) *Chem. Pharm. Bull.* **31**, 156.
38. Escoubas, P., Lajide, L. and Mizutani, J. (1993) *Entomol. Exp. Appl.* **66**, 99.
39. Alkofahi, A., Rupprecht, J. K., Anderson, J. E., McLaughlin, J. L., Mikolajczak, K. L. and Scott, B. A. (1989) In *Search for New Pesticides from Higher Plants* (Arnason, J. T., Philogene, B. J. R. and Morand P., eds), pp. 25–43. *Insecticides of Plant Origin*, ACS Symposium Series No. 387. American Chemical Society, Washington DC.