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TERMITE ANTIFEEDANT ACTIVITY IN *DETARIUM MICROCARPUM**

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Key Word Index—*Detarium microcarpum*; Leguminosae; leaves; *Reticulitermes speratus*; Isoptera; antifeedants; clerodane diterpenes.

Abstract—A methanol extract of *Detarium microcarpum* leaves exhibited strong feeding deterrent activity against workers of the subterranean termite, *Reticulitermes speratus* in a paper disk choice bioassay. Bioassay directed fractionation led to the isolation of four clerodane diterpenes, 3,13*E*-clerodien-15-oic acid, 4(18), 13*E*-clerodien-15-oic acid, 18-oxo-3,13*E*-clerodien-15-oic acid and 2-oxo-3,13*E*-clerodien-15-oic acid as the active compounds. With the exception of the latter, this is the first report of these compounds from *D. microcarpum*. The four compounds possessed strong antifeedant activity at 1% and their structures were confirmed by spectroscopic methods.

INTRODUCTION

Extractives from termite-resistant woods have been under investigation for many years and offer some promise as wood preservatives [2, 3]. Naturally occurring toxicants and semiochemicals (e.g. repellents and feeding deterrents) may offer safer alternatives to the current use of synthetic pesticides [4]. In our search for naturally occurring insect feeding deterrents, we have screened a large number of tropical plants from Nigeria. Extracts of the leaves of *Detarium microcarpum*, an underutilized tropical leguminous tree, demonstrated strong antifeedant activity against *Reticulitermes speratus* in our paper disk choice bioassay. The genus *Detarium* has already been investigated in the past, since it is the source of a Nigerian crude drug used for its diuretic and anti-inflammatory properties [5]. The gum resin is also reported to be used as an anti-insect fumigant for garments and houses in Africa [6]. Catechins and tetranorditerpenes have recently been isolated from *D. microcarpum* [6]. In this report, the identification of termite antifeedant compounds in a *D. microcarpum* leaf extract is discussed.

RESULTS AND DISCUSSION

The crude extract of *D. microcarpum* exhibited strong termite antifeedant activity against workers of *Reticulitermes speratus* and was initially separated into ethyl acetate- and n-butanol-soluble fractions. The ethyl acetate layer was separated by silica gel column chromatography using a *n*-hexane–ethyl acetate gradient. Eluates were monitored by TLC and pooled into five fractions (1–5) corresponding to zones A–E on a silica gel TLC plate with R_f values of 0.87, 0.77, 0.56, 0.50 and 0.45, respectively. The *n*-butanol-soluble fraction was concentrated *in vacuo*, redissolved in methanol and separated by preparative TLC on silica gel into three fractions (6–8) corresponding to zones F–H on TLC with R_f values of 0.40, 0.25 and 0.10, respectively. Fractions 1–8 (at 1%) were then used in the paper disk choice bioassay against workers of *R. speratus*, for two weeks. All but fractions 1 and 8 possessed strong termite antifeedant activity (Fig. 1). Using preparative TLC, the major active compounds from each zone were separated and identified by spectroscopic methods and comparison with previous literature data for the compounds. Pure compounds were later tested at only one concentration (1%) owing to non-availability of termite workers at the time of the test.

Two major compounds were isolated from zone B (R_f 0.77). Both were diterpenes with the molecular formula $C_{20}H_{32}O_2$. The ^1H and ^{13}C NMR spectra of one of the compound possessed a pair of trisubstituted double bond signals (δ5.72, *br s*, 5.68, *br s*; 114.3, *d*, 120.2, *d*, 144.2*s* and 164.4 *s*), two vinyl methyl signals (δ1.58 and 2.17), two tertiary methyl signals (δ0.75 and 1.07) and a secondary

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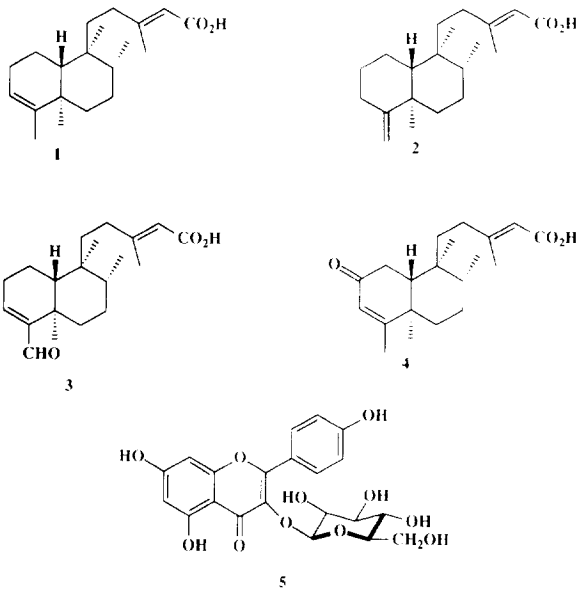

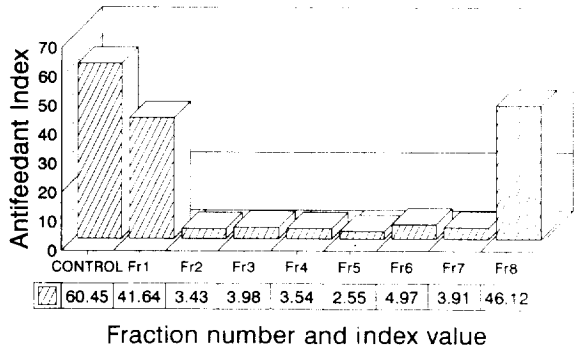

Fig. 1. Antifeedant Index values at 1% for fractions 1–8 of *Detarium microcarpum* leaf extract.

methyl group (0.81, *d*, $J = 6$ Hz), suggesting that the compound had a clerodane skeleton. Comparison of ^{1}H and ^{13}C NMR data with literature value 5 [8], confirmed it to be ent-3,13*E*-clerodien-15-oic acid (**1**).

The ^{1}H and ^{13}C NMR spectral data of the second compound in this fraction revealed the presence of a single trisubstituted double bond (δ5.66 1H *br s*, 113.8, *d*), a vinyl double bond (δ164.9), an exomethylene double bond (δ4.50, 2H *d*, $J = 1.3$ Hz, 102.0, *t*, 157.5 *s*), a vinyl methyl (δ2.16 *d*, $J = 1.2$ Hz), two tertiary methyl groups (δ0.75, 3H, *s*; 1.08 3H, *s*) and one secondary methyl (δ0.81, 3H, *d*, $J = 6$ Hz) and, thus, also suggested a clerodane skeleton for the compound. The exomethylene group could be placed either at C-4 or at C-8. By comparison with reported literature data [9], and coupled with the small geminal-coupling constant ($J = 1.3$ Hz), the location of the exomethylene group at C-4 is most probable. The coupling constant of similar clerodane diterpenes with an exomethylene group at C-8 [10], has $J = 14$ Hz. A differential NOE spectrum was positive for C-5, C-17 and C-20 methyl groups. The compound was found to be identical to ent-4(18), 13*E*-clerodien-15-oic acid (**2**), previously isolated from *Auracaria sp.* [9].

The ^{1}H NMR spectrum of the major compound from zone C (R_f 0.56) was similar to **1** except for the absence of a C-18 methyl signal at δ1.58 and the presence of a new aldehydic proton at δ9.96 (1H, *s*). The double bond signal at δ5.72 and 157.9, (*d*) indicated an α,β-unsaturated aldehyde (δ137.5, *s*, 189.1, *d*). The structure of the compound was further confirmed by the mass spectrum which gave *m/z* 318 $[M]^+$ ($C_{20}H_{30}O_3$) and the fragmentation ion *m/z* 205 ($C_{14}H_{21}O$, [M − side chain]$^+$). Although compounds with various degrees of oxidation of the methyl groups in clerodane diterpenes have been isolated [10–12], to our knowledge, this is the first report of **3** as a natural product.

The molecular formula of the major compound from zones D and E (R_f 0.45–0.5) was $C_{20}H_{30}O_3$. The spectroscopic data for the compound were in good agreement with 2-oxo-3,13*E*-clerodien-15-oic acid (**4**) which has been previously isolated from other species [13] and from the stem bark of *D. microcarpum* [6].

Purification of zone F (R_f 0.40) led to the isolation of the known kaempferol 3-*O*-β-glucopyranoside (**5**) [14]. The ^{1}H NMR spectrum of the major band from zone G (R_f 0.25) showed the presence of flavonoid glycosides but was not purified further owing to paucity of sample.

Compounds **1–5** gave Antifeedant Index values of less than 5, thus indicating a strong feeding deterrent activity against *R. speratus* at 1% (80 μg cm^{-2}).

Clerodane diterpenes have been shown to possess various activities, including insecticidal [15], and are well known antifeedant compounds for lepidopteran larvae [16–18]. However, to our knowledge, this is the first report of antifeedant activity against termites for this class of compounds. Similarly, flavones, whether substituted [17] or unsubstituted [18], have been implicated in various anti-insect properties, such as growth-inhibition or feeding deterrency, whilst isoflavonoids are well known insect feeding inhibitors [20, 21]. Additionally, flavones have been shown to be major resistance factors in wood against wood-destroying insects and pathogens [22–24]. Although the major class of compounds responsible for the antifeedant effect of *D. microcarpum* on termites, seems to be the clerodane diterpenes, the combination with flavonoids may provide this tree with a broad-based defence mechanism against a variety of predators and pathogens.

EXPERIMENTAL

Plant material. Detarium microcarpum was collected in Bauchi, Nigeria and identified by Dr M. O. Akanbi of the Forestry Research Institute of Nigeria, Shelter Belt Station, Kano.

Extraction and isolation. Dried leaves (30 g) were extracted with 5% aq. MeOH to obtain 9.8 g of crude extract, which was partitioned into EtOAc-solubles (3.4 g) and *n*-BuOH-solubles (4.5 g). The EtOAc fr. was subjected to silica gel CC using a gradient solvent system

(*n*-hexane–EtOAc) increasing the amount of EtOAc from 5 to 20%. Eluates monitored by TLC (silica gel, *n*-hexane–EtOAc–$CHCl_3$–MeOH, 10:5:5:2) were pooled to give frs 1–5, corresponding to zones A–E on TLC plates. The *n*-BuOH-soluble extract was concd and redissolved in MeOH, after which it was sepd into frs 6–8 corresponding to zones F–G by prep. TLC (MeOH–$CHCl_3$, 1:9). Pure compounds were sepd by repeated prep. TLC (silica gel, Merck PF_{254}) from each zone and then identified by ^1H and ^{13}C NMR, ^1H–^1H COSY, DIFNOE, ^{13}C DEPT, ^{13}C–^1H COSY and MS analyses and, if known, with data previously reported for the compound in lit. for comparison.

Zone B yielded ent-3,13*E*-clerodien-15-oic acid (**1**) (8 mg), spectroscopic data identical in all respect to values given in ref. [8], and ent-4(18), 13*E*-clerodien-15-oic acid, (**2**) (12 mg); oil, $[\alpha]_D$ + 10° ($CHCl_3$; *c* 0.004), lit. $[\alpha]_D$ + 9°, [9]. ^1H NMR ($CDCl_3$): δ0.75 (3H, *s*, Me-20), 0.81 (3H, *d*, J = 6.5 Hz, Me-17), 1.05 (3H, *s*, Me-19), 2.15 (3H, *d*, J = 1 Hz, Me-16), 4.59 (2H, *br s*, CH_2-18), 5.72 (1H, *br s*, H-14). HR-MS: Found *m/z* $[M]^+$ 304.2371, $C_{20}H_{32}O_2$ calc. 304.2375. Zone C yielded 18-oxo-3, 13*E*-clerodien-15-oic acid, (**3**) (18 mg). C oil. $[\alpha]_D$ + 7° ($CHCl_3$ *c* 0.004). ^1H NMR ($CDCl_3$): δ0.75 (3H, *s*, Me-20), 0.82 (3H, *d*, J = 6.5 Hz, Me-17), 1.06 (3H, *s*, Me-19), 5.66 (1H, *br s*, H-14), 5.72 (1H, *t*, J = 9.3 Hz, H-3), 9.96 (1H, *s*, CHO). HR-MS: Found *m/z* $[M]^+$ 318.2187, $C_{20}H_{30}O_3$ calc. 318.2188. Zones D and E gave 2-oxo-3, 13*E*-clerodien-15-oic acid (**4**) (25 mg). Data identical in all respects to those previously reported [7, 13]. Zone F afforded kaempferol-3-*O*-β-glucopyranoside (**5**) (7 mg). Data were in good agreement with reported values [13]. ^{13}C NMR data for **2** and **3** have not been reported before and are given in Table 1 together with data for **1** [7] and **4** [13, 25] for comparison.

Table 1. ^{13}C NMR spectral data for compounds **1**–**4** ($CDCl_3$, δ, 67 MHz)

C	1	2	3	4 [13, 25]
1	27.1 *t*	32.5	26.6	35.9
2	18.1 *t*	28.1	28.7	200.5 *s*
3	120.2 *d*	38.8 *t*	157.9	126.3
4	144.2 *s*	157.5	137.5	171.8
5[a]	38.0 *s*	41.6	38.2	40.3
6[b]	36.5 *t*	35.7	35.3	35.4
7	26.69 *t*	26.9	27.6	27.1
8[b]	36.7 *d*	36.8	36.6	36.4
9[a]	39.8 *s*	39.5	38.5	39.2
10	46.6 *d*	48.2	49.0	46.2
11[b]	34.7 *t*	34.7	34.5	34.7
12	36.2	36.2	37.8	36.0
13	164.4 *s*	164.9	164.3	163.6
14	114.2 *d*	113.8	114.8	114.9
15	172.2 *s*	170.0	170.5	172.8
16	19.4 *q*	19.4	18.5	19.4
17	15.9 *q*	15.5	15.5	16.2
18	17.9 *q*	102.1 *t*	189.1 *d*	18.8
19	19.4 *q*	20.3	19.9	19.9
20	17.9 *q*	17.6	17.6	18.3

[a,b] Values may be interchanged.

Multiplicities assigned by DEPT measurements and comparison with literature values.

Biological activity. The choice feeding bioassay on termite workers was carried out as described in ref. [28]. The Antifeedant Index values at 1% for the compounds isolated were **1** (0), **2** (0), **3** (0), **4** (3.0) and **5** (5.0). Treatment values were statistically different from controls (60.5) using the Mann–Whitney U-test, $P < 0.05$.

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