C_{18} Acetylenic Fatty Acids of *Ximenia americana* with Potential Pesticidal Activity

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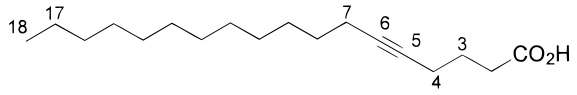
Bioactivity-driven fractionation of the $CHCl_3$ extract of the root of *Ximenia americana*, using the brine shrimp lethality test (BST) and hatchability test with *Clavigralla tomentosicollis* eggs, gave C_{18} acetylenic fatty acids **1** and **2**. **1** is octadeca-5-ynoic acid (tariric acid). **2** is a novel ene-ene-yne-ene acetylenic fatty acid (10*Z*,14*E*,16*E*-octadeca-10,14,16-triene-12-ynoic acid). The structures of **1** and **2** were assigned from the MS and NMR data. Fractions that are rich in acetylenic fatty acids inhibited the hatching of *C. tomentosicollis* eggs.

Keywords: *Ximenia; Olacaceae; Clavigralla; brine shrimp; hatchability; C_{18} acetylenic fatty acids; pesticidal*

INTRODUCTION

Olacaceous seed oils are a rich source of acetylenic lipids and unsaturated fatty acids (Spitzer et al., 1997; Badami and Patil, 1981). Acetylenic metabolites including polyacetylenes, acetylenic glyceryl ethers, and brominated acetylenic fatty acids show insecticidal (Jacobson, 1971), cytotoxic (Marles and Farnsworth, 1989), and antifungal (Fusetani et al., 1993) activities. They are also inhibitors of prostaglandin biosynthesis (Nugteren and Christ-Hazelhof, 1987), HIV reverse transcriptase, and H,K-ATPase (Ishiyama et al., 1997). In the course of our investigation of plants for biopesticides, we observed that the $CHCl_3$ extract of the root and twigs of *Ximenia americana* L. (Olacaceae) that are rich in acetylenic fatty acids inhibited the hatching of *Clavigralla tomentosicollis* Stal (Hemiptera:Coreidae) eggs. *C. tomentosicollis* is a pod-sucking bug of the cowpea, *Vigna unguiculata* L. Walp. Aphids, legume pod-borers, flower bud thrips, and pod-sucking bugs are the most damaging field pests of cowpea in the semiarid region of sub-Saharan Africa (Singh and Jackai, 1995). They are effectively controlled with synthetic insecticides (Jackai and Daoust, 1986). We embarked on this project to find fractions of cultivated plants that could be processed and used as garden pesticides. *X. americana* is a shrub of the African savanna. It is propagated by seed and could produce fruits in the third year (Maydell, 1990). Traditionally, *X. americana* is used for treating cancer and mouth infections, and its fruits are eaten as condiments. Oleanolic acid (D'agostino et al., 1994), unsaturated fatty acids (Hatt et al., 1959, 1960), and phenolic compounds (Nwangi et al., 1993) have been isolated from *X. americana*. The pesticidal potentials of *Ximenia* metabolites have not been investigated. The present study describes bioassay-guided isolation, structural identification, and bioeffects of extracts and acetylenic fatty acids **1** and **2** on *C. tomentosicollis*.



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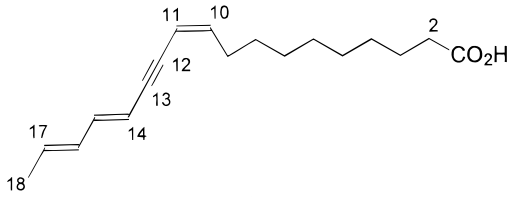

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EXPERIMENTAL PROCEDURES

Apparatus and Reagents. IR spectra were measured on a Nicolet FT-IR spectrometer. UV spectra were run on a UV−visible HP-8453 spectrophotometer; ^1H and ^{13}C NMR (DEPT) were run on a JEOL JNM-EX400 spectrometer, and mass spectra were obtained with a JEOL JMS-SX102A [EIMS, 70 eV; CIMS (isobutane), gun high 3.0 kV]. Open column chromatography was performed on Kieselgel S (Riedel-deHaen) 70−230 mesh, TLC on Whatman precoated silica gel (60A K6F) plates, and preparative TLC on plates coated with silica gel $G6F_{254}$ (BDH). TLC bands were visualized under a UV lamp or by exposure to iodine vapor.

Plant Material. The root of *X. americana* was collected at Yako village, ~40 km from Kano, Nigeria. The material was

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authenticated at the Herbarium of Bayero University, Kano. The dried root was milled.

Brine Shrimp Lethality Test (BST). Extracts, fractions, and isolated compounds were evaluated for lethality to brine shrimp larvae (Meyer et al., 1982; McLaughlin, 1991). In this test, a drop of DMSO was added to test and control vials to enhance the solubility of test materials.

Hatchability Test. Fresh pods of cowpea (*V. unguiculata* L. Walp) were supplied to field-collected egg-laying *C. tomentosicollis* (maintained by routine transfer on cowpea pods under ambient laboratory condition). Twenty-four hours after supply of pods, the pods were examined for eggs under a binocular microscope. Pods containing eggs were cut into pieces corresponding to 15−20 eggs per piece. The pieces were sprayed with a solution of sample in 5% aqueous Tween 20. The sprayed pods (test and control) were placed in separate Petri dishes, and the nymphs from hatched eggs were counted after 6 days. Each sample was tested in triplicate at 4×10^4 μg/mL. The percentage inhibition of hatching was computed, correcting for unhatched eggs in the control using Abbott's formula:

$$\% \text{ control} = [(\% \text{ unhatched of treated group} - \% \text{ unhatched of untreated group})/(100 - \% \text{ unhatched of untreated group})] \times 100$$

Extraction and Isolation. Dried root of *X. americana* (4 kg) was extracted by maceration with chloroform. The chloroform extract was laboriously fractionated between H_2O (F002) and $CHCl_3$ (F003). The residue of F003 was partitioned between petroleum ether and 10% H_2O in MeOH to give the petroleum ether soluble fraction (F006), the 10% H_2O in MeOH soluble fraction (F007), and the interface between F006 and F007, which was labeled F005. Twenty-four grams of combined fractions F005 and F006 was chromatographed on silica gel (130 g) and eluted in this order with petroleum ether, chloroform, chloroform/ethyl acetate (1:1), ethyl acetate, and ethyl acetate/methanol (1:1).

The chloroform eluants were collected in six fractions and in portions of 500 mL. The active fraction 2 (7.6 g) [BST LC_{50} 49 (88−27) μg/mL] was further resolved on another silica gel (100 g) column, eluting with a gradient of petroleum ether/chloroform in portions of 400 mL. Fraction 10, eluted with petroleum ether/chloroform (1:3), gave a brownish yellow residue (519 mg), which was further purified on preparative TLC, eluting with chloroform to give 234 mg of a yellow oil from which **1** was obtained as a major component.

Fractions 4−6 were pooled (460 mg) and purified on preparative TLC, eluting with chloroform/ethyl acetate (4:1) to give **2** (128 mg) [BST LC_{50} 20 (39−9) μg/mL] as the most polar fraction.

Octadeca-5-ynoic acid (1) was obtained as a yellow oil (234 mg); IR (film) (cm^{-1}) 2915, 2851, 2220, 1713, 1463, 761; CI-MS (isobutane) *m/z* (relative intensity) 281 (80) $[MH]^+$, 263 (85) $[MH - H_2O]^+$, 222 (100) $[MH - CH_2CO_2H]^+$, 171 (50); HREI-MS *m/z* 280.2378 $[M^+]$ (calcd 280.2402 for $C_{18}H_{32}O_2$); ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 1.

10*Z*,14*E*,16*E*-Octadeca-10,14,16-triene-12-ynoic acid (2) was obtained as a yellow oil (128 mg); IR (film) (cm^{-1}) 2924, 2853, 2232, 1702, 1650, 1462, 954, 728; UV λ_{max} (MeOH) 229 (log ϵ 3.84), 240 (log ϵ 3.52), 253 (3.44), 267 (log ϵ 3.87), 283 (log ϵ 3.29), 367 (log ϵ 3.29); EI-MS *m/z* (relative intensity) $[M^+]$ 274 (20), 256 (20) $[M - H_2O\}^+$, 187 (12) $[M - CH_2(CH_2)_2CO_2H]^+$, 145 (30) $[M - CH_2(CH_2)_5CO_2H]^+$, 131 (55) $[M - CH_2(CH_2)_6CO_2H]^+$, 117 (70), 91 (100), 41 (60); HREI-MS *m/z* 274.2021 $[M^+]$ (calcd 274.1933 for $C_{18}H_{26}O_2$); ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 1.

RESULTS AND DISCUSSION

During a routine screening of plant extracts for pesticidal effects, we observed that the less polar extracts of the twigs and root of *X. americana* that are active in the BST (Meyer et al., 1982; McLaughlin, 1991) suppressed the hatchability of the eggs of *C. tomentosicollis*. BST-directed fractionation of the $CHCl_3$ extract of the root of *X. americana* between solvents gave F005 and F006 as the most active fractions [F005, BST LC_{50} 78 (129−48) μg/mL; F006, BST LC_{50} 76 (121−49) μg/mL]. F005 and F006 are also similar, as evaluated by TLC analysis (petroleum ether/ethyl acetate, 1:1). A combination of F005 and F006 was submitted to hatchability test (inhibition of hatching = 68% of control), and successive BST-directed fractionation on silica gel column and preparative TLC to give white semisolids and **1** and **2** as yellow oils.

Table 1. ^{1}H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR Data for 1 and 2

	1H				^{13}C	
	1		**2**		**1**	**2**
position	δ_H	J^a	δ_H	J^a	δ_C	δ_C
1					180.1	179.3
2	2.35 t	7.3	2.34 t	6.8	34.1 CH_2	34.0 CH_2
3	2.19 m		1.25−1.59 m		22.7 CH_2	24.6 CH_2
4	2.32 t	7.8	1.25−1.59 m		18.7 CH_2	29.7 CH_2
5			1.25−1.59 m		80.4	30.7 CH_2
6			1.25−1.59 m		80.1	31.3 CH_2
7	2.13 m		1.25−1.59 m		18.8 CH_2	31.9 CH_2
8	1.63−1.26 m		1.25−1.59 m		29.1 CH_2	31.9 CH_2
9	1.63−1.26 m		2.10 m		29.2 CH_2	28.2 CH_2
10	1.63−1.26 m		6.29 dt	8.8; 7.3	29.5 CH_2	148.2 CH
11	1.63−1.26 m		6.26 d	8.8	31.7 CH_2	108.3 CH
12	1.63−1.26 m				31.8 CH_2	74.1
13	1.63−1.26 m				31.9 CH_2	83.4
14	1.63−1.26 m		5.49 d	15.6	31.9 CH_2	109.3 CH
15	1.63−1.26 m		6.46 dd	15.6; 9.3	29.7 CH_2	140.9 CH
16	1.63−1.26 m		6.05 dd	15.6; 9.3	32.9 CH_2	136.9 CH
17	1.33 m		5.73 dq	15.4; 7.3	22.6 CH_2	129.8 CH
18	0.88 t	6.3	1.63 d	7.3	14.1 CH_3	18.8 CH_3
COOH	8.7 b		7.9 b			

[a] *J* values given in hertz.

The yellow fraction that contains **2** suppressed the hatchability of *C. tomentosicollis* eggs at 92% of control when tested at 4×10^4 μg/mL (correcting for unhatched eggs in the control using Abbott's formula). **2** is, however, not lethal to nymphs and adult bugs at 4×10^4 μg/mL. The white semisolids containing C_{18} saturated fatty acids, oleanene palmitates, and β-sitosterol are relatively inactive in the BST assay.

Compound **1** had a molecular formula of $C_{18}H_{32}O_2$. The molecular ion was indicated by a dominant peak at *m/z* 281 $[MH]^+$ in CI-MS. The HREI-MS gave *m/z* 280.2378 (calcd 280.2402) for $[M^+]$. The IR spectrum displayed an acetylenic band at 2220 cm^{-1} and an acid band at 1713 cm^{-1}. The ^{1}H and ^{13}C NMR spectral data revealed signals in the table. The presence of three contiguous C_2-C_4 methylene groups between the acetylenic carbon and a carboxylic acid carbon was supported by ^{13}C NMR signals at δ 34.1, 22.7, and 18.8 and by ^{1}H NMR signals at δ 2.36 (2H, t, J = 7.3 Hz, H-2), 2.19 (2H, m, H-3), and 2.32 (2H, t, J = 7.8 Hz, H-4). The placement of the acetylenic bond between C_5 and C_6 was further supported by the mass spectral peaks at *m/z* 170 (50), 222 (100), and 263 (80) (see Figure 1), corresponding to the loss of 111, 59, and 18 mass units from the molecular ion $[MH^+]$. The relatively high field resonance values of C-4 (δ 18.7) and C-7 (δ 18.8) imply that they are adjacent to an acetylenic group (Kalinowski et al., 1984). The ^{13}C NMR spectral data (see Table 1) further supported the identification of **1** as tariric acid (Gunstone et al., 1976).

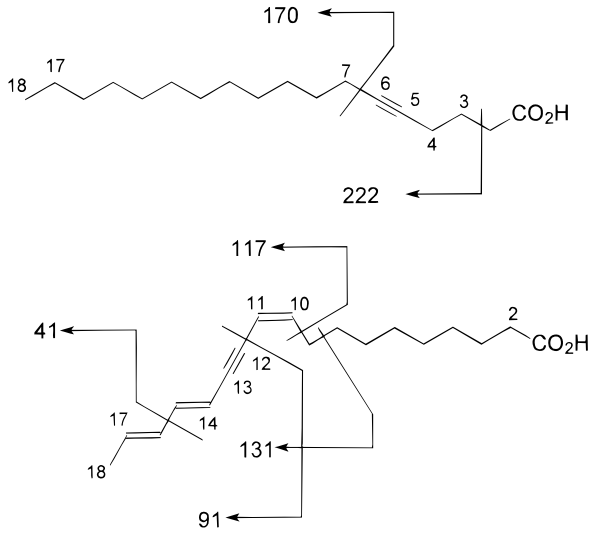

Figure 1.

The molecular formula of **2** was revealed as $C_{18}H_{26}O_2$ by HREI-MS that gave a molecular ion peak at *m/z* 274.2021 (calcd 274.1933). IR spectra contained an acetylenic absorption at 2232 cm^{-1}, a carboxylic acid band at 1702 cm^{-1}, and an olefinic band at 1650 cm^{-1}. The existence of conjugated unsaturated bonds was inferred from the UV λ_{max} (MeOH) absorptions at 229, 240, 253, 267, 283, and 367. The ^{1}H NMR spectra of **2** showed resonances at δ 6.46 (dd, H-15), 6.29 (dd, H-10), 6.26 (d, H-11), 6.05 (dd, H-16), 5.73 (dq, H-17), and 5.49 (d, H-14). The ^{13}C NMR also show six resonances at δ 148.2 (C-10), 140.9 (C-15), 136.9 (C-16), 129.8 (C-17), 109.3 (C-14), and 108.6 (C-11), revealing the presence of three double bonds. Two disubstituted acetylenic carbons were also quickly discerned in the ^{13}C NMR at δ 83.4 and 74.1. The ^{1}H NMR signal at δ 6.46 (dd, J = 15.6, 9.3 Hz) is coupled to the signals at δ 5.49 (d, J = 15.6 Hz) and 6.05 (dd, J = 15.60, 9.3 Hz). The latter is also coupled to the signal at δ 5.73 (dq, J = 15.4, 7.3 Hz). The coupling constant values established an *E*,*E* $CH_3CH{=}CH{-}CH{=}CH{-}$ unit for two of the three double bonds. The third double bond showed signals at δ 6.29 (dt, J = 8.8, 7.3 Hz) and δ 6.26 (d, J = 8.8 Hz), suggesting the presence of a *Z* $-CH{=}CH{-}CH_2-$ unit. The acetylenic carbons at δ 83.4 and 74.1 must be allylic to the *Z* and *E* olefinic bonds, and the carbon resonance at δ 28.2 (see Table 1) is expected of an allylic carbon near a cis double bond.

The presence of the peaks *m/z* 256 (20), 131 (55), and 117 (70) in the EI-MS of **2** (see Figure 1) further showed that the ene-ene-yne-ene unit is connected to a carboxylic acid function by eight methylene groups. The *m/z* 117 (70) also suggested that a double bond connects the carbon−carbon triple bond to the methylene groups. The peak at *m/z* 41 (60) further supported the presence of a $CH_3CH{=}CH{-}$ unit.

BST active fractions of *X. americana* are not lethal to nymphs and adult bugs. They could be useful in suppressing the population of colonizing pod-sucking bugs through destruction of viable eggs.

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