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Spectral Determination of Components Isolated from the Root of *Ximenia Americana* Linn

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Abstract One steroid, β-sitosterol (I), and two carboxylic fatty acids stearic (2), and *trans*-4-octadecenoic (3) acids were isolated from the roots of *Ximenia americana* Linn. (Olacaceae). *Trans*-l4-octadecenoic acid exhibited *in-vitro* cytotoxicity against brine shrimp larvae of *Artemia salina* (LC_{50} = 79.µg/ml). Compounds 1 and 2 showed no activity in brine shrimp lethality test (LC_{50}> 1000µg/ml). ^{1}H and ^{13}C NMR, IR and MS, spectroscopy have been used jointly to determine the conformation of the three compounds.

Keywords: *Ximenia americana, β-sitosterol, fatty acids, Artemia salina, brine shrimp*

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1. Introduction

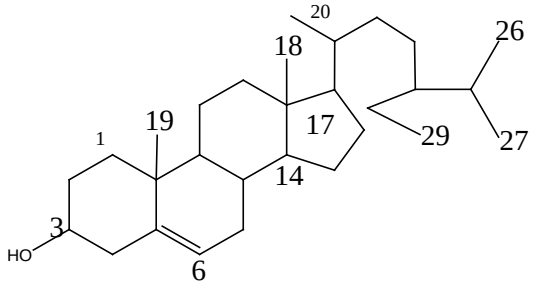
Ximenia americana 'Tsada" (Hausa) and "Chabulli" (Fulfulde) is a shrub with white flowers which grows mainly in the African savanna [4,7,8].

The root of the plant is used to treat leprosy, dysentery, and mental sickness. The Hausawa and Fulani of northern Nigeria use the roots of *Ximenia americana* and that of *Annona senegalensis* to treat trypanosomiasis, and also eye and ear infections. In Angola the crushed bark is applied to sores of domestic animals [13].

Sambunigirin was isolated from the leaves of *Ximenia americana* [3]. The following acids have also been detected in the seed oil of *Ximenia* plant: Oleic (60.8%). cerotic (15.21%), ximenic (14.6%), linoleic (6.7%), and stearic acid (1.2%) [11]. *Trans*-13-octadecen-9, 11-dynoic acid, *trans*-l l-octadecen-9-ynoic acid (Ximeninic acid), trans-l 1-trans- 13-octa decadien-9-ynoic acid and oleanolic acid saponin have been isolated from the roots [1,5].

Early pharmacological report on the plant have concluded that the roots have no antimalarial activity and the extracts of the fruits, twigs and leaves showed no significant insecticidal property [6,14].

Herein we report on the bioactivity-guided isolation and the identification of two fatty acids (2) and (3) in the roots of *Ximenia americana*. The identity of β-sitosterol (1) was confirmed' by comparison of its spectral data with those reported in the literature.



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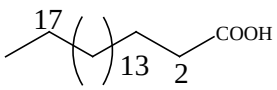

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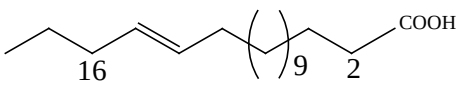

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2. Experimental Procedure

Apparatus and Reagents: Solvents were used without further purification except some ethanol which was redistilled before use. Column chromatography was performed on silica gel (Merck 70 - 30 mesh, bet surface area 500m^2/g, pore volume 0.75cm^2). Thin layer chromatography (TLC) was performed on plates coated with silica gel (Merck, TLC grade, with gypsum binder

and fluorescent indicator). TLC bands were visualized under UV light (at 254 nm and 365 nm) or by exposure to iodine. Brine shrimp eggs *(Artemia. Inc.*, California) and instant ocean see salt were purchased from Aquarium systems, Ohio, USA.

EIMS were obtained on JEOL IMS - 5 x 102 A spectrometer. ^{1}H and ^{I3}C NMR were recorded on JEOL JNM - EX 400 spectrophotometer and Bruker Avance in CDL_3, TMS was used as standard in NMR measurements. IR was visible HP-8453 spectrophotometer.

Plant Material. The root of *X. americana* was collected at Yako Village, 40 KM from Kano. northern Nigeria. The material was authenticated by Baba Ali Garko of the Herbarium of Bayero University, Kano. The root was dried and crushed into a fine powder.

Brine Shrimp Lethality (BST). Extracts, fractions and isolated compounds were evaluated for lethality to brine shrimp larvae [9,10]. In this test, a drop of DMSO was added to test and control vials to enhance the solubility of test materials.

Extraction and Isolation. Dried and ground root of *X. americana* (3.355kg) was percolated with 95% ethanol/chloroform (1:1) for two weeks, after the extract was drained off and evaporated in vacuum at 40°C. The marc was percolated with 6.5 litres of 95% ethanol/chloroform (1:1) for another one week and then drained and evaporated. The combined residue, 86.49g, reddish-brown labeled FOO1 was stored in a freezer until used. The column fractions of the crude extract (FOO1) of the root of *X. americana* was carried out by methods described by Fatope *et al.*, [2], In this method three portions (25g each) of the crude extract (FOO1) were separately chromatographed on silica gel columns using different solvent mixtures. Fractions were pooled and screened against brine shrimp larvae. Identical eluents were combined based on their TLC patterns and activity in BST.

Activity guided fractionation led to the isolation of fraction XA-9-4 (1.1588g) which was also inactive in BST (LC_{50} > 1000µg/ml). The fraction, a colourless oily substance was loaded on smaller column (length = 25cm, id = 2cm) packed with 30.5g silica gel. The column was eluted in the following order and collecting fractions in portions of 50ml: (50ml) petroleum ether, (1:1050ml) petroleum ether and chloroform. (1:15, 50ml) petroleum ether and chloroform (50ml) chloroform. (1:1, 50ml) chloroform and ethylacetate, and (50ml) ethyl acetate.

Eluents were pooled into nine fractions. Fractions XA-9-4-2. XA-9-4-3, XA-9-4-5, XA-9-4-7, XA-9-4-8 and XA-9-4-9 were pale yellow and XA-9-4-4 was a colorless oily fraction. TLC analysis revealed that XA-9-4-2 and XA-9-4-3 are the same compound at different levels of purity. Spectral data revealed that the dominant product in the two fractions is oleanene hydrocarbon and ester.

Further purification of (XA-9-4-4) gave colorless oil fractions. One of the fractions is coded XA-9-4-4-1. 0.1300g of fraction XA-9-4-4-1, a colourless semi solid, was loaded on a preparative TLC and developed in petroleum ether: ethyl acetate (15:1). Three major bands were obtained. The top XA-9-4-4-4-1A [35mg, R_f 0.36 in petroleum ether: ethyl acetate (15:1)] and the middle XA-9-4-4-IB (41 mg. R, 0.24 in petroleum ether: ethyl acetate (15:1)] were single products: and the bottom (XA-9-4-4-1C) a mixture of several compounds. Fractions XA-9-4-4-1A and XA-4-4-1B are inactive in BST (LC_{50} > 1000µg/ml).

Six fractions obtained from XA-9-5 (0.9509g) were pooled and purified on preparative TLC, eluting with chloroform: ethyl acetate (1:1), to give **2** (XA-9-5-5B). Fraction XA-9-5-6 (0.1232g) was loaded on preparative TLC and developed in chloroform: ethyl acetate (1:1). When the plate was visualized under UV light at 254 nm. two major bands were observed and the top band was compound **3** (XA-9-5-6A) scraped off, dissolved in ethyl acetate, filtered and dried. **3** (44mg) was a yellow oil.

β-sitosterol (1) (XA-9-4-4-1 A) was obtained as a white semi solid (35mg);EI-MS/mz (relative intensity) 414 (78) $[M^+]$, 396 (70) $[M\text{-}H_2O]^+$, 381(30) $[M\text{-}CH_3]^+$. 329 (30). 107 (49), 105 (40), 95 (59). 93 (36), 91 (33). 81 (61), 67 (36); HR El-MS m/z 414. 3854 (for C_{29} H_{50} O);^{1} H NMR ($CDCL_3$ 400 MHz) gave signals at δ1,007 (3H. d. J =6.8 Hz. 4H-21), 0.845 (3H. t. J =7.2 Hz, 3H, 29, 0.836 (3H, J = 6.8 Hz 3H - 26), 0.815 (3H, d, J = 6.8 Hz. 3H - 27); ^{13}C NMR ($CDC1_3$, 100MHz) data see Table 1. The ^{1}H NMR spectral data were consistent with reported values [12].

Stearic Acid (2) was obtained as a yellow oil (4lmg); IR (film) (cm^{-1}) 2926. 2857. 2230, 1714, 1465, 1242, El-MS m/z (relative intensity) $[M^+]$ 284 (51), 256 (100) $[M\text{-}CO]^+$, 99 (100) $[M\text{-}CH_2(CH_2)_{10}CO_2H]^+$, 71 (100) $[M\text{-}CH_2(CH_2)_{11}CO,H]^+$, 57(30) $[M\text{-}CH_2(CH_2)_{12}CO_2H]^+$, 43 (100) $[M\text{-}CH_2(CH_2)_{13}CO_2H)^+$; HR El-MS m/z 284.27l9$[M^+]$ (calcd 284.2715 for $C_{18}H_{36}O_2$); ^{1}HNMR($CDC1_3$.400MHz.) data see Table 2 and Figure 1.

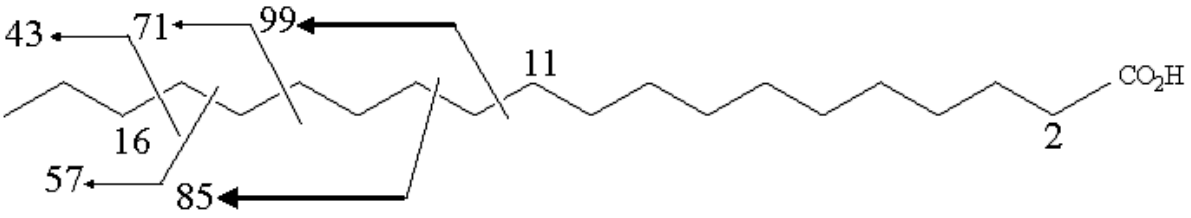

Figure 1

14-octadecenoic Acid (3) was obtained as a yellow oil (44mg), IR (film) (Cm^{-1}) 2935, 2820, 1723, 1474, 1294, 950, 718, El-MS m/z (relative intensity) $[M^+]$ 282 (25), 264 (63) $[M\text{-}H_20]^+$, 11(76) $[M\text{-}CH_2(CH_2)_8CO_2H]^+$ 83 (93) $[M\text{-}CH_2(CH_2)_{10}CO_2H]^+$, 69 (80) $[M\text{-}CH_2(CH_2)_{11}CO_2H^+]^+$, 43(100) $[M\text{-}CH_2\ (CH_2)_{13}\ CO_2H]^+$, HR El-MS m/z 282.2554 $[M^+]$ (calcd 282.2514 for $C_{18}H_{34}O_2$). ^{1}H NMR (CDI_3, 400 MHz) and ^{13}CNMR ($CDC1_3$, 100MHz) data see Table 2 and Figure 1.

Table 1. ^{13}CNMR (100 MHz, CDl_3) data for compound XA-9-4-4-1A

Carbon	δ_c	A Multiplicity
C-l	21.07	(r)
C-2	28.22	(t)
C-3	71.72	(d)
C-4	42.29	(s)
C-3	140.71	(t)
C-6	121.59	(s)
C-7	31.63	(d)
C-8	56.75	(t)
C-9	50.12	(d)
C-10	36.48	(d)
C-11	39.77	(s)
C-12	37.25	(t)
C-13	42.29	(t)
C-14	56.05	(s)
C-15	33.95	(d)
C-16	31.89	(t)
C-17	43.83	(t)
C-18	11.85	(d)
C-19	11.96	(q)
C-20	36.12	(q)
C-21	19.35	(d)
C-22	26.12	(t)
C-23	233.07	(t)
C-24	31.89	(d)
C-25	29.18	(d)
C-26	19.06	(q)
C-27	18.77	(q)
C-28	24.28	(t)
C-29	19.79	(q)

[a]Proton attachments determined via DEPT are shown in parentheses

Table 2. ^{1}H (400 MHz. $CDCl_3$ and ^{13}C (100MHz. $CDCI_3$) NMR Data for 2 and 3

Position	2 δH	J^a	^{1}H 3 δH	J^a	2 δC	^{13}C 3
1.					179.9	180.10
2.	2.34 (t)	7.2	2.34t	7.6	34.2 CH_2	34.10 CH_2
3.	1.63-1.31m		1.28-1.62m		24.6 CH_2	29.70 CH_2
4.	1.63-1.31m		1.28-1.62m		29.7 CH_2	29.80 CH_2
5.	1.63-1.31m		1.28-1.62m		29.8 CH_2	31.50 CH_2
6.	1.63-1.31m		1.28-1.62m		29.9 CH_2	31.60 CH_2
7.	1.63-1.31m		1.28-1.62m		29.8 CH_2	31.93 CH_2
8.	1.63-1.31m		1.28-1.62m		29.9 CH_2	32.90 CH_2
9.	1.63-1.31m		1.28-1.62m		31.8 CH_2	32.90 CH_2
10.	1.63-1.31m		1.28-1.62m		31.9 CH_2	24.60 CH_2
11.	1.63-1.31m		1.28-1.62m		31.9 CH_2	24.60 CH_2
12.	1.63-1.31m		1.28-1.62m		31.9 CH_2	130.21 CH_2
13.	1.63-1.31m		5.34m		31.9 CH_2	29.61 CH_2
14.	1.63-1.31m		5.34m		31.9 CH_2	130.21 CH_2
15.	1.63-1.31m		2.13m		34.1 CH_2	27.18 CH_2
16.	1.63-1.31m		2.05m		22.7 CH_2	22.60 CH_2
17.	1.63-1.31m		2.05m		22.7 CH_2	22.60 CH_2
18.	0.88t	6.8	6.86t	7.2	14.1 CH_2	14.20 CH_2
COOH	7.27s		7.3s			

[a]J values given in hertz

3. Results and Discussion

β-sitosterol **(1)** in had a molecular formula of C_{29} H_{50} O. The molecular ion was indicated by a dominant peak at m/z 414 [M^+] in El-MS. The peaks at m/z (relative intensity) 396 (70) and 381 (30) correspond to the loss of a water molecular ion. The El-MS also showed some characteristics peaks at m/z 107 (49), 105 (95), 93 (36), 91 (33), 81 (61) and 67 (36). The HR El-MS gave m/z 414.3854 and unsaturarion number of 5.0 for [M^+].

The ^{1}H NMR spectrum displayed diagnostic signals due to the influence of the ring system on the side chain methyl groups in the compound: 3H singlets at δ 1.007 and 0.68 (each 3H, S, CH_3 x 2) assigned to 3H - 26: 3H doublet at δ 0.815 (3H, J = 6.8 Hz) assigned to 3H - 27. The summary of ^{13}C NMR assignment is presented as in Table 1.

Stearic acid **(2)** had a molecular formula of C_{18} H_{34} O_2. The molecular ion was indicated by the peak at m/z 284 [M+] in El-MS. It also showed some peaks at m/z 99 (100). 85 (100), 85 (100), 71 (100), 57 (30), and 43 (100) corresponding to the loss of 185, 199, 213, 227 and 241 mass units from the molecular ion [M+]. The IR spectrum displayed an acid band at 1714cm^{-1}. The ^{1}H NMR showed the presence of the following easily recognized peaks: 2H triplet at δ 2.34 (2H, t, J = 7.2Hz) assigned to H-2: triplet at δ. 0.88 (3H. t, J = 6.8Hz) assigned to H-I8:2H multiple at δ 1.63-1.31 (2H, m) assigned to 15 equivalent methylene hydrogens.

The summary of ^{13}C NMR (see Figure-I) assigned is presented as in Table 2.

Trans-14-octadecenoic acid (3) had a molecular formular of C_{18} H_{34} O_2. The molecular ion was indicated by the peak at m/z 282 (M+) in El-MS. The HR El-MS gave rn/z 282.2554 (calcd 282.2514) for [M+] and showed unsaturation number of 2.0. The IR displayed an acid band at 1723cm and unsaturation band (alkene C-H def) at 950cm^{-1}.

The presence of the double between C-14 and C-15 was supported by ^{13}C NMR signals at δ 2.06 (2H, m), 2.13 (2H. m) and 5.34 (IH. m). The placement of the double bond between C-14 and C-15 was further supported by the mass spectra peaks at m/z 43 (100), 69 (80), and 97 (100), corresponding to the loss of 239, 213 and 185 mass units from the molecular ion [M^+]. The summary of ^{13}C NMR spectral data as in Table 2.

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