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Antibacterial Activities of 2-O-butyl-1-O-(2'-ethylhexyl) benzene-1,8-dicarboxylate and 1-phenyl-1,4-pentanedione Isolated from *Vitellaria paradoxa* Root Bark.

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ABSTRACT

A new antibacterial ester (1), 2-O-butyl-1-O-(2'-ethylhexyl) benzene-1,8-dicarboxylate and a ketone (2), 1-phenyl-1,4-pentanedione was isolated from *Vitellaria paradoxa* (G.Don) heper by directing the fractionation of an ethanol extract of the air dried root bark with bacterial sensitivity assay. The structures of 1 and 2 were determined from ^{13}C-NMR, ^{1}H-NMR, DEPT, IR and GC-MS spectral data. The two compounds were found to be active against gram positive *Bacillus subtilis* and *Staphylococcus aureus* and gram negative *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa*. The isolation, structural elucidation, NMR spectral assignment and bioactivities of compounds 1 and 2 are reported.

Key words: *Vitellaria paradoxa*, antibacterial activity, brine shrimp lethality test, structure elucidation

INTRODUCTION

Vitellaria paradoxa (G.Don) Heper. Commonly called 'Shea butter tree' in English, belongs to the family Sapotaceae. It is a plant that grows naturally in the wild in the dry savannah belt of West Africa from Senegal in the west to Sudan in the east and is locally abundant in Nigeria (NRC, 2006; FAO, 2007). It is a small to medium size tree which grows up to a height of 25 m. The tree is much branched, dense and spread in form of hemispherical crown. It consist of one of the most affordable and widely used vegetable fats in the Sahel and the nuts are important internationally where they are being sold to European and Japanese food industries. (Akhter *et al*., 2008).

Vitellaria paradoxa is reported to have a great medicinal value particularly in the preparation of skin ointment. The shea butter which is the fat extracted from the kernel is reported to contain a high level of UV-absorbing triterpenes ester (Wiesman *et al*., 2003; Brucken *et al*., 2008). Its anti-oxidant properties have led to its use in the protection of skin from sun burn, eczema and as a skin rejuvenator (Badifu, 1989; Alender, 2004; Akhter *et al*., 2008). Analysis of the kernel revealed the presence of phenolic compounds such as gallic acid, catechin, epicachin, epigallocachin gallete as well as quercetin and transcinnamic acid (Steven and Nissim, 2003; Maranz *et al*., 2004). The plant is used to treat inflammation, rashes in children, dermatitis, chapping, irritation, ulcer and rheumatism (Hong *et al*., 1996). Leaf decoctions are used for the treatment of stomach ache,

head ache and as an eye lotion. The paste of the root bark is taken orally to cure jaundice as well as diarrhea and stomach ache in humans and applied topically to treat chronic sores and girth sores in horses (Mallogo, 1989). Its stem bark decoction is used in a bath to facilitate child birth, encourage lactation after delivery, treatment of leprosy, neutralization of venom of spitting cobra and for gastric problems as well as for diarrhea and dysentery (Vining, 1992; Von Maydell, 1986). The immense applications of *Vitellaria paradoxa* to the treatments of many bacterial diseases motivated us to investigate the plant for antibacterial components.

MATERIALS AND METHODS

Plant collection: The roots of *Vitellaria paradoxa* (G.Don) heper were collected in August 2006 at Nigerian Defence Academy, Afaka, Kaduna state, Nigeria. The plant was authenticated at the herbarium section of Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. A specimen of the root is preserved at the Herbarium.

Extraction and isolation: The air dried root bark of *Vitellaria paradoxa* (500 g) was milled and extracted at room temperature by percolation with ethanol (2.5 L) for two weeks. The extract was concentrated using rotary evaporator at 40°C to give a residue (150 g). Twenty gram of the ethanolic residue was chromatographed on silica gel column (silica gel, 235 g i.d. 4.5 cm). The column was eluted successively with solvents of increasing polarity to give fractions and residue as shown in parenthesis: petroleum ether (0.5 g); $CHCl_3$ (3.9 g); hexane:EtOAc, 1:1, (0.8 g); $CHCl_3$-EtOAc, 1:1, (0.9 g); EtOAc (0.7 g); EtOAc-MeOH, 1:3, (12.9 g) and EtOAc-MeOH, 1:1, (2.9 g). The $CHCl_3$ fraction a light brown oil (3.5 g) which was found to be highly active against both the shrimp larvae and the targeted bacteria was further chromatographed on silica gel column (silica gel, 100 g, i.d. 2.5 cm) and eluted with a solvent mixture of $CHCl_3$-EtOAc, 1:1, to give 6 fractions. The first fraction after elution with $CHCl_3$-EtOAc, was found be very active and was therefore further chromatographed and eluted with $CHCl_3$ to give the ester (1) (0.98 g) with R_f value of 0.75. The refractive index, viscosity and color of the oil were determined using standard method (AOAC, 1997) and are summerized in Table 4. Fraction (F18) eluted with EtOAc-MeOH, 1:3, (4.3 g) was further chromatographed and eluted with a solvent mixture of $CHCl_3$-EtOAc-MeOH (1:2:1) to give fractions F1 to F15. Fraction 5 which was found to be highly active was coated on a preparatory TLC plate and developed in EtOAc-MeOH (3:1) to obtained three bands; the top band gave the ketone 2 (400 mg) with R_f value of 0.65 and melting point of 23°C.

Brine shrimp lethality test (BST): Fractions were evaluated for lethality to brine shrimp using standard methods (Meyer *et al.*, 1982; McLaughlin, 1991; Solis *et al.*, 1993). In this test a drop of DMSO was added to vials of the test and control substances to enhance the solubility of test materials.

Antibacterial assay: The antibacterial activity of the crude/isolated fractions were determined by paper disc diffusion method (Navarro *et al.*, 1996; Pelczar *et al.*, 1993; Okeke *et al.*, 2001; Ayandele and Adebiyi, 2007) using nutrient agar. A stock solution of each extract was prepared by dissolving 20 mg in 5 cm^3 of the respective solvents of extraction to give 4000 $\mu g\ cm^{-3}$ of the stock solution. Using a micropipette, 0.35, 0.3, 0.25 and 0.2 cm^3 of the solution were separately drawn into

vial and the volumes adjusted to 2 cm^3 to give approximate concentrations of 7×10^2, 6×10^2, 5×10^2 and 4×10^2 μg cm^{-3}, respectively. Filter paper was carefully labeled and cut into sizes of 0.5 cm diameter and separately introduced in each vial containing the prepared solution. They were dried at 40°C. A control was similarly set up using distilled water and ethanol.

Nutrient agar was used as the growth medium for the microbes. Each medium was prepared by dissolving 38 g of the agar in 1000 cm^3 of distilled water, heated to dissolve and autoclaved at 120°C for 15 min. It was then cooled and poured into a petri dish to solidify. Isolates of *S. aureus, S. typhi, E. coli, P. aeruginosa* and *B. subtilis* were separately cultured on each nutrient agar plate; sterile paper disc incorporated with the extract, were placed on each agar and incubated at 37°C 18 to 24 h. All tests were performed in triplicate and the zones of inhibition diameter were measured with the aid of a plastic ruler and the Minimum Inhibitory Concentrations (MIC) determined.

RESULTS AND DISCUSSION

The chromatograhed chloroform fraction which was found to be lethal to the shrimp larvae (BST LC_{50} = 23.20 μg cm^{-3}) was also active against *S. aureus, S. typhi, E. coli, P. aeruginosa* and *B. subtilis* at 700 μg cm^{-3}. The fraction after been further chromatographed yielded six fractions. Three of the fractions viz: F2 (0.49 g), F3 (0.31 g) and F4 (0.13 g) were similar and pure (R_f value 0.75 and BST LC_{50} = 23.7814 μg cm^{-3}) and gave the yellow-orange ester. The refractive index of the isolated ester does not differ much from the refractive index of palm oil while the viscosity obtained fall within category of most fluid (AOAC, 1997). They were highly active against all the test bacteria. They recorded zones of inhibition diameter of 28 and 26 mm against the gram positive bacteria *B. subtilis* and *S. aureus* respectively and 25 mm against the gram negative bacteria *E. coli, S. typhi* and *P. aeruginosa* (Table 1-3).

The preparatory TLC carried out on fraction 5 of F18, gave three pure components (F5:1 (0.40 g), F5:2 (0.04 g) and F5:3 (0.03 g) which have R_f values of 0.65, 0.75 and 0.85 g, respectively. Activity tests showed that TLC fraction F18:5:1 (BST LC_{50} = 100 μg cm^{-3}) was active against *B. subtilis, P. aeruginosa, E. coli, S. aureus* and *S. typhi* (zones of inhibition diameter range of 20-28 mm) and was elucidated as a ketone (2).

The isolated compounds (1 and 2) showed high activities (zones of inhibition diameter range of 25-28 mm) against the gram positive *S. aureus* and *B. subtilis* at the concentration of 7×10^2 μg cm^{-3}. Similarly the activities of compounds 1 and 2 against gram negative *E. coli, S. typhi* and *P. aeruginosa* were high, (zone of inhibition range from 25-28 mm) at the concentration of 7×10^2 μg cm^{-3}.

Comparisons of the antimicrobial efficacies of the compounds with those of reference standard (Amoxicillin, Erythromycin, Chloramphenicol and Gentamycin) indicated that the activities of the compounds were slightly lower than those of the reference standards (Table 1 and 2). For instance, Amoxicillin and compound 2 exhibited zones of inhibition diameter of 30 and 28 mm, respectively against *B. subtilis* at the concentration of 7×10^2 μ cm^{-3}. Similarly, gentamycin and compound 2 recorded zones of inhibition of 30 and 28 mm, respectively against *P. aeruginosa* at the same concentration. This is expected in view of the higher activities exhibited by synthetic drugs compared to their counterparts obtained from natural sources (Bhavanani and Ballow, 2000; Chung *et al.*, 2004; Nair and Chanda, 2004; Borris, 1996; Essawi and Srour, 2005).

Table 1: Activity of isolated compounds against gram positive bacteria

	Zone of inhibition diameter (mm)		
Test sample	Conc ($\times 10^3$ μg mL^{-1})	*Bacillus subtilis*	*Staphylococcus aureus*
Amoxicillin	7	30	33
	6	26	28
	5	21	22
	4	16	17
	C	NI	NI
Erythromycin	7	35	30
	6	30	26
	5	25	22
	4	17	18
	C	NI	NI
Compound 1	7	26	28
	6	22	20
	5	18	14
	4	15	10
	C	NI	NI
Compound 2	7	28	22
	6	23	19
	5	15	13
	4	10	08
	C	NI	NI

NI: No inhibition, C: Control

Table 2: Activity of isolated compounds against gram negative bacteria

	Zone of inhibition diameter (mm)			
Test sample	Concn. ($\times 10^3$ μg mL^{-1})	*Escherichia coli*	*Salmonella typhi*	*Pseudomons aeruginosa*
Gentamycine	7	35	25	30
	6	27	20	23
	5	22	15	18
	4	17	10	12
	C	NI	NI	NI
Chloramphenicol				
	7	33	25	30
	6	26	20	26
	5	20	16	20
	4	14	11	16
	C	NI	NI	NI
Compound 1	7	25	20	25
	6	20	16	19
	5	18	12	13
	4	10	08	09
	C	NI	NI	NI
Compound 2	7	28	22	28
	6	22	18	20
	5	18	12	15
	4	12	08	10
	C	NI	NI	NI

NI: No inhibition, C: Control

Table 3: ^{13}CNMR for compounds 1 and 2

Entry	1	2
1	167.81	198.00
2	132.70	29.58
3	131.15	22.55
4	129.08	190.0
5	129.08	13.28
6	131.15	
7	132.70	
8	167.75	
1'	68.40	130.08
2'	39.00	128.60
3'	66.45	115.18
4'	64.59	102.50
5'	35.67	115.18
6'	30.62	128.60
7'	68.40	
8'	66.45	
9'	64.59	
10'	19.86	
1"	24.02	
2"	14.30	

Table 4: Physical properties of the isolated compounds

Compound	1	2
Refractive index	1.267±0.0001	
Viscosity	2.2±0.2	
Colour	Yellow-orange	Light brown solid
Melting point		23°C

The ^{1}HNMR, ^{13}CNMR, APT and DEPT of compound 1 and 2 were obtained by analyzing the samples on Mercury BB 200 MHZ. The ^{1}HNMR of compound 1 recorded signals at d 7.65 (1H, d, H-3 and H-6), 7.55 (1H, d, H-4 and H-5) which are aromatic protons, 4.55 (2H, t, H-1' and 7'), 4.00 (2H, d, H-8') are methelene protons, 1.75 (1H, d, H-2') a methine proton, 1.55 (3H, q, H-6') and 0.85 (1H, s, H-10') are methyl protons.

The ^{13}CNMR spectrum had signals recorded at d 167.81 (C-1) and 167.75 (C-8) which are carbonyl carbons, d 132.70 (C-2 and C-7) are aromatic quaternary carbons (Table 3) as well as 131.15 (C-3 and C-6) and 129.08 (C-4 and C-5) which are aromatic carbons. The signals at d 68.40 (C-1' and C-7'), 66.45 (C-8'), 64.59 (C-9'), 35.67 (C-3'), 30.62 (C-4'), 29.19 (C-5') and 24.02 (C-1") are methylene groups. Those at d 19.86 (C-10'), 14.30 (C-2") and 11.23 (C-6') are methyl groups. The signal at d 39.00 (C-2') is a methine group (Fig. 1). The IR spectrum showed a strong absorption at 1729.31 cm^{-1} which is due to carbonyl stretching (C=O), 1127.53 cm^{-1} due to the presence of C-O-Cstr of an ester, 1588.28 cm^{-1} due to the presence of C=C of aromatic ring, 1460.31 cm^{-1} due to the presence of methyl and methylene groups and the presence of methyl group was further confirmed by the presence of a peak at 1379.36 (Fig. 2) (Robert and Robert, 1992). The GC/MS gave the molecular weight of the compound as 334, the signal at m/z 267 correspond to the loss of $C_5H_7^+$ from the molecular ion, the signal at m/z 223 corresponds to the loss

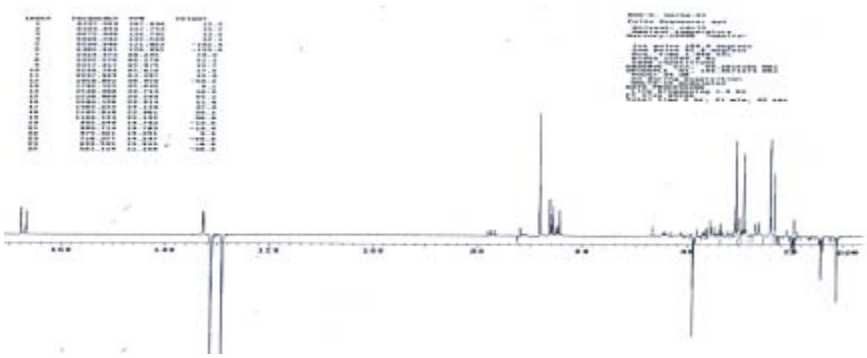


Fig. 1: ^{13}Cnmr of compound 1

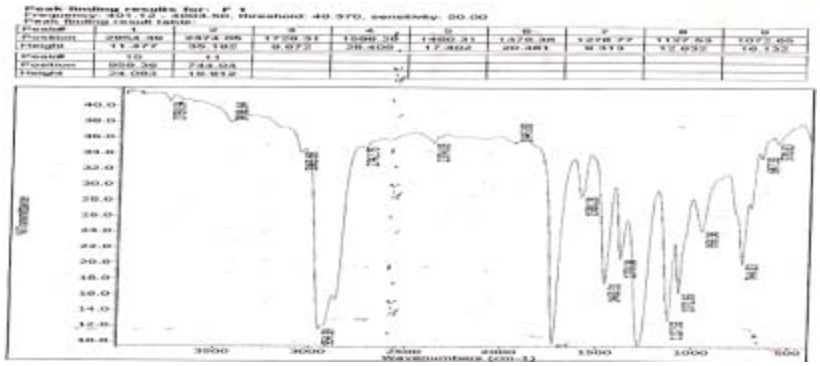
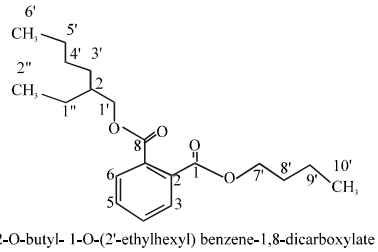


Fig. 2: IR spectra of compound 1

of $C_3H_7^+$ and the signal at m/z 205 corresponds to the loss of H_2O molecule from the ester molecule. The signal at m/z 160 is due to the loss of $CH_3CH_2O^{\cdot}$ radical, the signal at 149 corresponds to the base peak and is due to the loss $C_8H_4O_3^+$. The signal at m/z 76 corresponds to the loss of $C_6H_4^+$ a substituted benzene ring from the molecule. Taking all the above information into consideration the ester isolated from the $CHCl_3$ fractions is interpreted as 2-O-butyl- 1-O-(2'-ethylhexyl) benzene-1,8-dicarboxylate (1).



2-O-butyl- 1-O-(2'-ethylhexyl) benzene-1,8-dicarboxylate

Fractions F18:5:1 had ^{1}HNMR signals recorded at d 8.60 (1H, s, H-2' and H-6'), 8.20 (1H, d, H-3' and H-5') and 7.60 (1H, d, H-4'), are aromatic protons. The one at d 3.60 (2H, t, H-2), 1.20 (2H, d, H-3), are methylene protons. The signal at 2.0 (1H, s, H-5) a methyl group proton. The ^{13}CNMR spectrum showed signals at d 98.0 (C-1) and 190.0 (C-4) which are carbonyl carbons. Those at d 130.08 (C-1'), 128.60 (C-2' and C-6'), 115.18 (C-3' and C-5'), 102.50 (C-4') are aromatic carbons. The signals at d 29.58 (C-2), 22.55 (C-3) are methylene groups and the one at 13.28 (C-5) is a methyl group (Fig. 3).

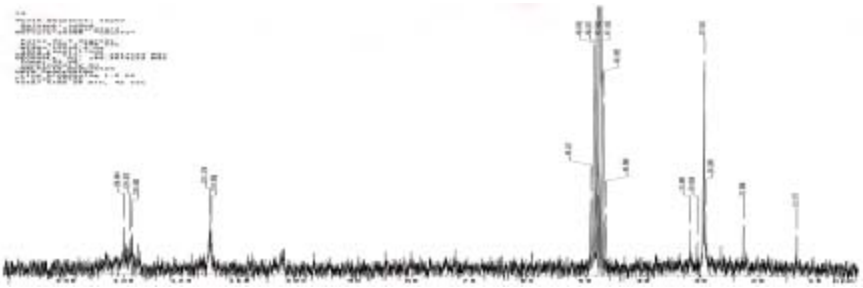


Fig. 3: 13Cnmr of compound 2

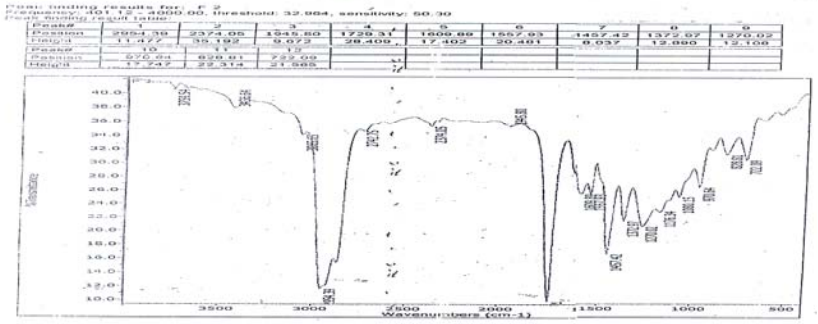
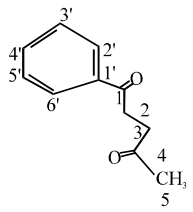


Fig. 4: IR spectra of compound 2

The IR spectrum showed no OH absorption indicating the absence of OH group. The peak at 1729.31 cm^{-1} is due to the presence of carbonyl carbon. The peak at 1557.93 cm^{-1} is due to aromatic C=C ring stretching vibration and those at 828.81 and 722.09 cm^{-1} are due to aromatic out of plane C=C bending vibrations (Fig. 4).

The GC/MS gave the molecular weight of the compound as 176. The signal at m/z 161 is due to the loss of CH_3 group from the molecular ion, the signal at m/z 133 corresponds to the loss of carbonyl group (C=O) from the molecule. The signal at m/z 105 is due to the loss of benzene ring which is the base peak and the signal at m/z 77 is due to the loss of $C_2H_3^+$ from the molecule. Taking all the information's into consideration fraction F18:5:1 is interpreted as 1-phenyl-1,4-pentanedione (2).



1-phenyl-1,4-pentanedione

CONCLUSIONS

2-O-butyl-1-O-(2'-ethylhexyl)benzene-1,8-dicarboxylate and 1-phenyl-1,4-pentanedione isolated from *Vitellaria paradoxa* (root) showed high activity against *S. aureus*, *S. typhi*,

P. aeruginosa, *B. subtilis* and *E. coli*, suggesting that the two compounds may be employed as broad spectrum antibiotics against these organisms.

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