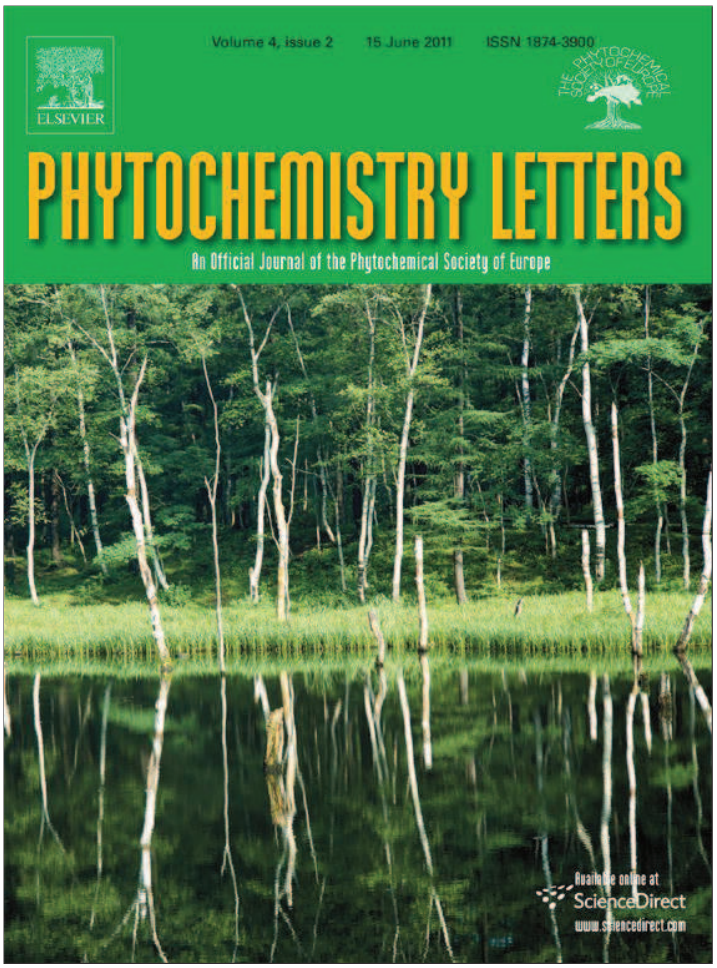

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7α-Acetylugandensolide and antimicrobial properties of *Warburgia ugandensis* extracts and isolates against sweet potato pathogens

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ABSTRACT

7α-Acetylugandensolide together with thirteen known drimane-type sesquiterpenes have been isolated from *Warbugia ugandensis* bark extracts. Their structures were established by spectroscopic and physical methods as well as comparison with data available in the literature. The *in vitro* antimicrobial activities of the extracts and isolates were investigated against fungi and bacteria which infect sweet potato. Ethyl acetate extract exhibited the highest ($P \leq 0.05$) antifungal and antibacterial activities compared to n-hexane and methanol extracts. *Rhizopus stolonifer* was more susceptible to ethyl acetate extract (inhibition zone = 24.5 mm) than Blitox which was used as a positive control. The minimum inhibitory concentration (MIC) for the isolates ranged between 12.5 and 200 μg/ml with the lowest MIC value of 12.5 μg/ml being observed with polygodial against *Fusarium solani*; warburganal against *Aspergillus niger* and *F. solani*; and mukaadial against *A. niger*.

1. Introduction

Sweet potato is an important food crop worldwide since it is drought tolerant and acts as a famine relief crop (Gibson et al., 1997). However, its production is limited by viral, fungal and bacterial infections (Clark and Hoy, 1994). The use of synthetic antimicrobial chemicals to manage infections in crops cause environmental pollution and carcinogenic risk (Cameron and Julian, 1984). There is therefore need to search for environmentally safe antimicrobial agents. The objective of this study was to evaluate the antimicrobial activity of *Warburgia ugandensis* extracts and its sesquiterpenes against fungi and bacteria which infect sweet potato.

W. ugandensis (Canellaceae) is a famous Kenyan medicinal plant used as a remedy for stomachache, constipation, toothache and malaria (Kokwaro, 2009; Dharani and Yenesew, 2010). It is also used for treatment of sexually transmitted diseases, diarrhoea, cough and internal wounds/ulcers (Kiringe, 2006). A number of drimane-type sesquiterpenes (Kubo et al., 1976; Brooks and Draffan, 1969; Kioy et al., 1990; Wube et al., 2005) and flavonoids (Manguro et al., 2003a,b) have been reported from various parts of the plant. *In vitro* pharmacological studies on this plant have also confirmed the presence of insect antifeedant (Kubo et al., 1979), molluscicidal (Kubo et al., 1983), antimicrobial (Mbwambo et al., 2009; Wube et al., 2005; Lee et al., 1999) and antileishmanial (Ngure et al., 2009) active sesquiterpenes.

In the present communication, we document the isolation and structural elucidation of 7α-aceylugandensolide (**1**) and the ^{13}C NMR data of bemadienolide (**2**) from the stem bark of the plant. We also report the isolation of drimenin (**3**) for the first time from *Warburgia genera* and the antimicrobial activity of crude extracts and pure isolates from the plant stem bark.

2. Results and discussion

Compound **1** was obtained as a colorless gummy material with $[\alpha]_D$+24^0 (MeOH, c 0.01). Its structure and molecular formula $C_{19}H_{26}O_6$ was deduced by a combination of high resolution mass spectrum (70 eV) (*m/z* 350.1154, $[M]^+$), ^1H, ^{13}C and DEPT NMR. The IR spectrum showed the presence of an ester carbonyl (1732 cm^{-1}), γ-lactone carbonyl (1680 cm^{-1}) and olefinic bond (1632 cm^{-1}). In the ^1H NMR spectrum, the resonances of three quaternary methyls (δ 1.03, 1.05 and 1.51, each singlet), two oxymethine protons (δ 5.54 br s, *J* = 1.7 Hz and 5.15, br d, *J* = 1.3 Hz, H-7) and oxymethylene protons (δ 4.71, dd, *J* = 17.8, 1.1 Hz and 4.61, d, *J* = 17.7 Hz) could be ascribed to a drimane skeleton (Brooks and Draffan, 1969; Kioy et al., 1990). The ^1H NMR spectral features of **1** closely resembled those of ugandensolide (Brooks and Draffan,

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Table 1
1D and 2D NMR data of compound **1**.

Position	^{1}H (δ_H, mult, J in Hz)[a]	^{13}C (δ_C, mult)[a]	^{1}H–^{1}H COSY	HMBC ($^3J_{CH}$)	NOESY
1_{eq}	2.56 d d d (13.1, 3.5, 1.6)	36.3 t	H-1_{eq}, H-2_{eq}, H-2_{eq}, Me- 15	C-3, C-5, C-9 C-9, C-3,	H-2_{eq}, Me-15
1_{ax}	1.19 t d (13.2, 3.8, 0.8)		H-1_{ax}, H-2_{ax}, H-2_{ax}, Me-15		H-3_{ax}, H-5
2_{eq}	1.80 q t (14.2, 3.7)	18.3 t	H-1_{ax}, H-2_{ax}, H-3_{eq}, H-1_{eq}, H-3_{eq}		H-3_{eq}
2_{ax}	1.61 m		H-1_{ax}, H-2_{eq}, H- 3_{ax}, H-3_{eq}	C-4, C-10	Me-15, Me-14
3_{eq}	1.53 m	43.0 t	H-2_{ax}, H-2_{eq}, H- 3_{ax}		H-2_{eq}
3_{ax}	1.29 t dd (13.6, 4.1, 0.8)		H-2_{ax}, H-2_{eq}, H- 3_{eq}	C-1, C-5, C-14, C-13	H-1_{eq}, H-5
4		35.4 s			
5	1.54 d (1.7)	50.6 d	H-6	C-1, C-3, C-7, C-9, C-15	H-1_{ax}, H-6, H-3_{ax}
6	5.54 br s (1.7)	69.6 d	H-5, H-7	C-10, C-4, C-8	H-5, Me-15
7	5.15 br d (1.3)	66.6 d	H-6	C-5, C-9, C-12.	H-12_{eq}
8		150.6 s			
9		140.3 s			
10		33.3 s			
11		171.0 s			
12_{eq}	4.71 d (17.8)	70.2 t	H-12_{ax}	C-8, C-6	H-7eq
12_{ax}	4.61 d (17.7)		H-12_{eq}		
Me-13	1.03 s	33.2 q		C-3, C-5, C-14	Me-14, H-6, H-5
Me-14	1.05 s	22.9 q		C-1, C-9, C-5	H-2_{ax}, Me-15
Me-15	1.51 s	20.6 q		C-1, C-5, C-9	Me-14, H-1_{ax}, 6-OAc
6-OAc	2.08 s	171.1 s			
6-OAc Me		21.3 q			
7-OAc	2.13 s	169.8 s			
7-OAc Me		20.8 q			

[a] ^{1}H and ^{13}C NMR spectra were run at 500 and 125 MHz, respectively in $CDCl_3$.

1969) except for an additional acetyl group signal evidenced by a peak at δ_H 2.13 with corresponding ^{13}C NMR peaks centered at δ_C 169.8 and 20.8. Also, the presence of 17 carbon signals for ugandensolide isolated from *W. ugandensis* were also observed in the ^{13}C NMR spectrum, thus suggesting that compound **1** may be related to ugandensolide. Unequivocal information on substitution mode in **1** was established from ^{1}H and ^{13}C NMR, ^{1}H–^{1}H COSY and HMBC data (Table 1). Comparison of the ^{1}H NMR data of **1** with those of ugandensolide showed an oxymethine peak relatively downfield at δ 5.15 suggesting that the second acetyl group could be at C-7. On the other hand, interpretation of the ^{1}H–^{1}H COSY data revealed the presence of two spatial structures: –CH_2–CH_2–CH_2– and –CH–CHO–CHO–. In fact, in the COSY spectrum, H-6 was the only proton that correlated with the C-7 methine proton, thus allowing the unambiguous placement of the second acetyl group at C-7. This interpretation was confirmed by HMBC long range correlations ($^3J_{CH}$) between H-7 and C-9 (δ 140.3) and in turn with C-5 (δ 50.6). Similarly, the H-7 peak directly correlated with the ^{13}C NMR peak at δ 66.6 (C-7) in the HSQC spectrum. Also, the observation of HMBC correlations between the two protons at δ 4.61 and 4.70 with C-7 and C-9 allowed for the location of oxymethylene at C-12 forming an α, β-unsaturated γ-lactone ring. The relative configurations particularly at positions C-6 and C-7 were deduced by ^{1}H NMR coupling constants and NOESY experiments. The doublets at δ 4.61 and 4.70 were assigned to H-12_{ax} and H-12_{eq}, respectively, based on NOE correlation observed between H-12_{eq} and H-7 in the NOESY spectrum. A broadened singlet at δ 5.54 attributed to H-6 showed a NOESY correlation with H-5 (δ 1.54) and also in turn with Me-14 but not with Me-15, suggesting that H-6 occupied a position equatorial to the axial Me-15 group at C-10, leaving the acetyl group to be β-oriented. This is in agreement with the observed coupling constants for H-6 (J = 1.7 Hz) with H-5. The H-6 proton was however observed not to correlate with H-7 in the NOESY spectrum, thus suggesting that the two vicinal oxygenated methine carbons have a trans-stereochemistry. This was confirmed by coupling constant data (J = 1.3 Hz) for H-7 with H-6 in the ^{1}H NMR spectrum. In fact, if the stereochemistry of the two oxygenated methine carbons is *cis* then the coupling constant between H-6 and H-7 would be a doublet of approximately J = 10 Hz (Harinantenaina et al., 2007). Thus on the basis of accrued spectroscopic data, compound **1** was concluded to be 7α-acetylugandensolide.

2.1. Antimicrobial activity of crude extracts and isolates

Results of the antimicrobial activities of MeOH, EtOAc and n-hexane extracts of *W. ugandensis* bark are shown in Table 2. The extracts were tested against fungi (*Alternaria* spp, *Aspergillus niger*, *Fusarium oxysporum*, *F. solani* and *Rhizopus stolonifer*) and bacteria (*Ralstonia solanacearum* and *Steptomyces ipomoeae*). The extracts showed activity against the fungi and bacteria tested with ethyl acetate extract exhibiting the highest ($P \leq 0.05$) inhibitory effects against the microorganisms. Methanol extract exhibited the lowest activity. In our observation, the fungus *R. stolonifer* were found to be the most susceptible to the ethyl acetate extract with inhibition zone of 24.5 mm, respectively, while *F. solani* was the least susceptible (inhibition zone, 9.4 mm). The antifungal activity of ethyl acetate extract against *R. stolonifer* was significantly ($P \leq 0.05$) higher than that of Blitox which was used as a positive control. Similarly, the bacteria *R. solanacearum* used in this study

Table 2
Antimicrobial activity of crude extracts.

Microorganism	Zone of growth inhibition in mm[a] (5 mm diameter)				
	Extracts			Standard drugs	
	EtOAc	n-hexane	Methanol	Blitox	Streptocycline
Fungi					
Alternaria spp.	17.1	14.1	5.4	22.1	ND
A. niger	21.1	11.5	3.1	28.0	ND
F. oxysporum	13.4	8.1	1.0	16.9	ND
F. solani	9.4	4.0	2.5	25.1	ND
R. stolonifer	24.5	13.7	5.6	18.3	ND
Bacteria					
R. solanacearum	16.2	12.3	5.0	ND	18.8
S. ipomoeae	12.3	10.2	6.2	ND	14.4
Mean	16.3	10.2	6.2	22.1	16.6

[a] Values are means of three replicates; ND, not done.

Table 3
Minimum inhibitory concentration (MIC, μg/ml) of isolated compounds.

Compound	MIC, μg/ml of isolated compounds						
	Test fungi					Test bacteria	
	Alter spp.	*A. nig*	*F. oxy*	*F. sol*	*R. sto*	*R. sola*	*S. ipo*
7α-Acetoxyugandensolide (**1**)	>200	>200	>200	>200	>200	200	>200
Bemadienolide (**2**)	>200	>200	>200	>200	>200	>200	100
Drimenin (**3**)	>200	>200	>200	>200	>200	>200	>200
Polygodial	25	50	50	12.5	50	25	50
Warburganal	50	12.5	25	12.5	25	50	50
Mukaadial	25	12.5	100	25	50	25	50
Ugandensidial	50	25	50	100	25	100	25
Muzigadial	50	50	25	100	50	25	50
6α-Hydroxymuzigadial	200	>200	100	>200	>200	100	>200
9-Deoxymuzigadial	>200	>200	>200	>200	>200	>200	>200
Ugandensolide	50	50	100	200	100	>200	100
Deacetoxyugandensolide	50	>200	>200	100	200	100	200
Cinnamolide	100	100	>200	200	>200	>200	>200
3β-Acetoxycinnamolide	>200	>200	>200	>200	>200	>200	>200
Blitox	50	6.25	12.5	6.25	12.5	ND	ND
Streptocycline	ND	ND	ND	ND	ND	25	12.5

ND, not done; *Alter* spp, *Alternaria* spp; *A. nig*, *Aspergillus niger*; *F. oxy*, *Fusarium oxysporum*; *F. sol*, *Fusarium solani*, *R. sto*, *Rhizopus stolonifer*; *R. sola*, *Ralstonia solanacearum*; *S. ipo*, *Steptomyces ipomoeae*.

showed moderate susceptibility to EtOAc extract with inhibition zones of 16.2 mm.

Fractionation of n-hexane and EtOAc extracts led to the isolation of 14 drimane sesquiterpenes of which ten showed activity against one or more of the tested microorganisms while four compounds (1,3,9-deoxymuzigadial and 3β-acetoxycinnamolide) were not (Table 3). Polygodial has been previously reported to exhibit antimicrobial activity (Taniguchi et al., 1978, 1988). In the present work, the compound inhibited the growth of *F. solani* (MIC = 12.5 μg/ml) while with both *Alternaria* spp and *R. solanacearum* a MIC = 25 μg/ml was afforded. Mukaadial was another isolate with promising antimicrobial results. The compound was observed to inhibit the growth of *A. niger* with MIC = 12.5 μg/ml. With this compound, the microorganisms *Alternaria* spp, *F. solani* and *R. solanacearum* afforded a MIC value of 25 μg/ml. Antimicrobial activity of drimane sesquiterpene dialdehydes has been attributed to α- and β-unsaturated aldehydes (Taniguchi et al., 1988), it is therefore worth noting that warburganal, which possesses this structural feature appeared to be the most active principle. The compound inhibited the growth of *A. niger* and *F. solani* with MIC value of 12.5 μg/ml and both *F. oxysporum* and *R. stolonifer* with MIC = 25 μg/ml. Another dialdehyde, ugandensidial was found to be effective against *A. niger*, *R. stolonifer* and *S. ipomoeae* with MIC value 25 μg/ml. Within the range of activity of drimane sesquiterpenes, it is noted that a structure in which one of the methyls at C-4 shifts to C-3 leaving exocyclic methylene group as in the case of muzigadial does not enhance the antimicrobial activity. Muzigadial was found to inhibit the growth of both *F. oxysporium* and *R. solanocearum* with MIC = 25 μg/ml.

Compounds polygodial, warbuganal and mukaadial exhibited comparable antimicrobial activity against one or more of the tested microorganisms (MIC ≤ 12.5 μg/ml) with reference to Blitox and Sterptocycline which were standard antibiotics. The rest of the active compounds gave MIC values ≤200 μg/ml.

This study revealed that extracts of *W. ugandensis* have antimicrobial activity against *F. oxysporum*, *F. solani*, *Alternaria* spp, *R. stolonifer*, *A. niger*, *R. solanacearum* and *S. ipomoeae* which are soil pathogens associated with rotting of sweet potato and other root crops (Ristaino, 1993). This suggests that the pathogens can be managed using herbal extracts as had also been observed in other studies (Okigbo and Nmeka, 2005). The herbal extracts are more environmentally safe compared to the synthetic antimicrobial drugs currently used (Masuduzzaman et al., 2008; Siva et al., 2008). Extracts from *W. ugandensis* are not only active against fungi and bacteria that cause disease in animals/man (Kubo and Nakanish, 1979; Ndalut et al., 2006; Mbwambo et al., 2009) but are also active against plant pathogens thus suggesting that the antimicrobial principles in the plant have broad spectrum activity. Most of the compounds isolated from the plant exhibited lower antimicrobial activity compared to the extracts. This observation suggests that the metabolites possibly exert synergistic effects or that there could be other more active minor compounds that were not isolated in this study.

3. Experimental

3.1. General experimental procedure

Melting points were determined on a Gallenkamp (Loughborough, UK) melting point apparatus and are uncorrected. The UV spectra were run on Pye Unicam SP8-150 UV–vis spectrophotometer (Cambridge, UK) using acetonitrile. IR data were recorded on a PerkinElmer FTIR 600 series spectrophotometer (Waltham, MA, USA) as KBr pellet. The ^{1}H and ^{13}C NMR data were measured in $CDCl_3$ and $CDCl_3$–DMSO-d_6 on a Bruker NMR Ultrashield TM (Darmstadt, Germany) operating at 500 and 125 MHz, respectively. The MS data were obtained on a Varian MAT 8200A instrument (Bremen, Germany).

3.2. Plant materials

The stem bark of *W. ugandensis* was collected near St Mary's Hospital along the Nakuru-Gilgil highway (latitude 0°24′42.49″S and longitude 36°15′10.59″E) in August 2008 and voucher specimen (2008/08/01/SAO/CHEMMK) was identified at the Kenya National Museum herbarium after comparison with authentic samples. The plant materials were chopped into smaller pieces, air-dried in the open and reduced to a powder using a mill.

3.3. Extraction and isolation of compounds

Powdered plant material (2 kg) was sequentially extracted with n-hexane, EtOAc and MeOH in the cold for seven days each with occasional shaking. The macerate was filtered and the filtrate concentrated under vacuum using rotary evaporator to afford 20 g,

105 g and 215 g of n-hexane, ethyl acetate and methanol extracts, respectively.

3.4. Fractionation of n-hexane extract

Approximately 15 g of the extract was subjected to chromatography over silica gel column (3 cm × 60 cm, SiO_2 250 g, pressure = 1 bar), eluted with n-hexane, n-hexane–EtOAc (95:5; 9:1 and 4:1) to give three major fractions (F1–F3). Fraction F1 (eluants 15–75, 5 g) yellowish-brown oil was kept for GC–MS analysis. Fraction F2 (eluants 78–127) crystallized out and was purified by recrystallization in n-hexane–EtOAc (9:1) to give polygodial in 150 mg. The last fraction F3 (eluants 130–151) also contained single spot R_f = 0.35, was similarly purified by recrystallization in n-hexane–EtOAc, 4:1 to give cinnamolide (15 mg).

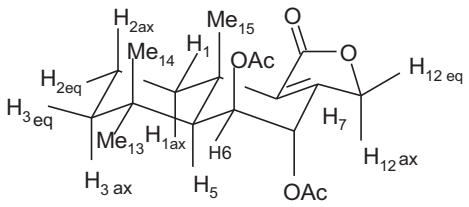
3.5. Fractionation of EtOAc extract

Approximately 80 g of ethyl acetate extract was dissolved in minimum amount of ethyl acetate and adsorbed into silica gel before evaporation to dryness. The resulting dark brown material was loaded on top of silica gel packed glass column (5 cm × 60 cm, SiO_2 1200 g) and eluted with increasing concentration of EtOAc in n-hexane, EtOAc, CH_2Cl_2–MeOH mixture with increasing concentration of MeOH and elution was finally concluded with MeOH. The whole process was done under medium pressure chromatography (pressure = 1.5 bar). A total of 400 fractions (each 20 ml) were sampled and their homogeneity monitored by TLC using solvent systems: n-hexane–ethyl acetate (9:1, 4:1, 3:2, 2:1 and 1:1) and CH_2Cl_2–MeOH (95:5 and 9:1). The eluants were combined depending on TLC profiles to give the six pools (I–VI).

Pool I, fractions 15–60 (n-hexane–EtOAc 95:5 eluates, 15 g) was an oily substance with an aromatic smell and was similarly kept for GC–MS analysis. Pool II, fractions 64–100 (n-hexane–EtOAc 9:1, and 4:1 eluates, 10 g) showed four spots of R_f values 0.53 (major component), 0.42, 0.35 and 0.22, which turned bluish-purple with anisaldehyde–conc. H_2SO_4 mixture after spraying and heating at 100 °C. The fractions were combined and from this pool crystallized out colorless needles which were filtered and further purified by recrystallization (n-hexane–EtOAc, 95:5) to give polygodial, R_f = 0.53 (n-hexane–EtOAc, 9:1, 120 mg). The mother liquor was evaporated under reduced pressure and upon further purification using (n-hexane–EtOAc, 9:1) followed by the same solvent system in the ratios 19:3 and 4:1 afforded further polygodial in 25 mg, cinnamolide (R_f = 0.42, n-hexane–EtOAc, 4:1, 73 mg), warbuganal (R_f = 0.35, n-hexane–EtOAc 4:1, 65 mg) and 9-deoxymuzigadial (n-hexane–EtOAc 4:1, R_f = 0.22, 45 mg). Pool III, fractions 102–220 (n-hexane–EtOAc 4:1, 3:2 and 1:1, 15 g) contained one major spot R_f = 0.33 (n-hexane–EtOAc 3:2) which masked other spots. This pool also crystallized out to give an amorphous powder which was further purified by re-crystallization in n-hexane–EtOAc, 19:3 to afford colorless plate-like material, muzigadial (2000 mg). The mother liquor was further purified by repeated flash chromatography (3 cm × 60 cm, 300 g) using n-hexane–EtOAc (9:1, 4:1 and 3:2), collecting 10 ml each to give a further polygodial (15 mg), warburganal (20 mg) and muzigadial (68 mg). The other secondary metabolites isolated from this eluate included bemadienolide (**2**, R_f = 0.22, n-hexane–EtOAc 3:2, 55 mg) and drimenin (**3**, R_f = 0.18, n-hexane–EtOAc 3:2, 64 mg).

Fractions 225–300 which constituted pool IV (11 mg) afforded five spots of R_f values 0.36, 0.32, 0.29, 0.26 and 0.18 using eluant: n-hexane–EtOAc, 1:1. The spots upon spraying with anisaldehyde–conc. H_2SO_4 mixture and heating turned bluish-purple. A change of developing reagent to iodine vapor revealed an additional spot of R_f value 0.34. This pool was resolved into individual compounds by fractionation over silica gel column (3.5 cm × 60 cm, SiO_2 250 g) using n-hexane–EtOAc (3:2) to give further warbuganal, muzigadial and 2 in 16, 29 and 10 mg, respectively. Also isolated from this fraction were 3β-acetoxycinnamolide (R_f = 0.26, 65 mg), 7α-acetylugandensolide (1, R_f = 0.34, 85 mg) and ugandensidial (R_f = 0.18, 54 mg). Pool V (fractions 310–360, 9 g) showed three spots R_f values 0.61 (major), 0.59 and 0.46 using CH_2Cl_2–MeOH, 97:3 as the developing solvent. Subjecting this pool to repeated fractionation over silica gel column (2.5 cm × 60 cm, 210 g) elution with CH_2Cl_2–MeOH (99:1) and collecting 10 ml each afforded mukaadial (R_f = 0.61, 100 mg), 6α-hydroxymuzigadial (R_f = 0.59, 55 mg) and ugandensolide (R_f = 0.46, 72 mg). Pool VI (fractions 365–400, 8 g) showed one component with R_f = 0.41 after development with CH_2Cl_2–MeOH (95:5) and spraying with anisaldehyde–conc. H_2SO_4 mixture. The compound was obtained in pure form by re-crystallization in n-hexane–CH_2Cl_2–MeOH (97:2.5:0.5) to give colorless crystals, deacetoxyugandensolide (45 mg).

7α-Acetylugandensolide (**1**). Colorless gummy material, $[\alpha]_D + 24^0$ (MeOH, c, 0.001); mp 228–230 °C; IR ν_{max} (KBr) cm^{-1}: 1: 2966, 2915, 1758, 1732, 1680, 1632, 1458, 1373, 1245, 1196, 1095, 1043, 1009, 964, 742; ^1H and ^{13}C NMR δ: data see Table 1; EIMS *m/z* (rel. int.): 350 $[M]^+$ (2), 307 (23), 290 (65), 274 (15), 248 (18), 247 (100), 232 (35), 206 (22), 42 (84); ESI-MS *m/z*: 373 $[M+Na]^+$.



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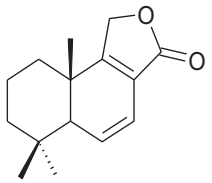

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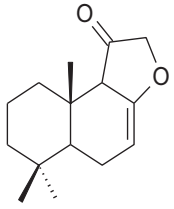

3

Bemadienolide (**2**). Colorless crystals (n-hexane–ethyl acetate, 3:2), mp 124–126 °C; IR ν_{max} (KBr) cm^{-1}: 2978, 1760, 1640, 1461, 1398, 1246; ^1H NMR (500 MHz, $CDCl_3$) δ_H: 6.32 (1H, d, *J* = 10.0 Hz, H-7), 6.01 (1H, d, *J* = 10.1 Hz, H-6), 4.83 (1H, d, *J* = 17.2 Hz, H-11α), 4.77 (1H, d, *J* = 17.1 Hz, H-11β), 2.22 (1H, m, H-5), 1.04, 1.01, 0.99 (9H, 3 s, 13, 14, 15-Me); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C: 33.5 (C-1), 17.9 (C-2), 40.5 (C-3), 32.7 (C-4), 52.3 (C-5), 117.6 (C-6), 131.7 (C-7), 122.3 (C-8), 171.7 (C-9), 36.8 (C-10), 67.7 (C-11), 170.2 (C-12), 32.3 (C-13), 22.5 (C-14), 14.97 (C-15); EIMS *m/z* (rel. int.): 232 $[M]^+$ (27), 217 (10), 203 (18), 176 (15), 173 (18), 161 (26), 149 (39), 147 (100), 131 (20), 119 (32), 91 (28), 41 (28); ESI-MS *m/z*: 255 $[M+Na]^+$.

Drimenin (**3**). Colorless crystals (n-hexane–ethyl acetate, 3:2), mp 131–133 °C; IR ν_{max} (KBr) cm^{-1}: 2923, 2848, 1761, 1706, 1487, 1385, 1365, 1274, 1172, 1134, 1003, 985, 933, 861, 809, 715, 560; ^1H NMR (500 MHz, $CDCl_3$) δ_H: 5.74 (1H, d, *J* = 4.0 Hz, H-7), 4.66 (2H, br s, H-12), 2.78 (1H, s, H-9), 2.48 (1H, dd, *J* = 12.6, 4.1 Hz, H-6α),

1.78 (1H, d, J = 12.8 Hz, H-6β), 1.98 (1H, m, H-5), 0.92 (3H, 3s, 14-Me), 0.89 (3H, 3s, 15-Me), 0.87 (3H, 3s, 13-Me); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C: 23.3 (C-1), 18.2 (C-2), 38.37 (C-3), 34.3 (C-4), 54.6 (C-5), 42.3 (C-6), 121.1 (C-7), 129.7 (C-8), 49.6 (C-9), 41.5 (C-10), 175.3 (C-11), 69.8 (C-12), 32.9 (C-13), 21.3 (C-14), 13.9 (C-15); EIMS m/z (rel. int.): 235 $[M]^+$ (100), 191 (10), 189 (10), 179 (7), 139 (6), 124 (28), 111 (25), 95 (12), 91 (5); ESI-MS m/z: 258 $[M+Na]^+$.

3.6. Isolation of test organisms

Five fungi and two bacteria isolated from infected sweet potato roots were used in this study. Sterilized pieces of infected sweet potato were incubated in nutrient agar (NA) and potato dextrose agar (PDA) at room temperature for up to 5 days and fungal and bacterial growth associated with rot affected tissues were identified with the aid of the appropriate taxonomic keys (Ainsworth et al., 1973). The isolates were maintained on NA and PDA slants.

3.7. Antimicrobial assay of crude extracts

Antimicrobial activities of the methanol, ethyl acetate and n-hexane extracts of *W. ugandensis* were evaluated by the agar diffusion method (Barry et al., 1979). The tests were performed in sterile Petri dishes (90 mm diameter) containing 20 ml PDA and NA for fungi and bacteria, respectively. The PDA and NA media were prepared by suspending 39 and 28 g in 1 l of distilled water and heated to dissolve completely. The media were sterilized by autoclaving at 120 °C for 20 min. Inoculation was done by spreading 0.5 ml of spore suspension (1×10^5 cfu/ml) of the test pathogen on the surface of the solidified agar (Kariba et al., 2001). Paper disc (Whatmann No. 1, 5 mm diameter) were impregnated with 100 μl of the plant extracts (5 mg/ml) using a sterile micropipette and left for 30 min to dry in the hood. The dried discs were placed on the surface of the solidified inoculated agar and incubated at 28 °C for 48 h for fungi and 37 °C for 24 h for bacteria. Blitox and streptocycline (10 μg/ml) were used as positive controls while DMSO without plant extract was used as a negative control. All tests were done in triplicates for statistical purposes. The presence of zones of inhibition around the disc was interpreted as an indication of antimicrobial activity.

3.8. Antimicrobial assay of pure isolates and minimum inhibitory concentration

The minimum inhibitory concentrations (MICs) of pure isolates were determined as outlined by Kariba et al. (2001). The compounds were dissolved in DMSO and different concentrations ranging between 200 μg/ml and 1 μg/ml prepared. Sterile paper discs were impregnated with 100 μl of the reconstituted samples in DMSO. The dried discs were transferred aseptically into PDA an NA plates previously inoculated with test fungi and bacteria, respectively and MIC was regarded as the lowest concentration that produced a visible zone of inhibition.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phytol.2011.02.007.

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