

Isolation and Characterization of Sesquiterpenes from Stem Bark of *Warburgia ugandensis* Sprague

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Abstract

Warburgia ugandensis Sprague is one of the medicinal plants traditionally used to treat a number of diseases like asthma, cough, diarrhea, common cold, stomachache and toothache in Ethiopia. However, there is still insufficient information on the isolation and evaluation of bioactive compounds from this plant species. Extraction, purification and isolation of the stem bark of this plant by dichloromethane and chloroform as solvents afforded two sesquiterpenes; namely, WU-1 (ugandensidial) and WU-2 (cinnamolide-3 β -acetate) respectively. The structural elucidations of these bioactive compounds were accomplished by using a variety of spectroscopic methods (IR, UV and NMR). The spectroscopic results compared with the reported data in the literature.

Keywords: Medicinal plants; Natural products; Sesquiterpenes; *Warburgia ugandensis* Sprague.

INTRODUCTION

Natural products extracted from medicinal plants are the major sources of modern drugs that have been used for treatment of human pathogens (Sasidharan *et al.*, 2011). The studies of these medicinal plants were the basis of earliest medicines. Plant derived medicines constitute a substantial component of present day human healthcare systems (Gómez *et al.*, 2007, Butle 2004). According to World Health Organization (WHO), more than 60% of the world's population and over 80% of the people in low incoming/developing countries depend upon traditional medicine for their primary health care needs (Sukirtha *et al.*, 2012).

In recent years, medicinal plants as natural products have attracted attention due to their important bioactive content such as terpenes, alkaloids, anthraquinones, steroids, tannins, glycosides, saponins, volatile oils, resins, phenols and flavonoids that are deposited in their different parts (Saiprasanna *et al.*, 2012). The medicinal value of these plants also depends on bioactive phytochemical constituents that produce a definite physiologic action on the human body (Mohammed, 2018).

The genus *Warburgia* is one of the flowering plants belongs to Canellaceae family (Rabe *et al.*, 2000). The four species of *Warburgia* namely, *Warburgia elongate* Verdc, *Warburgia salutaris* (Bertol. f.) Chiov., *Warburgia stuhlmannii* Engl. and *Warburgia ugandensis* Sprague are distributed restrictly in the

eastern and southern Africa (Maroyi, 2013; Muchugi *et al.*, 2008).

Warburgia ugandensis Sprague is highly aromatic evergreen trees that has restricted distributions in the evergreen forests (Rabe *et al.*, 2000; Drage *et al.*, 2014) and widely used as traditional medicinal plant in many parts of East Africa (Kenya, Uganda and Ethiopia) (Drage *et al.*, 2014, Rajab *et al.*, 2000). The plant is known with different names: East African green wood, East African green heart, East African pepper bark tree, Kenya green heart, green heart, pepper-bark tree (English) (Maundu *et al.*, 2005; Wube *et al.*, 2005), Zogdom (Wube *et al.*, 2005) or Kenaffa (Amharic) (Dagne *et al.*, 2009) and Befti (Oromo language).

The stem bark and roots have been used for the treatment of various diseases such as diarrhea, cough, common cold, general muscular pains, internal wounds, loss of appetite, malaria, syphilis, gonorrhea, stomachache, toothache, colds, throat and chest infections, fever, weak joints and general body pains (Rabe *et al.*, 2000, Dharan *et al.*, 2008). The compounds isolated from this plant also possess antifeedant, antibacterial, antifungal, anti-mycobacterial, cytotoxic (Rabe *et al.*, 2000, Rajab *et al.*, 2000) anti-plasmodial, anti-trypanosomal (Wube *et al.*, 2010) anti-asthmatic (Karani *et al.*, 2013) and antileishmanial activities (Ngure *et al.*, 2009). However, the leaves of plant has weaker property compared to the stem bark and roots (Mbwambo *et al.*, 2009). In Ethiopia, particularly in Bale zone, stem bark of this plant is traditionally used

for treatment of a wide range of diseases, such as stomachache, toothache, malaria, tuberculosis, gonorrhoea, asthma (Wube *et al.*, 2010, Wube *et al.*, 2005), cough and rabies (Geyid *et al.*, 2005). It is also used to remove tapeworm from human body.

Many drimane and coloratane sesquiterpenes was isolated from the stem barks of *Warburgia ugandensis* Sprague. Some of these include waburganal (**1**), polygodial (**2**) (Kioy *et al.*, 1990; Xu *et al.*, 2009), mukaadial (**3**), warburgin (**4**) warburgiadione (**5**) (Wube *et al.*, 2005; Kioy *et al.*, 1990; Xu *et al.*, 2009), pereniporin B (**6**) (Rajab *et al.*, 2000), cinnamolide (**7**), cinnamolide-3 β -acetate (**8**), ugandensolide (**9**) (Wube *et al.*, 2005; Xu *et al.*, 2009), dendocarbin A (**10**), ugandenial A (**11**), 9 α ,11 α -dihydroxy, 6 β -acetyl-cinnamolide (**12**), dendocarbin L (**13**), dendocarbin M (**14**), 9 α -hydroxycinnamolide (**15**) (Xu *et al.*, 2009), muzigadial (**16**), muzigadiolide (**17**) (Wube *et al.*, 2005; Kioy *et al.*, 1990; Rukutt *et al.*, 2005), 4(13),7-coloratadien-12,11-olide (**18**), 6 α ,9 α -dihydroxy-4(13),7-coloratadien-11,12-dial (**19**) and 7 β -hydroxy-4(13),8-coloratadien-11,12-olide (**20**), 7 α -hydroxy-8-drimen-11,12-olide (**21**) (Wube *et al.*, 2005), cinnamolide-3 β -ol (**22**), deacetylugandensolide (**23**) (Xu *et al.*, 2009), 11 α -hydroxymuzigadiolide (**24**) (Rajab *et al.*, 2000; Wube *et al.*, 2005), ugandensidial (**25**) (Rajab *et al.*, 2000; Wube *et al.*, 2005; Kioy *et al.*, 1990).

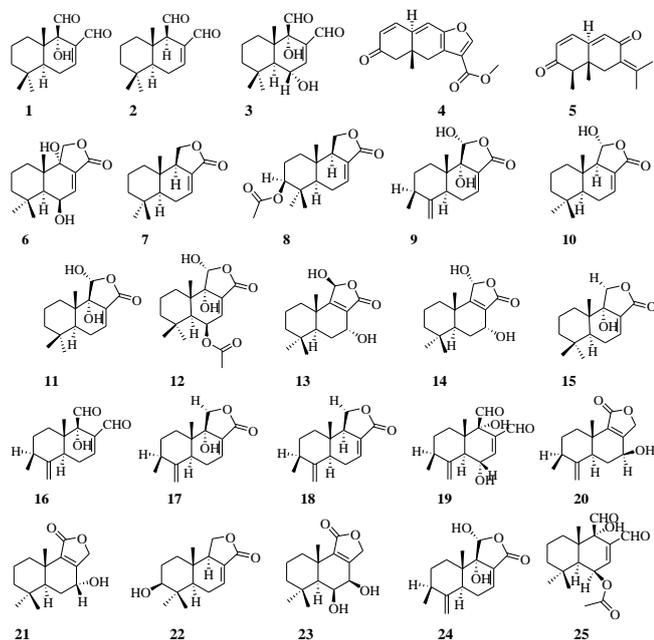


Figure 1. Drimane and coloratane sesquiterpenes isolated from stem bark of *Warburgia ugandensis* Sprague.

Many of sesquiterpenes have been extracted and isolated from this plant species. Despite of its medicinal uses, but still, extraction, isolation then characterization of active compounds from this medicinal plant is not sufficient. These, therefore, give emphasis on the choice

of appropriate solvents and selection of the plant part (stem bark) to isolate the phytochemical constituents and characterize with very visible spectroscopic data.

MATERIALS AND METHODS

Experimental materials

Freshly stem bark of *Warburgia ugandensis* Sprague (Canellaceae) was collected from Barbere wareda, Bale zone, South Eastern part of Ethiopia. The plant was then shade-dried at room temperature for one month. The dried stem barks were crushed to fine powder using an electrical grinder.

Chloroform, dichloromethane, EtOH, petroleum ether, n-hexane, deuterated chloroform (CDCl₃) were used. Analytical TLC was performed using 20 x 20 cm, 0.20 mm thick silica gel 60 with fluorescent indicator UV₂₅₄ to determine the number of components in a mixture and the purity of compounds. Kieselgel 40*, particle size of 0.063-0.200 mm and 70-230 mesh ASTM silica gel was used for column chromatography. Compounds on TLC were first detected by Ultraviolet lamp, multiband 254/366 nm and then sprayed with 1% vanillin in sulfuric acid.

Heidolph rotary evaporator was used for removal of solvent under reduced pressure. Melting points were determined by using Stuart SMP3 melting point apparatus. UV/Vis spectra were recorded on GENESY'S 2PC UV-Vis scanning spectrometer (200-800 nm) in CH₃CN. The 1D (¹H at 400.13 and ¹³C at 100.6 MHz) were recorded on a Bruker Advance 400 spectrometer in CDCl₃. The residual proton signal of the solvent is used as reference. IR spectra were recorded with KBr pellets on Perkin Elmer Bx Infrared Spectrometer in the range 400- 4000 cm⁻¹. Optical rotations were measured on Autopol IV automatic polarimeter at 20° in CHCl₃.

Experimental procedures

The air-dried and finely powdered stem bark (100 g) was soaked and extracted successively with 600 mL of n-hexane, dichloromethane and chloroform for 48hours. Each extract was filtered and the solvent was removed by rotary evaporator under reduced pressure.

The crude dichloromethane extract (5.5 g) was subjected to column chromatography on a silica gel using n-hexane (100%) as eluent. Out of 25 fractions collected, fractions 11 and 12 were combined, concentrated and further fractionated over a silica gel column using n-hexane/EtOAc 85:15 as eluent. Fractions 6 and 7 which showed a single spot with same R_f value were combined and concentrated to give 21 mg of white solid. The compound was coded as **WU-1**.

The crude chloroform extract (1.93 g) was applied on a column silica gel using 100% hexane as mobile phase. Fractions 11-15 were combined, concentrated and further purified on silica gel column using n-hexane/EtOAc 70:30. Fractions 10 and 11 showed a

single spot with the same R_f value and were combined and concentrated to afford 44mg of white solid. The compound was labeled as **WU-2**.

RESULTS AND DISCUSSIONS

Characterization of compound WU-1

WU-1 was isolated as a white crystalline solid with melting point of 131-134°C and R_f value 0.34 using n-hexane: ethyl acetate (3:2) as a solvent system. **WU-1** was reacted with 2,4-dinitrophenylhydrazine (DNP) in ethanol and H₂SO₄ gave yellow precipitate indicating the presence of carbonyl groups in the compound. The optical rotation ($[\alpha]_D^{20}$, CHCl₃) of **WU-1** measured to be -197° indicating that the compound is optically active. The UV(CH₃CN) spectrum (**Figure 2**) showed maximum absorption (λ_{max}) at 220nm indicating the presence of unsaturated group in the compound.

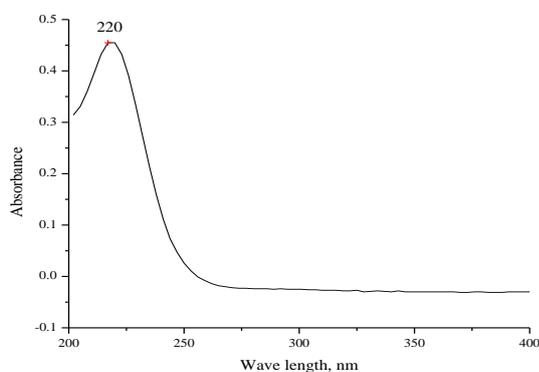


Figure 2. UV-Visible spectrum of **WU-1**.

The IR spectrum (**Figure 3**) displayed strong absorption band at 3431 cm⁻¹ due to vibrational stretching of O-H group. Absorption band at 2926 cm⁻¹ indicated the presence of C-H stretching of saturated group. IR spectrum demonstrated, in addition to strong absorption bands at 1744 and 1721 cm⁻¹ due to ester carbonyl and aldehyde, the presence of olefinic group at 1693 cm⁻¹.

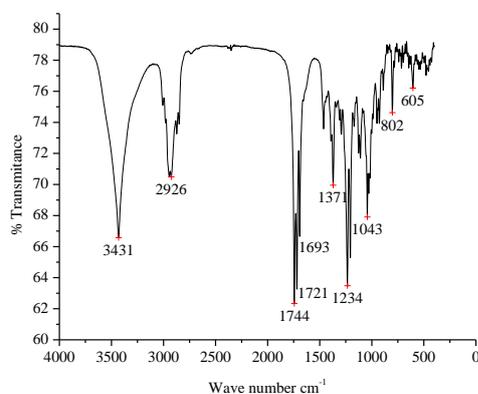


Figure 3. IR spectrum of **WU-1**.

The ¹H NMR spectrum (**Figure 4** and **Table 1**) demonstrated a doublet at δ 9.78 and a singlet at δ 9.5

each integrating for one proton due to two aldehyde protons. The doublet at δ 7.03 integrating for one proton corresponded to anolefinic proton. The triplet at δ 5.92 which integrated for one proton is due to a proton on a methine carbon attached to an electronegative atom whereas the peak at δ 4.10 indicated the presence of OH group. The spectrum also showed a singlet at δ 2.16 due to an acetyl methyl group and three methyl groups at δ 1.35, 1.18 and 1.04 attached to quaternary carbon atoms.

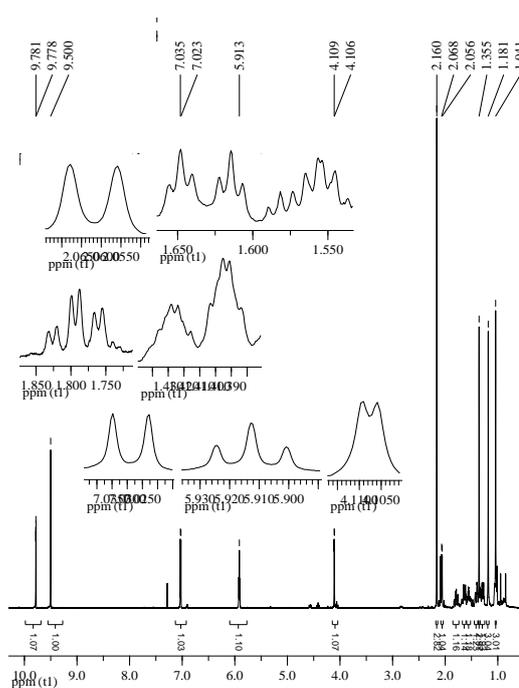


Figure 4. ¹H NMR spectrum of **WU-1**.

The ¹³C NMR spectrum (**Figure 5** of **WU-1** showed well-resolved resonances of the 17 carbon atoms. The ¹³C NMR and the DEPT135 (**Figure 6** and **Table 2**) spectra displayed four quaternary carbons, one ester carbonyl carbon (δ 170.09), three methine carbons, two aldehyde carbonyl carbons (δ 201.17 and 193.08), three methylene carbons and four methyl carbons.

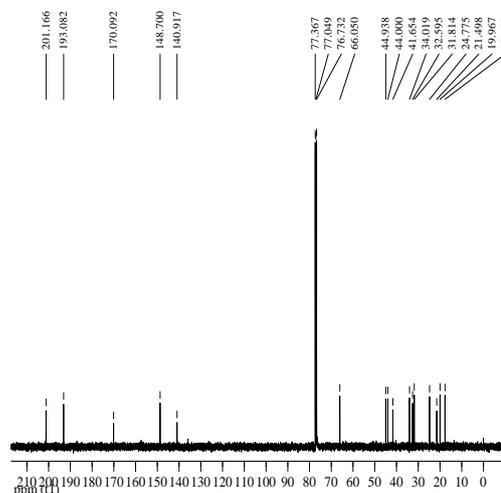


Figure 5. ¹³C NMR spectrum of **WU-1**.

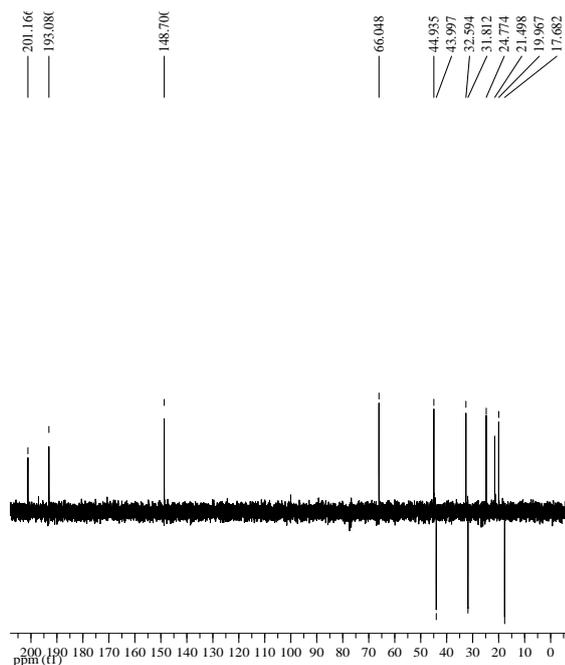


Figure 6. DEPT 135 spectrum of WU-1.

Table 1. Proton decoupled ^{13}C NMR and DEPT 135 (100.6 MHz, CDCl_3) spectroscopic data of WU-1.

Carbons No.	^{13}C NMR δ (ppm)	DEPT 135 δ (ppm)	Remark
C-1	31.81	31.81	CH_2
C-2	17.68	17.68	CH_2
C-3	44.00	44.00	CH_2
C-4	34.02	-	C (Quaternary carbon)
C-5	44.94	44.94	CH
C-6	66.05	66.05	CH
C-7	148.70	148.70	CH
C-8	140.92	-	C (Quaternary carbon)
C-9	-	-	-
C-10	41.65	-	C (Quaternary carbon)
C-11	201.17	201.17	CH
C-12	193.08	193.08	CH
C-13	32.60	32.60	CH_3
C-14	24.77	24.77	CH_3
C-15	19.97	19.97	CH_3
CH_3CO	21.50	21.50	CH_3
CH_3CO	170.09	-	C (Quaternary carbon)

The ^1H and ^{13}C NMR results obtained for WU-1 were comparable with ^1H NMR (Kioy *et al.*, 1990) and ^{13}C NMR (Rukutt *et al.*, 2005) spectral data of ugandesial (25) (Table 2).

Table 2. Comparison of ^1H (400.13, MHz, CDCl_3) and ^{13}C NMR (100.6 MHz, CDCl_3) spectroscopic data of WU-1 and ugandesial (25).

Position of carbons	WU-1		Ugandesial	
	δ ^{13}C NMR	^1H NMR δ (ppm) (Multiplicity, Integration)	δ ^{13}C NMR	^1H NMR δ (ppm) (Multiplicity, Integration)
C-1	31.81	1.83-1.76 (2H, m)	32.02	-
C-2	17.68	1.65-1.55 (2H, m)	17.88	-
C-3	44.00	1.43-1.40 (1H, m) 1.33 (1H, m)	44.21	-
C-4	34.02	-	34.21	-
C-5	44.94	2.09(1H, d, J= 4.8Hz)	45.16	2.04 (1H, d, J=4.7 Hz)
C-6	66.05	5.92 (1H, t, J= 4.8Hz)	66.26	5.89 (1H, t, J=4.7Hz)
C-7	148.70	7.03 (1H, d, J= 4.8Hz)	148.86	7.00 (1H, d, J= 4.7Hz)
C-8	140.92	-	141.14	-
C-9	-	4.10 (1H, d, J= 1.2 Hz)	-	4.10(1H,d, J=1.4Hz) = 1.4 Hz, 9-OH)= 1.4 Hz, 9-OH)
C-10	41.65	-	41.85	-
C-11	201.17	9.78 (1H, d, J= 1.2 Hz)	201.32	9.76 (1H, d, J= 1.4 Hz)
C-12	193.08	9.5(1H, s)	193.24	9.48(1H, s)
C-13	32.60	1.04 (3H, s)	32.79	1.03 (3H, s)
C-14	24.77	1.18 (3H, s)	24.96	1.17 (3H, s)
C-15	19.97	1.35(3H, s)	20.15	1.34 (3H, s)
CH_3CO	21.50	2.16 (3H, s)	21.67	2.14 (3H, s)
CH_3CO	170.09	-	170.27	-

In addition, the melting point of WU-1 (131-134°C) was in good agreement with the reported melting point of ugandesial (138-140°C) (Xu *et al.*, 2009). Based on the above data and in comparison with the literature (Kioy *et al.*, 1990), WU-1 (Figure 7) is most probably ugandesial.

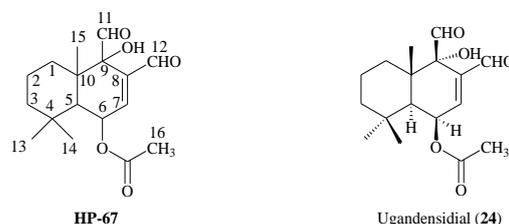


Figure 7. The structure of WU-1 and ugandesial (24).

Characterization of compound WU-2

WU-2 was obtained as a white crystalline solid (melting point 155–157°C) with Rf value 0.41 using n-hexane: ethyl acetate (3:2) as solvent systems. It is optically active with optical rotation ($[\alpha]_D^{20}$, (CHCl₃) +16. An absorption maximum (λ_{\max}) at 221 nm in the UV (CH₃CN) spectrum (Figure 8) was characteristic of a molecule possessing an unsaturated lactone structure.

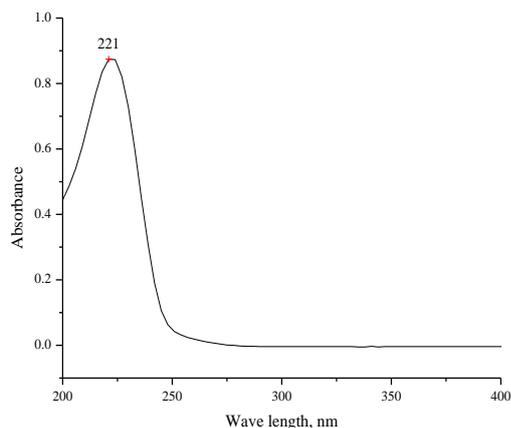


Figure 8. UV-Visible spectrum of WU-2.

The IR(KBr) spectrum (Figure 9) displayed an absorption band at 3439 cm⁻¹ due to the stretching vibration of O-H group whereas absorption bands at 2969 cm⁻¹ and 2914 cm⁻¹ demonstrated C-H stretching vibration of alkane. Strong absorption bands at 1760 cm⁻¹ and 1680 cm⁻¹ showed the presence of an α,β -unsaturated carbonyl group while absorption band at 1731 cm⁻¹ indicated the presence of ester carbonyl group. The strong absorption bands at 1246 cm⁻¹ and 1197 cm⁻¹ demonstrated C-O stretching.

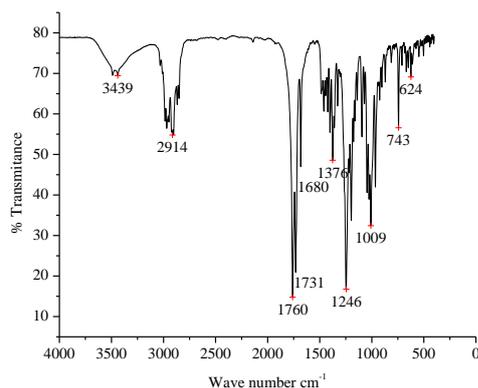


Figure 9. IR spectrum of WU-2.

The quartet at δ 6.9 observed in the ¹H NMR spectrum (Figure 10 and Table 4) of WU-2 integrating for one proton corresponded to an olefinic proton attached to C-7. The triplets at δ 4.41 and 4.05 integrated for one proton each due to oxymethyleneprotons on C-11. The doublet of doublet at δ 4.57 which integrated for one proton indicated oxymethine proton attached to C-3. The ¹H-NMR spectrum also showed three methyl protons signals at δ 1.00 (3H, s), 0.93 (3H, s) and 0.84

(3H, s) whereas the acetate methyl protons appeared at δ 2.08.

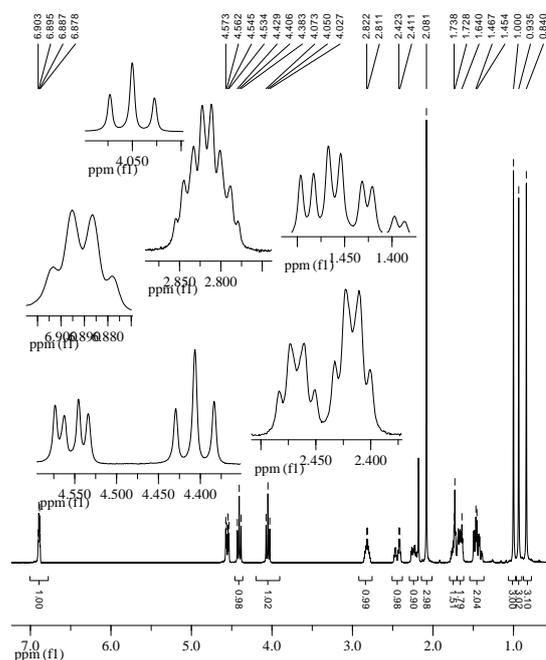


Figure 10. ¹H NMR spectrum of WU-2.

The ¹³C NMR (Figure 11) and DEPT135 experiments (Figure 12 and Table 3) displayed 17 carbon atom resonances comprising of two carbonyl carbons (δ 169.81 and 170.79), three quaternary carbons (δ 34.01, 37.63 and 127.16), four methine (δ 49.28, 50.57, oxymethine δ 80.13 and olefinic methine δ 135.82), four methylene (δ 23.52, 24.65, 36.67 and oxygenated methylene δ 66.95) four methyl carbons (δ 13.49, 15.99, 21.23 and 27.80).

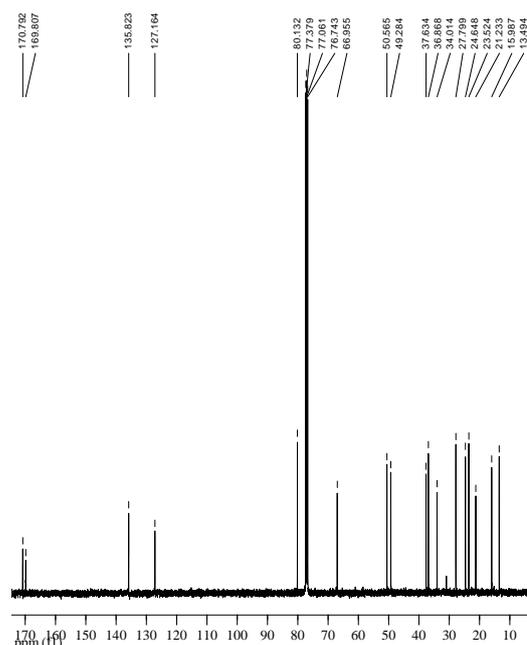


Figure 11. ¹³C NMR spectrum of WU-2.

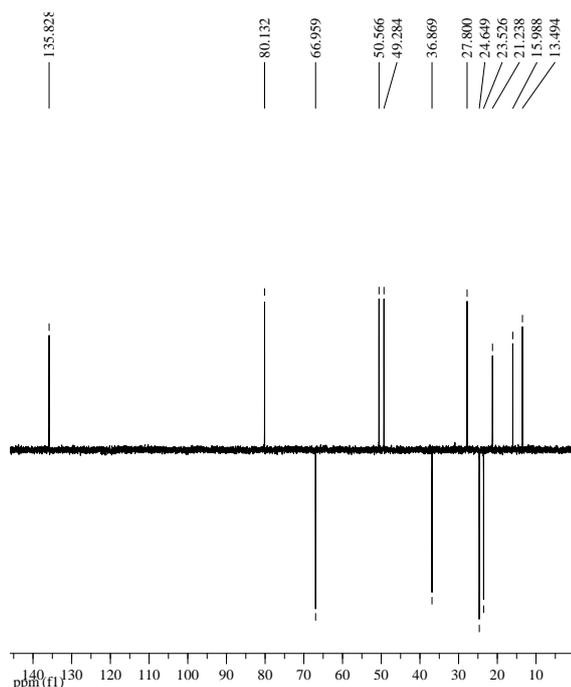


Figure 12. DEPT 135 spectrum of WU-2.

Table 3. Proton Decoupled ^{13}C NMR and DEPT 135 (100.6 MHz, CDCl_3) data of WU-2.

Carbons No.	^{13}C NMR δ (ppm)	DEPT 135 δ (ppm)	Remark
C-1	36.67	36.67	CH_2
C-2	23.52	23.52	CH_2
C-3	80.13	80.13	CH
C-4	37.63	-	C (Quaternary carbon)
C-5	49.28	49.28	CH
C-6	24.65	24.65	CH_2
C-7	135.82	135.82	CH
C-8	127.16	-	C (Quaternary carbon)
C-9	50.57	50.57	CH
C-10	34.01	-	C (Quaternary carbon)
C-11	66.95	66.95	CH_2
C-12	169.81	-	C (Quaternary carbon)
C-13	27.80	27.80	CH_3
C-14	15.99	15.99	CH_3
C-15	13.49	13.49	CH_3
C-16	21.23	21.23	CH_3
C-17	170.79	-	C (Quaternary carbon)

The ^1H NMR and ^{13}C NMR data obtained for WU-2 are comparable with data obtained for cinnamolide-3 β -acetate (**8**) (isolated and identified from the same plant) in the literature (Wube *et al.*, 2005). Moreover, the melting point of WU-2 (155–157°C) and cinnamolide-3 β -acetate (153–157°C) (Kioy *et al.*, 1990) are comparable.

Table 4. Comparison of ^1H (400.13, MHz, CDCl_3) and ^{13}C -NMR(100.6 MHz, CDCl_3) spectral data of WU-2 with those of cinnamolide-3 β -acetate (**8**).

Carbons No.	Observed NMR data of HP-81(ppm)		Cinnamolide-3 β -acetate (ppm)	
	δ ^{13}C NMR	δ ^1H NMR(ppm) (Multiplicity,Integration)	δ ^{13}C NMR	δ ^1H NMR(ppm) (Multiplicity, Integration)
1	36.67	1.42(1H, ddd, J= 13.6, 4Hz) 1.65 (1H, td, J= 11.6, 5.2 Hz)	36.9	1.42 (1H, ddd, J= 13.5, 4.0 Hz, α) 1.64 (1H, dt, J= 13.5, 3.5Hz, β)
2	23.52	1.73(2H, m)	23.5	1.68 (1H, ddd, J= 12.5, 1.5 Hz, β) 1.74 (1H, ddd, J= 13, 4 Hz, α)
3	80.13	4.57 (1H, dd, J= 11.2, 4.4Hz)	80.2	4.55 (1H, dd, J= 11.5, 4.5 Hz, α)
4	37.63	-	37.7	-
5	49.28	1.48 (1H, dd, J= 11.6, 5.2 Hz)	49.4	1.48 (1H, dd, J= 10.5, 4.5 Hz)
6	24.65	2.22(1H, td, J= 11.6, 4 Hz) 2.45 (1H, qd, 20, 4, 8.8 Hz)	24.7	2.22 (1H, ddq, J= 12.0, 3.5, 1.5 Hz, β) 2.44 (1H, dq, J= 20, 5, 4.0 Hz, α)
7	135.82	6.9 (1H, q, J= 3.2 Hz)	135.7	6.89 (1H, q, J= 3.5 Hz)
8	127.16	-	127.2	-
9	50.57	2.82 (1H, m)	50.6	2.82 (1H, m)
10	34.01	-	34.1	-
11	66.95	4.05 (1H, t, J= 9.2 Hz), 4.41 (1H, t, J= 9.2Hz)	66.9	4.05 (1H, t, J= 9.0 Hz, β), 4.41 (1H, t, J= 9.0 Hz, α)
12	169.81	-	169.7	-
13	27.80	0.93 (3H, s) (3H, s, H-13)	27.6	0.94 (3H, s) (3H, s, H-13)
14	15.99	1.00 (3H, s)	15.9	1.00 (3H, s)
15	13.49	0.84 (3H, s)	13.5	0.84 (3H, s)
16	21.23	2.08 (3H, s)	21.2	2.07 (3H, s)
17	170.79	-	170.7	-

Based on the above spectroscopic data and in comparison with literature data (Wube *et al.*, 2005), **WU-2** is proposed to be (+) cinnamolide-3 β -acetate (**8**).

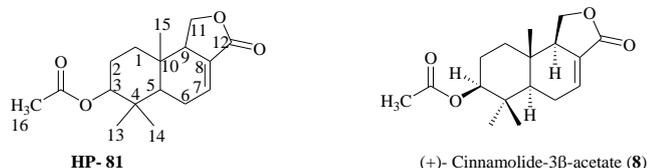


Figure 13. The structure of **WU-2** and of cinnamolide-3 β -acetate (**8**).

CONCLUSION

Phytochemical investigation of the stem bark of *Warburgia ugandensis* Sprague (Canellaceae) afforded two bioactive sesquiterpenes namely **WU-1** (ugandensidial) and **WU-2** (cinnamolide-3 β -acetate) isolated from dichloromethane and chloroform extracts respectively. Identification of these compounds was based on the melting point, array of spectroscopic data (UV/Visible and NMR) and comparison of their spectroscopic data with reported literature values. The traditional medicinal use of this plant may be attributed to its high content of these bioactive constituents.

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Conflict of interest: The author declares that there are no conflicts of interest concerning the publication of this article.

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