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Sesquiterpenes from *Warburgia ugandensis* and their antimycobacterial activity

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Abstract

The dichloromethane extract of the stem bark of *Warburgia ugandensis* afforded three new coloratane sesquiterpenes, namely: 6α,9α-dihydroxy-4(13),7-coloratadien-11,12-dial (**1**), 4(13),7-coloratadien-12,11-olide (**2**), and 7β-hydroxy-4(13),8-coloratadien-11,12-olide (**3**), together with nine known sesquiterpenes, i.e., cinnamolide-3β-acetate (**4**), muzigadial (**5**), muzigadiolide (**6**), 11α-hydroxymuzigadiolide (**7**), cinnamolide (**8**), 7α-hydroxy-8-drimen-11,12-olide (**9**), ugandensolide (**10**), mukaadial (**11**), ugandensidial (**12**), and linoleic acid (**13**). Their structures were assigned on the basis of 1D and 2D-NMR spectroscopic and GC-MS analysis.

The compounds were examined for their antimycobacterial activity against *Mycobacterium aurum*, *M. fortuitum*, *M. phlei* and *M. smegmatis*; and the active constituents showed MIC values ranged from 4 to128 μg/ml compared to the antibiotic drugs ethambutol (MIC ranged from 0.5 to 8 μg/ml) and isoniazid (MIC ranged from 1 to 4 μg/ml).

Keywords: *Warburgia ugandensis*; Canellaceae; Antimycobacterial activity; Drimane sesquiterpenes; Coloratane sesquiterpenes; 6α,9α-Dihydroxy-4(13),7-coloratadien-11,12-dial; 4(13),7-Coloratadien-12,11-olide; 7β-Hydroxy-4(13),8-coloratadien-11,12-olide

1. Introduction

Warburgia ugandensis Sprague (Canellaceae), which is commonly known as *zogdom* in Amharic, is characterized by its bitter and peppery taste. The stem bark has been widely used in East African ethnomedicine for the treatment of stomach-ache, constipation, toothache, cough, fever, muscle pains, weak joints and general body pains (Kokwaro, 1976; Watt and Breyer-Brandwijk, 1962). The Shinasha people in Ethiopia use the stem bark for the treatment of tuberculosis. Species of the genus *Warburgia* are known to be rich in sesquiterpenes of the drimane and coloratane skeletones (Kioy et al., 1990; Mashimbye et al., 1999), which have been shown to possess insect antifeedant, antimicrobial, antiulcer, molluscicidal (Kubo et al., 1983) and antifungal properties (Kubo and Taniguchi, 1988). Previous phytochemical investigation of *W. ugandensis* showed the presence of muzigadial, ugandensidal, pereniporin B, polygodial, mukaadial, warburganal, cinnamolide and 11α-hydroxymuzigadiolide in the stem bark; and ugandensolide, ugandensidial, warburgin and warburgiadione in the heart wood (Brooks and Draffan, 1969).

The drimanes, a group of sesquiterpenoides isolated from species of the genus *Warburgia*, are characterized by α,β-unsaturated carbonyl chromophores assembled around a *trans*-decalin ring system. As part of our search for antimycobacterial agents from Ethiopian medicinal plants, we identified three new and nine known sesquiterpenes along with a known unsaturated

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fatty acid from the stem bark of *W. ugandensis* and evaluated their antimycobacterial activity against four rapidly growing species of mycobacteria.

There are several reports on the constituents of this plant, but no report has been found on their antimycobacterial activity.

2. Results and discussion

Compound **1** was obtained as colourless needles in *n*-hexane/CH_2Cl_2 (see Fig. 1). A molecular formula $C_{15}H_{20}O_4$ was determined by HRMS (*m/z*; measured 287.1264 $[M + Na]^+$; calc. 287.1259). In addition, a prominent peak at *m/z* 235 $[M - CHO]^+$ was present in the EI-MS spectrum, corresponding to a molecular formula $C_{15}H_{20}O_4$, which is in agreement with 15 carbon signals observed in the ^{13}C NMR spectrum. A broad band absorption at 3406 cm^{-1} in the IR spectrum suggested the presence of hydroxyl groups. In addition, the IR spectrum showed carbonyl absorptions at 1725, 1683 cm^{-1} and an olefinic absorption at 1640 cm^{-1}. An absorption maximum at 234 nm in the UV spectrum was also indicative of an α,β-unsaturated lactone. The ^1H NMR spectrum (Table 1) of **1** showed characteristic signals of a coloratadiene sesquiterpene ring system (Ying et al., 1995) with signals at δ 5.03 and 5.13 attributable to two exocyclic methylene protons, as well as a one proton doublet at δ 7.10 for H-7. A singlet at δ 0.96 and a doublet at 1.11 for two methyl groups were also observed in the ^1H NMR spectrum. The signals for H-13a and H-13b protons were unambiguously assigned based on NOE correlations observed between CH_3-14 and H-13a and between H-6β and H-13b in the NOESY spectrum. The signals at δ 9.50 and 9.65 were attributed to two aldehyde groups which were further confirmed by the carbonyl signals at δ 192.6 and 200.5 in the ^{13}C spectrum. The remaining two broad singlets at δ 1.59 and 4.07 in the ^1H NMR spectrum, which did not exhibit any correlations in the HMQC spectrum, were assigned to two hydroxyl groups. The former was assigned to a hydroxyl group bonded to the tertiary carbon, C-6, whereas the downfield broad singlet at δ 4.07 was assigned to a hydroxyl group attached to the quaternary carbon, C-9. This was further supported by the downfield carbon resonances, δ 66.1 and 77.6, observed in the ^{13}C NMR spectrum of **1** for C-6 and C-9, respectively. The ^{13}C and DEPT analyses gave signals corresponding to two methyl, three methylene, six methine and four quaternary carbons further confirming the presence of a coloratane type sesquiterpene. Carbon resonances at δ 106.7, 139.3, 149.1 and 153.7 were assigned to four olefinic carbons, whereas carbon signals at δ 66.1 and 77.6 were assigned to methine and quaternary carbons bearing hydroxyl groups, respectively. The HMQC, HMBC and NOESY experiments allowed unambiguous assignment of the chemical shift values of the methylene protons at C-1 and C-2. Assignment of the relative stereochemistry of the two hydroxyl groups in **1** was accomplished by analyses of the coupling constants and NOESY spectrum. The proton H-6 showed NOE correlation (Fig. 2) to CH_3-15, thus H-6 occupied a position axial to the axial CH_3-15 group at C-10 leaving OH-6 α-oriented. This is in agreement with the observed coupling constants for H-6 ($J = 10.0$ Hz) with H-5 and ($J = 2.5$ Hz) with H-7. Similarly, a cross NOE peak was observed between the aldehyde proton H-11 and CH_3-15, indicating that the

Table 1
^1H NMR spectral data for compounds **1**–**3**[a] (500 Hz, $CDCl_3$)

Proton	1	2	3
1α	1.02 (*dt*) (13.5, 4.0)	1.54 (*dt*) (13.5, 4.0)	1.47 (*dt*) (13.5, 4.0)
1β	2.05 (*m*)	1.65 (*td*) (13.5, 3.0)	2.58 (*m*)
2α	1.12 (*m*)	1.25 (*m*)	1.33 (*m*)
2β	1.73 (*m*)	1.70 (*td*) (13.0, 3.0)	1.80 (*m*)
3	2.00 (*m*)	2.05 (*m*)	2.11 (*m*)
5	2.65 (*d*) (10.0)	2.18 (*m*)	2.38 (*bd*) (13.0)
6α		2.35 (*m*)	1.92 (*m*)
6β	4.70 (*dd*) (10.0, 2.5)	2.32 (*m*)	1.99 (*m*)
7	7.10 (*d*) (2.5)	6.91 (*q*) (3.5)	4.54 (*d*) (4.0)
9		2.99 (*m*)	
11α	9.65 (*s*)	4.48 (*t*) (9.0)	
11β		4.02 (*t*) (9.0)	
12α	9.50 (*s*)		4.68 (*d*) (17.0)
12β			4.96 (*d*) (17.0)
13α	5.13 (*s*)	4.90 (*s*)	4.86 (*s*)
13β	5.03 (*s*)	4.73 (*s*)	4.62 (*s*)
14	1.11 (*d*) (6.5)	1.11 (*d*) (6.5)	1.10 (*d*) (7.0)
15	0.96 (*s*)	0.65 (*s*)	0.89 (*s*)
6-OH	1.59 (*bs*)		
7-OH			1.94 (*s*)
9-OH	4.07 (*bs*)		

Coupling constant values (in parentheses) are in Hz.
[a] Chemical shifts are in ppm relative to TMS.

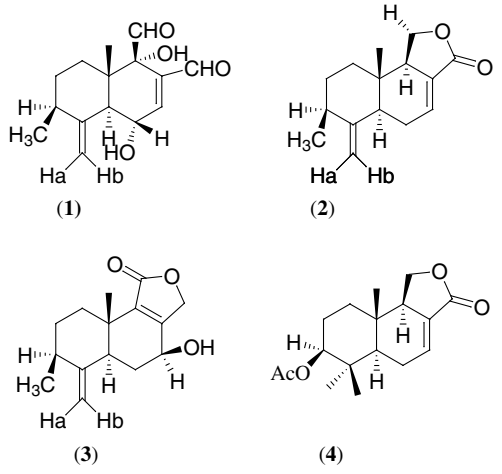

Fig. 1. The structure of compounds **1**–**4** isolated from *W. ugandensis* stem barks.

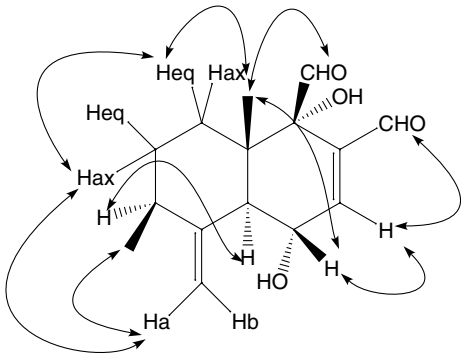

Fig. 2. NOE correlations in compounds **1**.

aldehyde at C-9 is located equatorial to the axial CH_3-15 leaving the hydroxyl at position 9 α-oriented. On the basis of these observations compound **1** was identified as 6α,9α-dihydroxy-4(13),7-coloratadien-11,12-dial.

Compound **2** was isolated as a white prism from CH_2Cl_2. A molecular formula of $C_{15}H_{20}O_2$ for **2** was deduced from the HRMS (*m/z*; measured 233.1542 $[M+H]^+$; calc. 233.1536). The EI-MS spectrum also gave *m/z* 232 $[M]^+$. The IR spectrum exhibited a strong absorption bands at 1757 and 1686 cm^{-1}, suggesting the presence of an α,β-unsaturated lactone and a band at 1639 cm^{-1} due to olefinic groups. The ^1H NMR spectrum of compound **2** differed from **1** in the absence of two aldehyde protons at C-11 and C-12. Instead, **2** exhibited mutually coupled methylene protons at δ 4.02 and 4.48 ($J = 9.0$ Hz), which were absent in **1**. The ^1H and ^{13}C NMR spectra were highly similar to that of **1**, except that **2** lacked the hydroxyl group at C-9 which was present in **1**. Of the two methyl signals observed in the ^1H NMR spectrum, the singlet at 0.65 was assigned to CH_3-15 and the doublet at δ 1.11 was assigned to CH_3-14. The protons of the latter methyl doublet gave HMBC correlation to the quaternary carbon C-4 and was therefore located at C-3. Three one proton multiplets at δ 2.05, 2.18 and 2.99 were assigned to the H-3, H-5 and H-9, respectively. The chemical shifts for the remaining methylene protons were assigned by a detailed analysis of HMQC and HMBC spectra. In the ^{13}C NMR spectrum, double bond carbon resonances at 127.0 (C-8) and 135.5 (C-7), and a lactone carbonyl carbon at 170.2 (C-12) suggested the presence of α,β-unsaturated lactone group (Table 2). The relative stereochemistry of the four asymmetric centres in the coloratadiene skeleton was determined by COSY and NOESY experiments (Fig. 3). Both the H-6α and H-6β resonances were seen to be coupled in the COSY spectrum to H-5 and H-7. The NOESY spectrum of **2** showed correlations between the protons of H-9 and H-5 and between the protons of H-9 and H-11α. These

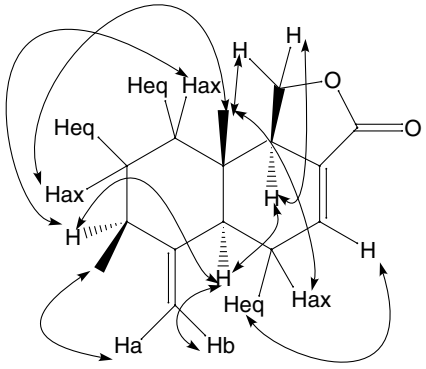

Fig. 3. Significant NOE correlations in compound **2**.

Table 2
^{13}C NMR and HMBC spectral data for compounds **1**–**4** (125.8 Hz, $CDCl_3$)

Carbon	**1**		**2**		**3**		**4**	
	δ	HMBC	δ	HMBC	δ	HMBC	δ	HMBC
1	31.7	H-15	39.7	H-15	33.7	H-15	36.9	H-2β, H-5, H-15
2	31.8	H-1α, H-1β	32.3	H-1α, H-1β, H-14	31.8	H-14	23.5	
3	38.8	H-13a, H-14	38.8	H-13a, H-14	38.5	H-13a, H-14	80.2	H-1α, H-2α, H-5 H-14H-13a, H-14
4	149.1	H-5, H-6, H-14	151.6	H-5, H-14	152.0	H-3α, H-5, H-14	37.7	H-14
5	50.4	H-7, H-13b, H-15	45.9	H-13b, H-15	44.2	H-6α, H-13b, H-15	49.4	H-1β, H-13b, H-14
6	66.1	H-5	26.9		31.5		24.7	H-5
7	153.7	H-6	135.5		62.3	H-6α	135.7	
8	139.3	H-12	127.0	H-11α	56.9	H-6α, H-12α, H-12β, 7-OH	127.2	
9	77.6	H-7, H-11. H-12, H-15	49.3	H-11α, H-11β, H-15	137.4	H-12α, H-12β, H-15	50.6	H-1α, H-5, H-11α, H-15
10	44.1	H-5, H-15	36.7	H-5, H-11β, H-15	36.8	H-6α, H-15	34.1	H-1α, H-2α, H-5, H-15
11	200.5		67.9		172.1		66.9	
12	192.6	H-7	170.2	H-11α	69.9		169.7	
13	106.7	H-5	105.5		104.7		27.6	H-5, H-14
14	18.2		18.6		18.1		15.9	H-3α, H-5 H-13
15	15.8	H-5	12.5	H-1α, H-5	16.4	H-1α	13.5	H-1α, H-5
CH_3–CO–							21.2	
CH_3–*C*O–							170.7	H-3, CH_3-CO

correlations require that H-9, H-5, and H-11α all be *cis* to each other, and therefore that the H-9 proton has an α-orientation. The ^{1}H–^{1}H coupling observed between H-9 and H_2-11 in the COSY spectrum confirmed the position of the methylene group to be at 11. The H-11β and H-11α protons were unambiguously distinguished by the NOESY spectrum, which displayed cross NOE peaks between the H-11β proton (δ 4.02) and the methyl H-15 protons (δ 0.65), as well as between the H-11α proton (δ 4.48) and the H-9α (δ 2.99) protons. The β-orientation of CH_3-14 was supported by a NOE cross-peak between CH_3-14 and one of the exocyclic methylene protons, Ha-13, at δ 4.90. This stereochemical assignment would be in agreement with that of other coloratadiene sesquiterpenes previously isolated from *Warburgia* species (Kioy et al., 1990; Rajab and Ndegwa, 2000). Therefore, the new compound **2** was established structurally as 4(13),7-coloratadien-12,11-olide.

Compound **3** was isolated as colorless prisms from CH_2Cl_2. A molecular formula $C_{15}H_{20}O_3$ was obtained from HRMS (*m*/*z*; measured 271.1302 $[M + Na]^+$; calc. 271.1310). Similarly, a prominent peak *m*/*z* 246 $[M - 2H]^+$ appeared in the EI-MS spectrum, corresponding to the molecular formula $C_{15}H_{20}O_3$, which was further supported by two methyl, five methylene, three methine, and five quaternary carbons signals observed in the ^{13}C and DEPT spectra. In addition, a fragment ion peak at *m*/*z* 215 indicated the facile loss of a hydroxyl group. The IR spectrum showed a hydroxyl stretch band at 3416 cm^{-1}, unsaturated lactone absorptions at 1735 and 1670 cm^{-1} and olefinic absorption at 1646 cm^{-1}. An absorption maximum at 230 nm in the UV spectrum was characteristic of a molecule possessing an unsaturated lactone structure. The ^{1}H NMR spectrum of **3** was very similar to that of **2** and exhibited two singlets at δ 4.62 and 4.86 that are typical of exocyclic methylene protons, and a three protons doublet at δ 1.10, which further characterized the compound as a rearranged drimane sesquiterpene in which one methyl group has migrated from C-4 to C-3. Furthermore, two one proton doublets at δ 2.38 and 4.54 were assigned to the H-5 and H-7, respectively. The doublets at δ 4.68 and 4.96 were unambiguously assigned to H-12α and H-12β, respectively, based on NOE correlations observed between H-12α and H-7 in the NOESY spectrum (Fig. 4). Similarly, the signals at δ 1.92 and 1.99 in the 1H NMR spectrum were ascribed to H-6α and H-6β, respectively, based on cross NOE peaks observed between H-6β and CH_3-15, and H-6β and H-13b. A broad singlet at 1.94 was attributed to a hydroxyl group at position 7. The position of the hydroxyl group at C-7 was determined by analysis of its HMBC spectrum, which showed a correlation between H-7 and C-8. This was also supported by the methine carbon resonance at δ 62.3 which suggested a hydroxyl substituted carbon, which was correlated in the HMBC to H-6 and thus assigned as C-7. The chemical shift values of the methyl protons at C-1 and C-2 were assigned based on HMQC, HMBC and NOESY experiments. When compared with the spectral data of **1**, the lack of two aldehyde groups was apparent from the ^{1}H NMR spectrum of **3**. The relative stereochemistry of the hydroxyl group was established by analysis of the NOESY spectrum. The H-7 resonance showed a NOESY correlation to H-5, which suggested an axial (α)-orientation for this H-7 proton, leaving the hydroxyl group with an equatorial (β)-orientation. These assignments were consistent with the coupling constant data ($J = 4.0$ Hz) for H-7. On the basis of these observations, the structure of **3** was assigned as 7β-hydroxy-4(13),8-coloratadien-11,12-olide.

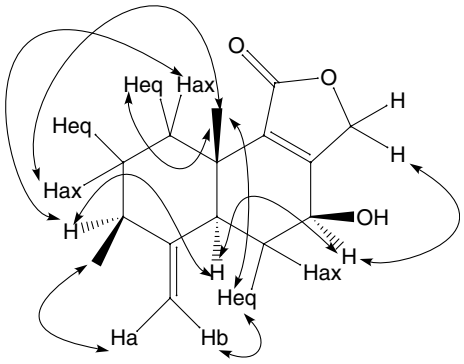

Fig. 4. Significant NOE correlations in compound **3**.

Compound **4** was obtained as colorless prisms from *n*-hexane–CH_2Cl_2 mixture. The EI-MS spectrum of **4** showed a molecular ion peak at *m*/*z* 291 $[M - H]^+$ and fragment ions at *m*/*z* 249 $[M - 43]^+$ and 232 $[M - 60]^+$ corresponding to the loss of an acetate and acetic acid groups, respectively. The IR spectrum displayed, in addition to α,β-unsaturated lactone at 1760 and 1683 cm^{-1}, the presence of an acetate group at 1731 cm^{-1}. The ^{1}H NMR data reported previously (Kioy et al., 1990; Ying et al., 1995) for this compound were highly similar to compound **4**, but the chemical shift values of each methylene proton were not assigned explicitly. Four three protons signals were observed in the ^{1}H NMR spectrum of **4**, of which the acetate methyl resonance appeared at δ 2.07. Of the remaining three methyl groups, the singlet at δ 0.84, which showed a HMBC correlation with the C-9 at δ 50.6, was located at C-10, while the remaining two methyl groups could be placed at C-4 on the basis of HMBC correlations. Although, the carbon resonances for C-1, C-6, C-13, C-15, C-12 and acetate carbonyl carbon reassigned by Ying et al. (1995) were very similar with the ^{13}C NMR spectrum of **4**, the carbon resonances for C-4 and C-10 still needed to be reversed as shown in the ^{13}C NMR and HMBC spectra (Table 2). The ^{13}C spectrum of **4** revealed resonances for C-7, C-8, C-9 and C-11 in close agreement with resonances for the corresponding carbons in **2**. The carbonyl carbon resonance of the ace-

tate group appeared at δ 170.7 and showed HMBC correlations with H-3 at δ 4.55, which indicated that the acetate group was attached at C-3. This was supported by the observation of NOE correlations between the CH_3–CO and CH_3-15. The HMBC spectrum showed connectivities between the H-11α proton (δ 4.41 *t*) and the carbonyl carbon of the lactone (δ 169.7), and between the H-3α proton (δ 4.55) and the carbonyl carbon of the acetate (δ 170.7), whereas the NOESY spectrum displayed NOE cross-peaks between the H-3α and H-5α protons, thus establishing the relative stereochemistry of the acetate group as β-oriented. Moreover, the acetate methyl proton (δ 2.07) showed an NOE correlation with the axial C-15 methyl protons (δ 0.84) but neither of the C-4 methyl protons (δ 0.94 and 1.00) showed an NOE with the H-3α proton. Although the optical rotation value determined for **4** was slightly different from previous report (Kioy et al., 1990), the above spectroscopic evidences strongly support the structural assignment of compound 4 as cinnamolide-3β-acetate. The full spectral data and unambiguous assignment of resonances are reported here for the first time.

Compounds **5**–**13** were identified as muzigadial, muzigadiolide, 11α-hydroxymuzigadiolide, cinnamolide, 7α-hydroxy-8-drimen-11,12-olide, ugandensolide, mukaadial, ugandensidial, and linoleic acid (*Z*, *Z*), respectively by spectroscopic analysis and comparison of their ^{1}H NMR, ^{13}C NMR, and mass spectral data with those in the literature (Kubo et al., 1976, 1983; Rajab and Ndegwa, 2000; Nakanishi and Kubo, 1977; Maurs et al., 1999). Although **9** has been reported from *Capsicodendron dinisii* (Mahmoud et al., 1980), this is the first time it has been found in the genus *Warburgia*. In addition, compound **13**, which is the constituent of most vegetable oils and animal fats, has been reported from the genus for the first time.

The antimycobacterial activity of the isolated compounds **1**–**13** was determined by the broth microtiter dilution method against fast growing strains of mycobacteria and their MICs are presented in Table 3.

Table 3
Antimycobacterial activity of compounds **1**–**13** and the antibiotic drugs[a]

Compound	MIC (μg/ml)			
	M. aurum	*M. fortuitum*	*M. phlei*	*M. smegmatis*
5	32	16	64	64
2	128	128	NA	128
6	128	128	64	128
3	NA	128	NA	NA
9	128	128	NA	NA
12	NA	128	NA	NA
13	4	8	4	16
Ethambutol	0.5	8	2	1
Isoniazid	4	1	4	4

[a] NA, not active; compounds **1**, **7**, **4**, **8**, **10**, and **11** showed no activity up to 128 μg/ml.

Among the compounds tested, compound **13** displayed the most potent inhibitory activity with MIC values of 4, 8, 4, 16 μg/ml against *M. aurum*, *M. fortuitum*, *M. phlei* and *M. smegmatis*, respectively. This is in agreement with previous reports (Stavri et al., 2004; Seidel and Taylor, 2004) about the antimycobacterial activity of linoleic acid. Interestingly, muzigadial (**5**) having the coloratadiene-dialdehyde structural feature displayed pronounced antimycobacterial activities with MICs ranging from 16 to 64 μg/ml. Previous studies done by Taniguchi et al. (1984) revealed that the dialdehyde moiety was responsible for the broad range of antimicrobial activity of muzigadial, which might have also contributed to enhancement of the antimycobacterial activity. In contrast, compound **1** having one more hydroxyl group compared to compound **5** showed no activity against the strains of mycobacteria in our assay. This suggests that compounds polarity seems to influence the in vitro antimycobacterial activity of drimane sesquiterpenes. Haemers et al. (1990) also reported that higher lipophilicity may play an important role in the antimycobacterial activity. This is due to the fact that the mycobacterial cell wall contains lipophilic substance such as mycolic acid, lipophilic compounds would therefore have the advantage of better penetration through the cell wall and inhibit the growth of mycobacteria. Among the sesquiterpene lactones compound **6** showed moderate activity, whereas the remaining sesquiterpenes failed to inhibit the growth of the four strains of mycobacteria up to 128 μg/ml. The use of *W. ugandensis* stem bark in Ethiopian folk medicine to treat tuberculosis can therefore be attributed to the presence of linoleic acid and the drimane sesquiterpenes.

3. Experimental

3.1. General experimental procedures

Melting points were determined with KOFLER microscope and are uncorrected. Optical rotations were measured on a Perkin–Elmer 241 MC polarimeter. Perkin–Elmer 881 infrared spectrophotometer was used in recording the IR. UV spectra were recorded on Shimadzu UV-160A spectrophotometer. Analytical TLC was performed on Merck silica gel 60 and plates were sprayed with 0.5% anisaldehyde sulphuric acid reagent. Semipreparative HPLC was performed using LiChrospher® RP-18 (10 μm, 250 × 10 mm i.d.) column and Hypercarb-S (100 × 4 mm i.d.), monitoring wavelength 214 nm. NMR spectra were recorded at 500 MHz for ^{1}H and 125 MHz for ^{13}C on a Bruker AVANCE 500 spectrometer. All spectra were measured in $CDCl_3$, except for compound **11** which was in pyridine-d_5. HRMS was determined with Micromass QTOF Ultima using the internal standard [Glu]-fibrinopeptide B which had

$[M + 2H]^{2+} = 785.8426$. Mass spectra were further obtained by EI-MS analysis on a 5890 Series II plus gas chromatograph interfaced to a 5989 B mass spectrometer (Hewlett-Packard). The analysis was performed on an HP5-MS (29 m × 0.25 mm i.d., 0.50 μm film thickness) fused silica capillary, carrier gas helium with a flow rate 1.0 ml/min; injection, interface and ion source temperatures: 250 °C and ionization at 70 eV. Strains of *M. aurum* (PI 104482), *M. fortuitum* (ATCC 6841), *M. phlei* (ATCC 19249) and *M. smegmatis* (ATCC 19420) were obtained from the American Type culture collection or the Pasteur Institute.

3.2. Plant material

Stem barks of *W. ugandensis* Sprague (Canellaceae) were collected from a single tree growing in Harena Forest, about 13 km from Dello Menna on the way to Goba, Ethiopia in April 2001 and identified by Mr. Melaku Wendafrash, the National Herbarium, Biology Department, Science Faculty, Addis Ababa University. A voucher specimen (collection No. 977) was deposited at the National Herbarium for further reference.

3.3. Extraction and isolation

The powdered stem barks of *W. ugandensis* (800 g) were extracted successively with *n*-hexane, dichloromethane and methanol in a Soxhlet apparatus. The dichloromethane extract was concentrated under vacuum to yield 35 g of yellowish residue, of which 25 g were subjected to vacuum column chromatography on silica gel eluting with *n*-hexane/EtOAc mixtures of increasing polarity and 18 fractions of 400 ml each were collected. Fractions 3 and 4 eluted with *n*-hexane/EtOAc (9:1) were combined and rechromatographed on silica gel eluting with *n*-hexane/EtOAc (95:5) and further purified on semi-prep. HPLC with Hypercarb S column using MeOH as eluent to yield **2** (5 mg). Fractions 5 and 6 eluted with *n*-hexane/EtOAc (8:2) were purified by semi-prep. HPLC using MeCN/H_2O (6:4 → 8:2) gradient elution for 50 min to afford **8** (9 mg). Fractions 7–13 were combined and applied to a Sephadex LH-20 column connected to a fraction collector using CH_2Cl_2 as eluent to give 120 subfractions of 15 ml each. Subfractions 111 and 112 afforded pure compound **7** (32 mg). Subfraction 14 gave **4** (60 mg) after multiple development on prep. TLC using *n*-hexane/EtOAc (7:3) as eluent. Subfractions 16–18 were further subjected to semi-prep. HPLC using MeCN/H_2O (44:56) isocratic elution for 70 min to give **5** (6 mg) and **12** (23 mg) at 56 and 66 min, respectively. Subfractions 49–54 were chromatographed on semi-prep. HPLC using MeCN/H_2O (45:55) isocratic elution to afford **1** (25 mg), **9** (4 mg) and **6** (10 mg) at 15, 35 and 41 min, respectively. Similarly, purification of subfractions 63 and 64 by semi-prep. HPLC eluting with MeCN/H_2O (45:55) isocratic system yielded **11** (24 mg), **3** (15 mg) and **10** (11 mg). Finally, compound **13** (35 mg) was purified from subfractions 33–37 by semi-prep. HPLC using MeCN/H_2O (25:75 → 9:1 for 30 min and MeCN/H_2O (9:1) isocratic system from 31 to 55 min.

3.4. Antimycobacterial assay

The antimycobacterial activities of the compounds were evaluated by the minimum inhibitory concentration assay method reported by Schinkovitz et al. (2003) in 96-well microtiter plates. The test compounds initially dissolved in DMSO were diluted in cation adjusted Mueller–Hinton broth (MHB) to give a concentration of 256 μg/ml. One hundred and twenty five microlitres of each test solution were added to each well containing 125 μl of MHB to achieve a starting concentration of 128 μg/ml. A twofold serial dilution of test solutions were prepared to get final concentrations ranging from 128 to 0.25 μg/ml. Mycobacterial strains were cultivated on Columbia blood agar supplemented with 7% defibrinated horse blood agar. Bacterial suspension with a turbidity of 0.5 on the MacFarland scale was made in 0.9% NaCl solution and diluted to give a final inoculum's density of 5×10^5 cfu/ml. After addition of 125 μl of bacterial inocula into all wells, except the blank the plates were incubated at 37 °C for 72 h. The lowest assay concentration of the test compounds that produce complete inhibition of the macroscopic growth (MIC) were detected by addition of 20 μl methanol solution of tetrazolium redox dye (MTT, 3.5 mg/ml) followed by incubation at 37 °C for 20 min. Tests were conducted three times in duplicate and ethambutol and isoniazid were used as positive controls.

3.5. 6α,9α-Dihydroxy-4(13),7-coloratadien-11,12-dial (*1*)

Colorless needles from *n*-hexane–CH_2Cl_2 mixture, m.p. 137–139 °C, $[\alpha]_D^{24}$ −40 (CH_2Cl_2; c1.6), UV$\lambda_{max}^{CH_2Cl_2}$ nm(log ε): 234(3.6), IR$\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 3406(−OH), 1725 and 1683 (CH=CHO)), 1640 and 796 (exocyclic methylene), ^1H NMR (500 MHz, $CDCl_3$) see Table 1, ^{13}C NMR (125.8 MHz, $CDCl_3$) see Table 2, HRMS Found 287.1264 $[M + Na]^+$; $C_{15}H_{20}O_4Na$, calc. 287.1259, EI-MS (70 eV) *m/z* (rel. int.): 246 $[M - H_2O]^+$(5), 235 $[M - CHO]^+$(100), 217 $[M - CHO - H_2O]^+$(26), 189 $[M - CHO - H_2O - CO]^+$(40), 175 $[M - CHO - OH - Me - CO]^+$(35), 105(60), 91(73), 77 (66), 55(81), 41(93).

3.6. 4(13),7-Coloratadien-12,11-olide (*2*)

White prism from CH_2Cl_2, m.p. 85–87 °C, $[\alpha]_D^{24}$ +84.7 (CH_2Cl_2; c1.5), UV$\lambda_{max}^{CH_2Cl_2}$ nm(log ε): 231

(3.7), IR $\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 1757 and 1686(CH=CHO), 1639 and 758 (exocyclic methylene), ^1H NMR (500 MHz, $CDCl_3$) see Table 1, ^{13}C NMR (125.8 MHz, $CDCl_3$) see Table 2, HRMS Found 233.1542 $[M+H]^+$; $C_{15}H_{21}O_2$, calc. 233.1536, EI-MS (70 eV) *m/z* (rel. int.): 232 $[M]^+$(22), 217 $[M-CH_3]^+$(17), 190 $[M-C_3H_6]^+$(10), 122(100), 107(58), 91(22), 41(12).

3.7. 7β-Hydroxy-4(13),8-coloratadien-11,12-olide (3)

Colorless prism from CH_2Cl_2, m.p. 139–142 °C, $[\alpha]_D^{25}$ + 242.1 (CH_2Cl_2; c1.9), UV $\lambda_{max}^{CH_2Cl_2}$ nm(log ε): 230 (3.3), IR $\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 3414(−OH), 1735 and 1670 (CH=CHO)), 1646 and 786 (exocyclic methylene), ^1H NMR (500 MHz, $CDCl_3$) see Table 1, ^{13}C NMR (125.8 MHz, $CDCl_3$) see Table 2, HRMS Found 271.1302 $[M+Na]^+$; $C_{15}H_{20}O_3Na$, calc. 271.1310, EI-MS (70 eV) *m/z* (rel. int.): 230 $[M-H_2O]^+$(100), 215 $[M-H_2O-CH_3]^+$(55), 201 $[M-OH-2CH_3]^+$(25), 185(45), 171(83), 91(90), 77 (74), 41(77).

3.8. Cinnamolide-3β-acetate (4)

Colorless prism from *n*-hexane–CH_2Cl_2 mixture, m.p. 147–149 °C, $[\alpha]_D^{25}$ − 2.4(CH_2Cl_2; c1.3), UV $\lambda_{max}^{CH_2Cl_2}$ nm (log ε): 232.5(3.6), IR $\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 1706 and 1683 (CH=CHO)), 1637 and 779 (exocyclic methylene), ^1H NMR (500 MHz, $CDCl_3$): δ 0.84 (3H, *s*, H-15), 0.94 (3H, *s*, H-13), 1.00 (3H, *s*, H-14), 1.42 (1H, *ddd*, *J* = 13.5, 4.0, H-1α), 1.48 (1H, *dd*, *J* = 10.5, 4.5 Hz, H-5), 1.64 (1H, *dt*, *J* = 13.5, 3.5 Hz, H-1β), 1.68 (1H, *ddd*, *J* = 12.5, 1.5 Hz, H-2β), 1.74 (1H, *ddd*, *J* = 13, 4 Hz, H-2α), 2.07 (3H, *s*, O–$COCH_3$), 2.22 (1H, *ddq*, *J* = 12.0, 3.5, 1.5 Hz, H-6β), 2.44 (1H, *dq*, *J* = 20, 5, 4.0 Hz, H-6α), 2.82 (1H, *m*, H-9α), 4.05 (1H, *t*, *J* = 9.0 Hz, H-11β), 4.41 (1H, *t*, *J* = 9.0 Hz, H-11α), 4.55 (1H, *dd*, *J* = 11.5, 4.5 Hz, H-3α), 6.89 (1H, *q*, *J* = 3.5 Hz, H-7), ^{13}C NMR (125.8 MHz, $CDCl_3$) see Table 2, EI-MS (70 eV) *m/z* (rel. int.): 291 $[M-H]^+$(5), 249 $[M-H-CH_3-CO]^+$(7), 232 $[M-CH_3-COOH]^+$(10), 217 $[M-CH_3-COOH-Me]^+$(8), 122(100), 107(65), 43(52).

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