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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.

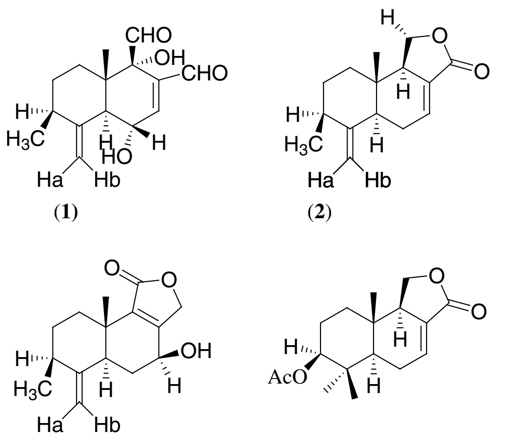

Fig. 1. The structure of compounds **1**–**4** isolated from *W. ugandensis* stem barks.