Secondary metabolites from *Tetracera potatoria* stem bark with antimycobacterial activity


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**ABSTRACT**

Ethnopharmacological relevance: *Tetracera potatoria* (Afzel. Ex. Don (Dilleniaceae) is a medicinal plant used traditionally in Africa for the treatment of tuberculosis related ailments and respiratory infections. The antibacterial activity of the medium polar extracts of *T. potatoria* leaves and stem bark was recently reported against *Mycobacterium smegmatis* (MIC 25 µg/mL) and *M. aurum* (65 µg/mL), two fast-growing *Mycobacterium* strains used as model micro-organisms for the more pathogenic strain *Mycobacterium tuberculosis* (Fomogne-Fodjo et al., 2014). The aim of this study was consequently to isolate the compounds possibly contributing to this activity, and which may therefore be promising precursors to be used for the development of novel anti-TB drugs.

**Materials and methods:** *T. potatoria* medium polar extract [MeOH/DCM (1:1, v/v)] was fractionated sequentially with petroleum ether to which EtOAc and MeOH were gradually added to increase the polarity. The examination of *T. potatoria* extract and its fractions was guided by bioassays for anti-mycobacterial activity against *M. smegmatis* (ATCC 23246) and *M. aurum* (NCTC 10437) using the minimum inhibitory concentration (MIC) method. All the isolated compounds were structurally elucidated using spectroscopic techniques and evaluated for their anti-mycobacterial activity.

**Results:** Two novel secondary metabolites (1, 2) named tetraceranoate and N-hydroxy imidate-tetracerane, together with five known compounds (β-stigmasterol (3), stigmast-5-en-3β-yl acetate (4), betulinic acid (5), betulin (6) and lupeol (7)) were isolated and identified. Tetraceranoate exhibited the best activity against *M. smegmatis* with a minimum inhibitory concentration (MIC) of 7.8 µg/mL, while β-stigmasterol, betulinic acid and betulin showed appreciable anti-mycobacterial activity against both strains (MIC 15 µg/mL).

**Conclusion:** Seven compounds were isolated from the medium polar extract [MeOH/DCM (1:1, v/v)] of *T. potatoria* stem bark. Only tetraceranoate one of the isolated compounds showed antibacterial activity against *M. smegmatis* having efficacy as high as rifampicin (one of a three drug regimen recommended in the initial phase short-course anti-tuberculosis therapy). Thus, tetraceranoate might be an interesting target for systematic testing of anti-TB treatment and management. This research supports the use of *T. potatoria* in African traditional medicine for the treatment of tuberculosis related symptoms.

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1. Introduction

Species from the *Tetracera* genus are commonly used for the treatment of various diseases and infections (Laval et al., 2011). *Tetracera potatoria*, also known as *liane à eau* in France and water tree in Sierra-leone (*Burkill, 1985*), is a scendent shrub or climber that may reach heights of up to 5 m. The leaves, stem bark, stem, and sap may be used in different ways to treat various ailments. For instance, the leaves or a portion of the stem are boiled in the sap of the plant and used as a reportedly powerful diuretic, vermifugal and purgative, as

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**Keywords:**

Dilleniaceae

Stem bark

Tuberculosis

Anti-microbial

Compounds

Tetraceranoate

N-hydroxy imidate-tetracerane

**Abbreviations:**

CFU/mL, Colony Forming units/millilitre; DMSO, Dimethyl Sulfoxide; MIC, Minimum Inhibitory Concentration; INT, 3-Iodonitrotetrazolium Chloride; ATTC, American Type Culture Collection; WHO, World Health Organization; MDR-TB, multi-drug resistant Tuberculosis; XDR-TB, Extensively Drug Resistant Tuberculosis; Cam, Cameroon; NCTC, National Collection of Types Cultures; DCM, Dichloromethane; EtOAc, Ethyl Acetate; s, singlet; m, multiplet; mp, multiplicity; br, broad; dd, doublet of doublet; t, triplet; δ, chemical shift

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well as for the treatment of gastrointestinal and other stomach complaints (Burkill, 1985). In Cameroon, a leaf decoction is taken orally for hemorrhoids while the sap is a well-known treatment for jaundice (Betti, 2004). Bakola pygmies who live in the littoral region of Cameroon cut the stem and drink the sap to quench their thirst (Tsabang, Personal Communication). The sap is also used for the treatment of toothache and cough (Oliver, 1960). In south-western Nigeria, the aqueous extract from the root is an active remedy for intestinal disorders (Adesanwo et al., 2003). Furthermore, T. potatoria stems may be macerated in palm wine and taken orally to ameliorate fever (Kerharo and Bouquet, 1950). Our attention was drawn to the fact that T. potatoria is used commonly for the traditional treatment of tuberculosis (TB) related ailments, respiratory infections and inflammation caused by such infections in central Africa (Cameroon) (Fomogne-Fodjo et al., 2014).

Health care systems around the world are faced with the challenge that bacteria are developing resistance to existing antimicrobial agents resulting in an increase in health care costs, morbidity and also mortality (Souza, 2009). The spread of tuberculosis (TB) and other infections caused by a variety of opportunistic micro-organisms is becoming rampant and in many instances is associated with the Human Immunodeficiency Virus and Acquired Immunoodeficiency Syndrome (HIV-Aids) (McGaw et al., 2008). Furthermore, several multi-drug resistant Tuberculosis (MDR-TB) and extensively drug-resistant Tuberculosis (XDR-TB) strains are emerging with no new drug against TB introduced over the past 40 years (Global Alliance for TB Drug Development, 2013). It is estimated that about six million new cases of TB were reported in 2014 across the globe (Global Tuberculosis Report, 2015). Tuberculosis is consequently a major public health concern in several countries, but especially in India, Indonesia, China, Nigeria, Pakistan and South Africa, where it is reported to have the highest number TB cases in the world (Global Tuberculosis Report, 2015). Consequently, the development of new anti-TB agents active through novel modes of action (Lawal et al., 2011) is crucial. A possible approach is the assessment of plants traditionally used for the treatment of TB related ailments. These plants may contain various compounds possibly active against the Mycobacterial species, or may contain active anti-bacterial compounds acting together synergistically in a multi-target fashion.

The anti-bacterial potency of medium polar extracts of T. potatoria leaves and stem bark was recently reported against M. smegmatis (MIC 25 µg/mL) and M. aurum (65 µg/mL), (Fomogne-Fodjo et al., 2014), two fast-growing Mycobacterium strains used as model micro-organism for the more pathogenic strain M. tuberculosis (McGaw et al., 2008; Chung et al., 1995). The focus of this study was consequently to isolate the compounds possibly contributing to this activity, and which may therefore be promising precursors to be used for the development of novel anti-TB drugs. We describe herein the isolation, structure elucidation and the anti-mycobacterial activity of two new compounds named tetraceranoate (1) and N-hydroxy imidate-tetracerane (2) along with that of five known terpenoids [β-stigmasterol (3), stigmast-5-en-3β-ol acetate (4), betulinic acid (5), betulin (6) and lupeol (7)] all isolated from the MeOH/DCM (1:1, v/v) extract of T. potatoria stem bark.

2. Materials and methods

2.1. Plant material

The stem bark of T. potatoria was collected from an uncultivated farmland on the Kala Mountain in the central province of Yaoundé in Cameroon in January 2010. Identification was made by Mr. Victor Nana, a botanist from the National Herbarium of Cameroon in Yaoundé, where a voucher specimen (N° 27918/SRF/Cam) has been deposited.

2.2. Extraction and isolation

The plant material (5 kg) was air-dried at 37 °C and ground to a powder using an industrial grinder. The finely ground powder obtained was extracted three times with MeOH: DCM (1:1, v/v) for 72 h at room temperature (5 kg plant material/7.5 L solvent). With this method of extraction, we anticipated to mimic the traditional use of palm wine for the preparation of decoctions and infusions of the different plant parts (Kerharo and Bouquet, 1950). It is also known that organic extracts of medium polarity are likely to contain phytochemicals such as terpenoids, polyphenols and alkaloids with antimicrobial activities (Cowan, 1999; Parekh et al., 2005; Kumar et al., 2009). The extract suspension was filtered through Whatman No 1 filter paper and the filtrate concentrated to dryness under reduced pressure. After the evaporation of the solvent, a crude extract (365 g) was obtained of which a part (355 g) was chromatographed on Si gel 60 (230–400 mesh) (1000 g) using petroleum ether (P.E.), P.E-ethyl acetate (EtOAc), EtOAc-methanol (MeOH) and MeOH in order of increasing polarity. A total of 113 fractions (200 mL each) were eluted. Fractions containing compounds with similar Rf values were combined into five main series [A–E]: A (8.94 g) (fractions 4–55) (containing most non-polar groups of compounds), B (8.81 g) (fractions 56–68), C (50 g) (fractions 69–84), D (50 g) (fractions 84–102) and E (21 g) (containing most polar groups of compounds).

Repeated column chromatography of series A (8.84 g) on Si gel 60 (70–230 mesh) using mixtures of hexane–DCM with increasing polarity yielded compounds 3 (30 mg), 4 (15.5 mg), 5 (30 mg), 6 (5 mg) and 7 (30 mg). Series E contained a high percentage of tannins which had to be removed prior to further isolation of the major compounds in the series. This was accomplished by reconstituting the series E fraction in MeOH and adding enough CHCl3 to obtain an 8% (v/v) MeOH/ CHCl3 solution. An immediate precipitation of tannins was observed. The resulting suspension was stored overnight at 4 °C to afford complete precipitation of tannins and the obtain mixture was filtered (Saucier et al., 2001). The filtrate was dried under reduced pressure to afford a fraction (21 g) which was subjected to column chromatography, first through Sephadex LH-20 CC eluted with H2O-MeOH (20:80), and then Si gel 60 C (70–230 mesh) using DCM/EtOAc/Acetic acid (60:50:1). This procedure afforded two purified compounds, i.e. compounds 1 (6 mg) and 2 (6 mg).

2.2.1. Tetraceranoate (1)

Yellow oil; Yield: 0.028%; [α]D 35 ~ -51.12° (CHCl3, c = 0.05); FT-IR νmax cm–1: 1729 (COOR), 3299 (OH), 1608 (C=O) (log e: 276.3 (0.5162); For 1H (600 MHz) and 13C (300 MHz) NMR spectroscopic data, see Table 1; ESIMS (Positive mode) m/z 405 [M + H]+; HR-ESIMS (Positive mode) m/z 405.2277 [M+H]+ (calc for C23H32O6 + H, 405.2286).

2.2.2. N-hydroxy imidate-tetracerane (2)

Yellow oil; Yield: 0.028%; [α]D 35 ~ -37.9° (MeOH, c = 0.09); FT-IR νmax cm–1: 3375 (OH), 1607 (C=O); UV (MeOH) λmax nm (log e) : 276.3 (2.0377); For 1H (600 MHz) and 13C (300 MHz) NMR spectroscopic data, see Table 2; ESIMS (Positive mode) m/z 420 [M + H]+; HR-ESIMS (Positive mode) m/z 422.2543 [M+H]+ (calc for C23H35NO6 + H, 445.2286).

2.2.3. β-Stigmasterol (3)

Colourless needles; Yield: 0.34%; mp 163–169 °C; FT-IR νmax cm–1: 3349 (OH), 2934, 1463, 1046; UV [MeOH] nm (log e): 257. 1H NMR (400 MHz, CDCl3, δ in ppm): δ 5.52 (1 H, br d, H-6), δ 5.13 (1 H, dd, J=14.4 Hz; 8.4 Hz, H-22), 4.98 (1 H, dd, J=14.4 Hz; 8.4 Hz, H-23), δ 3.49 (1 H, m, H-3), δ 0.98 (3 H, s, CH3-10), δ 0.89 (3 H, d, J=6.4 Hz, CH3-20), δ 0.82 (3 H, d, J=7.6 Hz, CH3-26), δ 0.79 (3 H, d, J=7.6 Hz, CH3-27),δ 0.77 (3 H, s, CH3-29), δ 0.65 (3 H, s, CH3-13).

The anti-bacterial activity of T. potatoria along with many other plants named tetraceranoate (1) and N-hydroxy imidate-tetracerane (2) along with that of five known terpenoids [β-stigmasterol (3), stigmast-5-en-3β-ol acetate (4), betulinic acid (5), betulin (6) and lupeol (7)] all isolated from the MeOH/DCM (1:1, v/v) extract of T. potatoria stem bark.
Table 1

<table>
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<th>Attribution</th>
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<th>DEPT</th>
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<tr>
<td>2</td>
<td>73.4</td>
<td>CH</td>
<td>3.45 (m)</td>
<td>C-1, OCH3, 2-14, 2-24</td>
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<tr>
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<td>70.2</td>
<td>CH</td>
<td>3.85 (br s)</td>
<td>C-4, C-2, C-1</td>
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<tr>
<td>4</td>
<td>63.3</td>
<td>CH2</td>
<td>3.48 (m); 3.51 (m)</td>
<td>C-3, C-6</td>
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<tr>
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<td>69.0</td>
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<td>C-1, C-2, C-4, C-12</td>
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<td>6</td>
<td>65.1</td>
<td>CH2</td>
<td>4.10 (dd, J= 4.5; 11.6 Hz); 4.12 (dd, J= 4.5; 11.6 Hz)</td>
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Table 2

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<td>3.56 (m); 3.57 (m)</td>
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<td>CH2</td>
<td>3.94 (dd, J= 9.7; 6 Hz)</td>
<td>4.01 (dd, J= 9.7; 4.5 Hz)</td>
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</table>

1H NMR, DEPT, 13C NMR, DEPT and HMBC data showing connectivity between compound 1 atoms (300 MHz 13C NMR, 600 MHz 1H NMR, CDCl3).

2.2.6. Betulin (6)

White powder; Yield: 0.056%; mp 256–257 °C; FT-IR v max (cm⁻¹): 3430 (OH), 1643 (C=O); UV [DMSO] nm (log ε): 316.

1H NMR (400 MHz, CDCl3, δ in ppm): δ 4.65 (1 H, br s, H-29a), δ 4.55 (1 H, br s, H-29b), δ 3.77 (1 H, d, J= 8 Hz, H-28b), δ 3.16 (1 H, dd, J= 12 Hz, 4 Hz), δ 2.35 (1 H, m), δ 1.79 (5 H, s, H-30, H-26), δ 0.99 (3 H, s, H-26), δ 0.95 (3 H, s, H-23), δ 0.94 (3 H, s, H-27), δ 0.80 (3 H, s, H-25), δ 0.73 (3 H, s, H-24).

2.2.7. Lupeol (7)

White powder; Yield: 0.34%; mp 210 °C; FT-IR v max (cm⁻¹): 3311, 1518, 1437.
4.67 (1 H, br s, H-29b), δ 3.17 (1 H, m, H-3), δ 1.23 (3 H, s, CH₃-30), δ 1.00 (3 H, s, CH₃-27), δ 0.94 (3 H, s, CH₃-26), δ 0.92 (3 H, s, CH₃-25), δ 0.82 (3 H, s, CH₃-24), δ 0.80 (3 H, s, CH₃-23), δ 0.73 (3 H, s, CH₃-28).

**13C NMR** (300 MHz, CDCl₃, δ in ppm): δ 183.78 (C-1), δ 26.00 (C-2), δ 78.91 (C-3), δ 31.84 (C-4), δ 55.35 (C-5), δ 18.02 (C-6), δ 34.33 (C-7), δ 40.87 (C-8), δ 50.48 (C-9), δ 38.10 (C-10), δ 20.87 (C-11), δ 25.17 (C-12), δ 38.64 (C-13), δ 42.39 (C-14), δ 26.40 (C-15), δ 29.68 (C-16), δ 47.88 (C-17), δ 48.35 (C-18), δ 64.68 (C-19), δ 150.42 (C-20), δ 29.68 (C-21), δ 33.78 (C-22), δ 27.33 (C-23), δ 15.35 (C-24), δ 16.07 (C-25), δ 15.67 (C-26), δ 14.66 (C-27), δ 55.28 (C-28), δ 109.6 (C-29), δ 19.13 (C-30).

By comparing the above spectral data with those reported in literature, Compounds 1, 3, 4, 5, 6 and 7 were identified as β-stigmasterol (Jain and Bar, 2009), stigmaster-5-en-3β-ol acetate (Muhit et al., 2010), 3β-hydroxy-lup-20(29)-en-28-oic acid or betulinic acid (Mahato and Kundu, 1994), 20(29)-lupene-3β,28-diol or betulin (Mahato and Kundu, 1994) and 20(29)-lupen-3β-ol or lupeol (Iman et al., 2007) respectively.

### 2.3. Analytical procedures

Melting Points (MPs) were determined using a Reichert Austria apparatus. Infra-Red (IR) spectroscopy was performed on a Perkin-Elmer FT-IR 100 spectrometer. The apparatus. Infra-Red (IR) spectroscopy was performed on a Perkin-

2.4. Determination of anti-mycobacterial activity

Each fraction was tested using the minimum inhibitory concentration (MIC) method. This was achieved by using a micro-titre plate method adapted from Eloff (1998). Under aseptic conditions, 100 µL of sterile water was added to each well of a 96 well micro-titre plate. Stock solutions of all the compounds (1000 µg/mL) were prepared in acetone or 25% DMSO/water (where selected compounds were insoluble in acetone). These were serially diluted with sterile distilled water. The final concentration of DMSO (2% or less) in the wells had no effect on the bacterial growth (Molina-Salinas et al., 2006). For anti-bacterial susceptibility testing, ethambutol (1 000 µg/mL), rifampicin (1 000 µg/mL), pyrazinamide (1 000 µg/mL) and isoniazid (1 000 µg/mL) were included as positive controls, while broth containing bacterial suspension was used to monitor the viability of the test organism. Thereafter, micro-titre plates were removed from the laminar flow unit and 100 µL of prepared culture was placed into each of the 96 wells.

The micro-titre plates were then sealed with sterile adhesive sealing film (AEC Amersham) and incubated overnight at 37 °C to stimulate bacterial growth. The remaining prepared culture was incubated along with another negative control, which was a streak plate of the culture used in the assay. This was carried out in order to determine the purity of the culture by the identification of single identical colonies. After incubation, 40 µL of a 200 µg/mL p-iodonitrotetrazolium violet (INT, Sigma-Aldrich) solution was added to all wells of the micro-titre plates and left to stand for 24 h. Bacterial growth is indicated by the appearance of a red/pink colour in the wells. The MIC was recorded as the lowest concentration at which there is no bacterial growth. Tests were performed in duplicate (or in triplicate, where resulting MIC values did not show congruency after the second replicate).

### 3. Results and discussion

#### 3.1. Isolation and characterization

The MeOH/DCM (1:1, v/v) extract of *T. potatoria* stem bark was subjected repeatedly to column chromatography on silica gel to afford seven compounds (1–7 Fig. 1). The known compounds (3–7) were identified by comparison of their spectroscopic data with those reported in literature.

Both compounds 1 and 2 are phenolic yellow oils with molecular formulae of C₂₃H₄₂O₆ and C₂₃H₄₃NO₆ respectively, as determined through the use of HR-TOF-ESI-MS and elemental analyses. Compound 1 exhibited a pseudomolecular ion [M+Na]+ at m/z 429.2159 corresponding to 7 unsaturation equivalents. The decoupled ¹³C NMR spectrum of 1 exhibited 23 carbon signals which could be classified as methyl (3), methylene (8), methine (8) and quaternary (4) carbons through distortionless enhancement by polarization transfer (DEPT) (Table 1). The presence of an ester carbonyl, four oxymethine, two oxymethylene and a methoxy moieties were confirmed by correlating the respective observed ¹³C chemical shifts (δ 174.4 (C-9), δ 73.4 (C-2), δ 70.2 (C-3), δ 69.0 (C-6), δ 68.8 (C-1), δ 65.1 (C-7), δ 63.3 (C-4) and δ 59.3 (OCH₃)) with the corresponding ¹H shifts. A broad singlet observed at δ 3.83 ppm was associated with a OH-moiety and were confirmed through IR, where the spectrum of 1 showed strong absorption bands associated with a hydroxyl (3299 cm⁻¹), together with those of the ester carbonyl (1729 cm⁻¹) and aromatic double bond (1608 cm⁻¹, 1511 cm⁻¹) functionalities. The presence of a benzene ring was confirmed by the UV spectrum with an absorbance at λmax 279 nm, and through 1D and 2D NMR indicating a typical AA’BB’ system [¹H NMR: δ 6.71 (2 H, d, J=8.6 Hz, H-2’; H-6’)] and δ 7.02 (2 H, d, J=8.6 Hz, H-3’, H-5’) suggesting a para-substituted benzene ring. The ¹³C NMR spectroscopic data indicated in the aromatic region a signal at
δ 156.3 (C-1’) appeared downfield from the other aromatic carbon. This deshielding effect was suggested to be due to the presence of an electronegative atom linking to C-1’. This was confirmed with the aid of HMBC spectrum (Fig. 2) showing the correlation between H-1 (δ 3.89) and C-1’ (δ 68.8). The correlations observed on the HMBC spectrum between H-6 (δ 4.05) and C-1 (δ 68.8), between C-4 (δ 63.3) and C-2 (δ 73.4), and between C-3 (δ 70.2) and C-1 (δ 68.8) confirmed the presence of the saturated six membered ring. The position of the OCH3-group on C-2 was also evident through correlation between H-2 and the -OCH3 carbon displayed on the HMBC spectrum. The NOESY cross peaks and C-1’. The correlations observed on the HMBC spectrum between H-6 (δ 4.05) and C-1 (δ 68.8), between C-4 (δ 63.3) and C-2 (δ 73.4), and between C-3 (δ 70.2) and C-1 (δ 68.8) confirmed the presence of the saturated six membered ring. The position of the OCH3-group on C-2 was also evident through correlation between H-2 and the -OCH3 carbon displayed on the HMBC spectrum. The NOESY cross peaks
between both protons H-1 and H-6, together with the coupling constant of H-1 (J=5.5 Hz) confirmed the axial orientation of H-1 (Ho-Chen et al., 2012). The connectivity between C-6 and C-7 was confirmed through the NOESY correlation between the axial H-4 and H-7 (Fig. 3). Correlations between H-16 (δ 1.52) and C-4’ (δ 143.5); H-3’/H-5’ (δ 7.02) and C-14 (δ 41.6); established that the C-14 moiety was connected to the benzene ring. Furthermore, long-range HMBC correlations (Table 1) between the H-7α/H-7β gem-protons (δ 4.12/4.10), H-10 (δ 2.24) and the carbonyl (δ 174.4) suggesting an ester group vicinal to the aliphatic H-10. The stereochemistry of the asymmetric carbon C-14 was not determined. In accordance to the above spectral data, compound 1 was established as 14, 15-p-benzeno-3-hydroxy-2-methoxy-14-methyl-9-oxo-14-propyl-5, 8, 16-trioctacyclo [9,4,01,6] henicosane, a new compound named tetraceranoate.

According to the elemental analysis of compound 2, nitrogen was present, i.e. the molecular formula was determined as C23H35NO6, and was confirmed through HR-TOF-ESI-MS in positive mode (m/z 422.2543 [M+H]+), also corresponding to 7 unsaturation equivalents. The presence of nitrogen in compound 2 could not be confirmed by 15N NMR analysis, which is not unusual. The number of published 15N NMR papers is disproportionally small relative to the importance of nitrogen; studies utilizing the 99.6% natural abundance 15N iso are even scarcer. The difficulty of 15N NMR stems from two main factors. First, the nucleus has a very low gyromagnetic ratio. In other words it is inherently less sensitive than other nuclei, and also suffers from equipment related issues such as acoustic ringing and limited radio frequency (rf) pulse powers. Secondly, and more importantly, it is a spin-1 nucleus, and while its electric quadrupole moment is not even scarcer. The difficulty of 15N NMR stems from two main factors. First, the nucleus has a very low gyromagnetic ratio. In other words it is inherently less sensitive than other nuclei, and also suffers from equipment related issues such as acoustic ringing and limited radio frequency (rf) pulse powers. Secondly, and more importantly, it is a spin-1 nucleus, and while its electric quadrupole moment is not particularly large, both Zeeman transitions experience significant perturbations due to the quadrupolar interaction (O’Dell, 2011).

Nevertheless, in most instances the 13C NMR spectrum of 2 was similar to that of compound 1 except that the carboxyl signal observed at δ 174.4 ppm on compound 1 spectrum appeared up field (δ 161.75 ppm) on the 13C NMR spectrum of 2. This shielding effect was suggested to be due to the substitution of an imidate group in 2 in place of the ester group in 1. Moreover, the presence of the imidate group in the molecule was confirmed by the long range HMBC correlation observed between H-11 (δ 1.30) and the one oxide (δ 161.7). Again, the presence of an AA’BB’ system was confirmed by means of NMR. As with compound 1, the presence of the hydroxyl and benzene moieties were confirmed through IR and UV respectively. The 1H–1H COSY and HSQC data presented one contiguous structural sequence with correlations from: H-13 to H-12, H-13 to H-11, H-12 to H-11 and H-11 to H-10, in accord with the presence of a spin system corresponding to a CH3-CCH2-CCH3-CCH2-CCH2-CCH-10 moiety. Similarly to compound 1, HMBC (Table 2, Fig. 4) data analysis allowed us to establish the position of the C-14 moiety (connected to the benzene ring), and that the arrangement of atoms in the six membered ring of O(5)– C(6)– C(1)–C(2)–C (3)–C(4) with its methoxy group at C-2. Protons H-1, H-2, H-3 and H-4 occupied an axial-orientation as determined by the NOESY Fig. 5 correlations and coupling constants values (J=10 Hz). The stereochemistry of the asymmetric carbon C-14 was not determined.

Based on this evidence, the structure of compound 2 was named N-hydroxy imidate-tetracran e and was determined to be 14,15-p-benzeno-3-hydroxy-2-methoxy-14-methyl-14-propyl-5,8,15-troixacyclo [9,4,01,6] henicosana-9-one oxime, a novel compound.

Through comparison between the spectroscopic data obtained for compounds 3–7 with published spectroscopic data, these known compounds were identified as two sterols [β-stigmasterol (3) (Jain and Bari, 2009) and stigmast-5-en-3β-yl acetate (4) (Mahat et al., 2010)], and three pentacyclic triterpenoids [betulinic acid (5) (Mahato and Kundu, 1994), betulin (6) (Mahato and Kundu, 1994) and lupeol (7) (Iman et al., 2007)]. Compounds 3, 4, 6 and 7 were isolated from T. potatoria for the first time.

3.2. Anti-mycobacterial activity

Traditionally in Cameroon, T. potatoria is used to treat TB related symptoms, respiratory infections and inflammations caused by such infections (Pomogne-Fodjo et al., 2014). All the seven compounds isolated from T. potatoria stem bark were evaluated for their anti-mycobacterial activity against M. smegmatis and M. aurum, which may be used to predict the efficacy of these compounds against a more pathogenic strain such as M. tuberculosis. As the latter is a slow-growing pathogenic strain, faster growing saprophytic species such as M. smegmatis (McGaw et al., 2008) and M. aurum (Chung et al., 1995) are used successfully as comparable screening pathogens. Our study was made more plausible by including clinically used anti-mycobacterial agents, i.e. ethambutol, rifampicin, pyrazinamide and isoniazid as positive controls. It should also be noted that anti-mycobacterial efficacies are considered noteworthy when isolated compounds achieve MIC values less or equal to 100 µg/mL (Gibbons, 2004; Rios and Recio, 2005). From the results in Table 3, it is shown that M. smegmatis may have been the more susceptible strain of the two, and that 57% of the MIC values displayed noteworthy activity against both test organisms (MIC values range between 7.8–63 µg/mL). These efficacies were better than or equal to the standard treatments for TB (i.e. the positive controls ethambutol, rifampicin, pyrazinamide and isoniazid). In this instance, the best activity (MIC 7.8 µg/mL) was exhibited by the newly isolated compound, 1, against M. smegmatis, and it is as active as rifampicin, one of the first line drugs used for TB treatment (Table 3). This activity may be explained by the fact that 1 is cyclic and has lipophilic domains. Lipophilicity of drug molecules may ameliorate their penetration through various biomembrane, consequently improving their permeation properties towards microbial cells (Mashoq, 2014). In addition, it is known that anti-mycobacterial activity is frequently enhanced by increased lipophilicity which helps the penetration through the highly lipophilic mycobacterial outer envelope and cell wall (Zitko et al., 2013). Another raised hypothesis explores the possibility that compound 1 and rifampicin might have the same mode of action by strongly inhibiting bacterial DNA-dependent RNA poly-
merase (Heinz and Tin-Wein, 2005). The slightly poorer activity exhibited by compound 2 against M. smegmatis could be explained by the difference in their structure suggesting that the presence of an ester function in the structure leads to an improved anti-mycobacterial effect. Sterol-containing compounds are also known to have the ability to interfere with membrane structures in bacteria (Mboossou et al., 2010). To this effect, both β-stigmasterol (3) and stigmasst-5-en-3β-yl acetate (4) displayed appreciable activity (MIC 15–250 µg/mL) against both strains tested. Terpenes are in turn known to disrupt cell membrane function by interfering with the efflux pumps (Ramalhete et al., 2011). In this instance β-stigmasterol (3), betulinic acid (5), betulin (6) and lupeol (7) showed good overall activity against both mycobacteria strains tested (MIC 15–63 µg/mL). Lupeol (7) activity observed in this study (Table 3; MIC 31–63 µg/mL) correlates well with previous report (Bibi et al., 2010) where it exhibited an MIC of 100 µg/mL against a stationary phase culture of M. tuberculosis (H37Rv). Agreement was also found with the activity of betulinic acid (5) and betulin (6) (MIC 15 µg/mL in each case) previously reported to inhibit the growth of M. tuberculosis (H37Rv) with an MIC value of 32 µM (Wächter et al., 1999). Despite the fact that standard TB treatment regimens require combination therapy of the control drugs used here, the efficacies of all the compounds tested (Table 3) are comparable to the controls, and look extremely promising.

4. Conclusion

Tetracera potatoria is used traditionally for the treatment of various ailments including respiratory ailments (cough) and TB related symptoms. Apart from the known compounds β-stigmasterol (3), stigmasst-5-en-3β-yl acetate (4), betulin (6) and lupeol (7) isolated here for the first time from this plant species; two new compounds 1 (6 mg; yield: 0.028%) and 2 (6 mg; yield: 0.028%) were also isolated. These novel compounds were named tetraceranoate (1) (14,15-p-benzeno-3-hydroxy-2-methoxy-14-methyl-9-oxo-14-propyl-5,8,16-trioxacyclo[9, 4, 0.61] henicosane) and N-hydroxy imidate tetracerane (2) (14,15-p-benzeno-3-hydroxy-2-methoxy-14-methyl-14-propyl-5,8,15-trioxacyclo[9,4,0.15] henicoso-9-one oxime). Compounds 1 displayed the best anti-mycobacterial activity against M. smegmatis (MIC 7.8 µg/mL), while compounds 2, 3, 4, 5, 6 and 7 displayed slightly weaker activity (MIC 31, 15, 31 and 31 µg/mL respectively). Based on the promising results obtained here for the seven isolated compounds against the fast-growing saprophytic species, M. smegmatis and M. aurum, a positive outcome may be expected in further studies against M. tuberculosis. These results could certainly be correlated with the traditional uses of this species for the treatment of respiratory and TB related ailments. Further studies to evaluate the toxicity of the isolated compounds and elucidate the mechanism of action of these compounds would be valuable.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jep.2016.11.027.

References


