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Nor-hopanes from *Zanha africana* root bark with toxicity to bruchid beetles

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ABSTRACT

Zanha africana (Radlk.) Exell (Sapindaceae) root bark is used by farmers throughout sub-Saharan Africa to protect stored grain from bruchid beetles, such as *Callosobruchus maculatus*. Chloroform, methanol and water extracts of *Z. africana* root bark inhibited oviposition and caused significantly higher mortality of *C. maculatus* at a rate of application equivalent to that applied by farmers compared to control insects. The chloroform extract contained *nor*-hopanes rarely found in plants of which seven were isolated, one of which was previously known. Two of the most abundant *nor*-hopanes 3β,6β-dihydroxy-7β-[(4-hydroxybenzoyl)oxy]-21αH-24-norhopa-4(23),22(29)-diene and 3β,6β-dihydroxy-7β-[(4-hydroxybenzoyl)oxy]-24-norhopa-4(23),17(21)-diene were toxic to and reduced oviposition of *C. maculatus* in a dose dependent manner. *Z. africana* root bark is rich in insecticidal compounds that account for its effective use by smallholder farmers as an alternative to conventional insecticides.

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1. Introduction

Zanha africana (Radlk.) Exell is a medium sized tree belonging to the Sapindaceae (Flora Zambesiaca) and occurs in the African savannah and distributed from Kenya southwards through Tanzania, Malawi, Mozambique, Zambia, Zimbabwe, southern Angola and Namibia (Beentje, 1994; Swanepoel, 2013). *Zanha* species have cultural importance across the range. For example, *Zanha golunguensis* is a source of medicine (Bruschi et al., 2011) with activity reported in bark against trypanosomiasis (Nibret et al., 2010), bacterial pathogens (Kambizi and Afolayan, 2001) and fungi (Fabry et al., 1996), and also has anti-inflammatory activity (Recio et al., 1995). *Z. africana* is rich in oleanane type saponins based on the zanhagenic triterpene skeleton (Kapundu et al., 1992) including zanhasaponins A, B and C isolated from the root bark which reportedly account for anti-inflammatory properties (Cuellar et al., 1997a,b). More recently, the structurally related compounds zanhasaponins D–H have also been reported from root bark of *Z. golunguensis*, the only other species in the genus growing in Africa (Lavaud et al., 2015).

Smallholder farmers in Tanzania use the root bark of *Z. africana* to protect stored grain from stored product pests (Mkoga et al., 2004) by pounding the stripped bark to a powder and admixing with their grain. The potential livelihood impact of wild, locally available plants in pest control is compelling if they can be sustainably sourced, particularly for poorly-resourced small-scale farmers (Grzywacz et al., 2014; Isman, 2006). The aim of this study was to analyse the chemistry of *Z. africana* root bark and identify components that might be responsible for any biological activity against insects. Understanding the chemical basis of activity in pesticidal plants provides tools necessary to explain temporal and spatial variation in efficacy (Belmain et al., 2012), inform about the occurrence of chemotypes (Stevenson et al., 2012), and enable the development of optimized field application (Stevenson et al., 2009).

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In this paper, the identity of seven *nor*-hopanes from the root bark of *Z. africana* was determined of which six are reported for the first time. These components explain, at least in part, the bio-efficacy of the root bark of *Z. africana* in protecting stored cowpeas from bruchid damage in smallholder farm stores.

2. Results and discussion

2.1. Identification of nor-hopanes in Z. africana

The chloroform extract of *Z. africana* root bark was shown to be toxic to a bruchid beetle, *Callosobruchus maculatus* (L.), in bioassays described in detail below (Sections 2.2 and 3.1). This extract was analysed using LC-UV-MS/MS and indicated the presence of numerous non-polar peaks with similar UV spectra. Seven compounds, **1–7** (Fig. 1), were isolated using semi-preparative HPLC and characterized using spectroscopic techniques.

Full assignment of the ^{1}H and ^{13}C NMR spectra of **1** in $CDCl_3$ was obtained using COSY, HSQC and HMBC data (Tables 1 and 2). The ^{13}C NMR assignments of **1** showed a good match with those for 3β,6β-dihydroxy-7β-[(4-hydroxybenzoyl)oxy]-21α*H*-24-norhopa-4(23),22(29)-diene (Chávez et al., 1997). Good agreement was also found between the ^{1}H NMR assignments and a partial dataset given by the latter authors, with the exception of the assignments of H-9 and H-13 which required revision (Table 1). A second NMR dataset for **1** was acquired in MeOH-d_4 because of the improved resolution of the multiplet structure of several key resonances including H-3 and H-6. A series of 1D site selective ROE experiments indicated that **1** had the same relative configuration as the published structure (Fig. 1). In particular, the α-configuration of H-21 was confirmed by an ROE correlation with 28-Me. Other key ROE correlations were between 28-Me and 27-Me, 27-Me and H-7 (confirming the β-configuration of the 7-(4-hydroxybenzoyl)oxy group), H-7 and H-5, H-6 and H-5 (confirming the β-configuration of the 6-OH group), H-5 and H-3 (confirming the β-configuration of the 3-OH group) and 25-Me and 26-Me. The optical rotation for **1** of α_D = +45.7 (*c* 0.54, MeOH) had the same sign as the literature value of α_D = +10 (*c* 0.79, $CHCl_3$).

The molecular formula of **2** established by HRESIMS as $C_{36}H_{50}O_6$ differed from that of **1** by the inclusion of one additional oxygen atom. Full assignment of the ^{1}H and ^{13}C NMR spectra of **2** was carried out in both $CDCl_3$ and MeOH-d_4. Comparison of the latter with the analogous assignments for **1** indicated that **2** possessed an oxygenated methine in place of a methylene group. In the COSY spectrum ($CDCl_3$), the oxygenated methine (δ_H 4.30) correlated with H-9 (δ_H 1.65) and 12-CH_2 (δ_H 1.81 and 1.59). Similarly, in the HMBC spectrum acquired in $CDCl_3$, correlations were observed from δ_H 4.30 to C-8 (δ_C 48.4), C-9 (δ_C 54.2), and C-10 (δ_C 39.8). The additional hydroxyl group was therefore located at C-11. NOE connectivities observed between both 25-Me and 26-Me with H-11 indicated that this hydrogen atom was β-oriented. The significant downfield shift ($\Delta\delta$ + 1.06 ppm) experienced by H-1β (δ_H 2.89 in $CDCl_3$) was also consistent with an α-configuration for 11—OH (Isaka et al., 2011). Compound **2** was therefore 3β,6β,11α-trihydroxy-7β-[(4-hydroxybenzoyl)oxy]-21α*H*-24-norhopa-4(23),22(29)-diene.

The ^{1}H NMR spectrum of **3** was similar to that of **2** with the exception that H-11 (δ_H 5.48 in MeOH-d_4) showed a significant downfield shift ($\Delta\delta$ + 1.28 ppm) and a 3H singlet at δ_H 2.03 was observed corresponding to an acetyl group (δ_C 172.1 and 22.1). In the HMBC spectrum, H-11 correlated with the acetyl carbonyl group at δ_C 172.1 and also with C-9 (δ_C 52.9). In the COSY spectrum, H-11 correlated with both H-9 and 12-CH_2 as expected. The magnitude of the coupling constant $J_{9,11}$ of 11.3 Hz indicated a diaxial relationship between these hydrogen atoms such that H-11 was β-oriented. Compound **3** was therefore 11α-acetoxy-3β,6β-dihydroxy-7β-[(4-hydroxybenzoyl)oxy]-21α*H*-24-norhopa-4(23),22(29)-diene.

The main difference between the ^{1}H NMR spectra of **4** and **2** was that the former contained resonances corresponding to two 4-hydroxybenzoyl groups rather than one. The first set of resonances was assigned to the 4-hydroxybenzoyl group at 7-OH. The second was placed at 11-OH on the basis of the large downfield shift ($\Delta\delta$ + 1.56 ppm) experienced by H-11 (δ_H 5.76) and the long-range correlation between this proton and the remaining 4-hydroxybenzoyl carbonyl at δ_C 167.3. As expected, H-11 correlated with H-9 and 12-CH_2 in the COSY spectrum. In common with **3**, $J_{9,11}$ for **4** was also 11.3 Hz, confirming a diaxial relationship between H-9 and H-11 with the latter β-oriented. Thus compound **4** was 3β,6β-dihydroxy-7β,11α-di[(4-hydroxybenzoyl)oxy]-21α*H*-24-norhopa-4(23),22(29)-diene.

A full set of ^{1}H and ^{13}C NMR resonance assignments was obtained for **5** using COSY, HSQC and HMBC data. Compound **5** was isomeric with **1** and could be readily identified as a 24-norhopadiene derivative. The difference between the two compounds resided in the structure of the E-ring. In the case of **5**, the E-ring was a fused cyclopentene with an isopropyl group at C-21, whereas **1** featured an isopropylidene group attached to C-21 of a fused cyclopentane moiety. The multiplet structure and *J*-values for H-3, H-6, and H-7 (MeOH-d_4) were similar for **5** and **1** indicating that the configurations of these atoms were conserved between the two compounds. Thus **5** was 3β,6β-dihydroxy-7β-[(4-hydroxybenzoyl)oxy]-24-norhopa-4(23),17(21)-diene.

The molecular formula of compound **6** differed from that of **5** by the inclusion of one additional oxygen atom, and it was also isomeric with **2**. Analysis of its ^{1}H and ^{13}C NMR spectra indicated that **6** was the 11α-hydroxyl derivative of **5**. Thus in the HMBC spectrum acquired in $CDCl_3$, H-11 (δ_H 4.25) correlated with C-8 (δ_C 48.5), C-9 (δ_C 54.5), C-10 (δ_C 39.8) and C-12 (δ_C 36.6). Similarly in the COSY spectrum, H-11 correlated with H-9 and 12—CH_2. The relative configuration of **6** was examined in a series of 1D site selective ROE experiments (Fig. 2). In particular, H-11 correlated with both 25-Me and 26-Me, as was also found with the isomeric **2**, allowing the β-orientation to be assigned. Thus **6** was 3β,6β,11α-trihydroxy-7β-[(4-hydroxybenzoyl)oxy]-24-norhopa-4(23),17(21)-diene.

Comparison of the ^{1}H NMR spectra of **7** with **6** indicated that 2-CH_2, H-5 and 23-CH_2 were all downfield shifted, and the doublet of doublets resonance of H-3 was lacking. In the ^{13}C NMR spectrum, there were 3 rather than 4 resonances attributable to oxygenated methines and a new resonance at δ_C 204.8 assigned to a carbonyl group. The location of the latter was readily established as C-3 from HMBC data, with correlations from 1-CH_2, 2-CH_2, H-5, and 23-CH_2 to δ_C 204.8 detected in the spectrum. Compound **7** was thus 6β,11α-dihydroxy-7β-[(4-hydroxybenzoyl)oxy]-3-oxo-24-norhopa-4(23),17(21)-diene.

2.2. Biological evaluation of compounds from Z. africana against bruchids

Water, methanol and chloroform extracts of *Z. africana* root bark (10% w/v) significantly reduced the number of eggs laid per female bruchid when compared to the solvent control both prior to and after the exposure to cowpeas (*Vigna unguiculata* L. (Walp)) (Table 4 and Fig. 2). None of the treatments at equivalent concentrations were more toxic than rotenone, the positive control. Water and chloroform extracts assayed as a 10% w/v extract of dry root bark also increased mortality of bruchids over a six day exposure period (Table 4). Prior to the addition of the cowpeas, the females actively probed on vials for suitable oviposition sites and left visible marks in the extract residues on the vial surface indicative of

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