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

Philip C. Stevenson[a,b*], Paul W.C. Green[b], Nigel C. Veitch[b1], Iain Farrell[b], Paul Kusolwa[c] and Steven R. Belmain[a].

[a] *Natural Resources Institute, University of Greenwich, Central Avenue, Chatham Maritime, Kent, ME4 4TB, UK*

[b] *Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3DS, UK*

[c] *Faculty of Agriculture, Sokoine University of Agriculture, Box 3005, Morogoro, Tanzania.*

[1] deceased

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.

* Corresponding author. Tel.: +44 208 332 5369; fax: +44 208 332 5310
E-mail address: p.stevenson@kew.org (P. C. Stevenson)

Keywords: *Zanha africana*, Sapindaceae, cowpea; Fabaceae; bruchid beetles; *Callosobruchus maculatus;* pesticidal plants, botanicals, stored product pests, post-harvest pest management, *Vigna unguiculata*.

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, *Z. 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; Cuellar et al., 1997b). More recently, the structurally related compounds zanhasaponins D-H have also been reported from root bark of *Zanha 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).

In this paper, the identity of seven *nor*-hopanes from the root bark of *Zanha africana* was determined of which six are reported for the first time. These components explain, at least in part, the bioefficacy 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** (Figure 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 (Figure 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 HIRESIMS as $C_{36}H_{50}O_6$ differed from that of **1** by the inclusion of one additional oxygen atom. Full assignment of the ^1H 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 (Δδ +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 ^1H 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 (Δδ +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 (Δδ +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 (Figure 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 Figure 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 this behaviour; they also deposited eggs. Probing is part of a sequence of behaviours leading to oviposition (Parr et al., 1996, 1998) and provides a route of absorption of toxins, where present, via ovipositors. Females should have laid eggs on the cowpeas when given the opportunity, but in the presence of all extracts they laid significantly

fewer eggs compared with the solvent control (Table 4). Similarly, a significantly reduced oviposition was recorded from insects in the presence of **1**and **5** at all concentrations evaluated (10, 100, 1000 ppm) in comparison to the solvent control and this effect was not significantly different to rotenone, the positive control (Table 4 and Figure 2). The effects of the two *nor*-hopanes on bruchid mortality were influenced by concentration and exposure period. Mortality observed after 1, 2, 3 and 6 days exposure were described by linear regression, with significantly increasing mortality observed with increasing exposure period and increasing concentration (S1). Although the addition of untreated beans after 72h provided a refuge from exposure to the extract and compounds, they did not prevent further mortality of insects. In fact, the mortality continued to increase after 72h (S1). These data suggest that the *nor*-hopanes are toxic to bruchids but are less toxic than other highly potent plant compounds such as rotenone and deguelin identified in *Tephrosia vogelii* that is used as a natural pesticide in East and Southern Africa (Belmain et al., 2012).

Hopanes, while rare, are previously known from plants, for example, from *Megacodon stylophorus*, (Liu et al., 2014) and are elsewhere reported to be insecticidal compounds produced by entomopathogenic fungi (Isaka et al., 2011). *Nor*-hopanes, however, have previously been of interest primarily due to their occurrence in crude oil albeit in highly reduced form (Prince et al., 1994) rather than the oxidised and benzoyloxy substituted products reported here and have not been reported before as insecticidal compounds. While **1** and **5** were not potent insecticidal compounds on their own compared to rotenone, the positive control, it is likely that they contribute to the insecticidal effects of *Z. africana* preparations that are used by farmers. *Zanha* species are also rich in saponins (Cuellar et al., 1997a; Cuellar et al., 1997b; Lavaud et al., 2015) which occur widely in other plants used for control of a agricultural pests (Jain and Tripathi, 1991; Mongalo et al., 2015; Nozzolillo et al., 1997; Shinoda et al., 2002) and, in some cases, also explain, biological

activities against storage pests including bruchids activity (Stevenson et al., 2009). Thus further work on may reveal additional value of saponins of this species but this present work provides scientific evidence that at least partially underpins the use of *Z. africana* by resource-poor farmers based on the occurrence *nor*-hopanes that reduce the survival and oviposition behaviour of storage pests.The commercial potential of plant materials as pesticides is constrained by regulatory hurdles and plants such as *Z. africana* are unlikely to replace synthetic products (Sola et al., 2014). Isman (2006), however, suggests that the value of pesticidal plants will be most important in developing countries by poorer farmers. But, this requires greater scientific information to help understand how use of pesticidal plants might be optimised. In this respect, the present work provides important knowledge for understanding more about chemical variability, persistence, residues and improving application.

3. Experimental Section

3.1 General Instrumentation.

HRESIMS data were recorded using a Thermo LTQ-Orbitrap XL mass spectrometer linked to a Thermo Accela LC system performing chromatographic separation of 5 µl injections on a Phenomenex Luna C18(2) column (150 mm × 3.0 mm i.d., 3 µm particle size) with a linear mobile phase gradient of MeOH:H_2O containing 0.1% HCO_2H (90% H_2O at t=0 min. to 100% MeOH at t=20 min). Spectra were recorded in either the positive or negative ion modes at 30,000 resolution

NMR spectra were acquired in $CDCl_3$ or MeOH-d_4 at 30 °C on either a Bruker 400 (Avance) MHz instrument or a Bruker 700 (Avance II+) MHz instrument equipped with a 5mm 1H/13C/15N triple-resonance PFG cryoprobe. Standard pulse sequences and parameters were used to obtain one-dimensional ^{1}H, ^{13}C, and site selective NOE or ROE, and two-dimensional gradient-enhanced COSY, HSQC, and HMBC spectra. Chemical shift referencing was carried out using TMS for

samples dissolved in $CDCl_3$ and the internal solvent resonances at δ_H 3.31 and δ_C 49.1 (calibrated to TMS at 0.00 ppm) for samples dissolved in MeOH-d_4.

Optical rotation measurements (sodium D line, λ 589 nm, 22 °C) were made using a Perkin-Elmer 141 polarimeter with a 10 cm light path cylindrical cell of 1 ml volume. UV and CD spectra (22 °C, MeOH, 0.25 mg/ml) were acquired on an Applied Photophysics Ltd., Chirascan spectropolarimeter with the following parameters: 1 nm bandwidth, 1 nm step-size and a 0.5 s instrument time-per-point sampling; 2 mm and 0.5 mm cell path lengths were employed in the wavelength range 500–200 nm and all spectra were solvent baseline subtracted.

3.2. Plant material and extraction.

Z. africana was collected from a field site in Dodoma, Tanzania and a voucher specimen (Ref SUA Kusolwa 1) is deposited at the national herbarium, at Arusha and verified by Dr. E-F. A. Njau. The root bark was removed and air dried in the dark. Root bark from *Z. africana* (155g) was coarsely ground into pieces (1-5mm) in a coffee-mill before extraction in *n*-hexane (1.55L). After 48h, the extract was filtered using a Büchner funnel and the plant material left in a fume-cupboard to allow any solvent residue to evaporate before adding $CHCl_3$ (1.55L). This process was repeated for extracts in MeOH and H_2O. Filtered extracts were dried under vacuum affording extract residues of 0.20 (*n*-hexane), 0.80 ($CHCl_3$), 35.84 (MeOH) and 10.10g (H_2O), respectively

3.3 Analysis and isolation of nor-*hopanes.*

The dried $CHCl_3$ extract was re-dissolved in MeOH to 50mg mL^{-1}. An HPLC system consisting of a Waters 2695 separations module linked to a 2996 photodiode array detector (PDAD) were used for visualization and isolation of the *nor*-hopanes. Aliquots (90μL) were injected onto a

Phenomenex Luna RP18 column (150 × 10 mm, length × i.d.; 10µm particle size, maintained at 30ºC) and eluted at 5mL min^{-1} using a non-linear gradient (curve=4) of 50%A: 40%B: 10%C (t=0) to 90%A: 0%B: 10%C (t=20-30min) returning to the starting conditions (t=31min), where A=HPLC-MeOH; B=H_2O and C=1% HCO_2H in CH_3CN. Automatic collection using a Waters fraction collector (WFC III) yielded five compounds **1** (8.1 mg; t_R = 12.1 min), **2** (2.6 mg; t_R = 7.8 min), **5** (12.5 mg; t_R = 12.8 min), **6** (7.2 mg; t_R = 8.2 min), **7** (2.4 mg; t_R = 8.7 min) while a sixth peak at t_R = 9.7 was subjected to further HPLC using the same method described above, but with CH_3CN in place of MeOH and a non-linear gradient (Waters curve=3) of 50% A: 40%; B 10%C (t=0) to 90%A: 0%B: 10%C (t=20-21 min) returning to the original conditions (t=22 min). This procedure yielded two further compounds: **3** (~0.5 mg; t_R = 5.9 min) and **4** (~0.5 mg; t_R = 5.2 min). All compounds were isolated to a purity of at least 95%.

The optical rotations of **1**-**3** and **5**-**7** were individually recorded on a Perkin-Elmer 343 spectrometer. The instrument was calibrated with D (+) sucrose (1.04 g/100ml, 1dm, $[\alpha]_{589}$ = + 66.6º). A 10cm cylindrical Quartz cell was employed (low volume). All samples were measured at room temperature (23ºC). There was insufficient material to obtain optical rotation data for **4**.

3.4 Insects and bioassays

Callosobruchus maculatus (Fabricius, 1775) originally collected in Ghana (Kestenholz et al., 2007) were maintained in 5 l glass culture jars with perforated lids in an unlit growth cabinet (28°C, 55% RH) to develop on cowpea seeds *Vigna unguiculata.* Under these conditions, adults emerged from cowpea seeds 24-28 days after oviposition. The insects used for bioassays were 3-5 days post-emergence.

Samples of dried extract were re-dissolved in aliquots (10mL) of solvent to concentrations that represented the proportions in a 10% extract of plant material. **1** (8.1mg) and **5** (12.1mg) were dissolved in CH_3CN to 1mg mL^{-1} (1000 ppm) and further diluted to 100 and 10ppm. The lowest concentration was chosen as it approximated the amounts of **1** (5.2ppm) and **5** (8.1ppm) in the 10% w/v chloroform extract. Aliquots (75 µL) of compounds or extracts were evaporated onto vials (25mL, nominal capacity) under a stream of air and with constant rotation of the vial. Insects (N=5-9) were added to the vials, ensuring a ratio of at least 1:1 (male to female). Replicates (N=25) were prepared in this wayand the negative control group, which consisted of vials from which 75µL aliquots each of water, methanol and chloroform has been sequentially evaporated (N=23). Rotenone, 1000, 100 or 10ppm, in MeOH, was used as a positive control (N=10, per concentration). After 24, 48 and 72h mortality was assessed, and cowpeas (N=5) were added to each vial. After a further 72h, mortality was recorded once more. The numbers of eggs laid on both the vials and the beans were counted and from these data the eggs laid per female were calculated.

*3.5. 3β,6β-dihydroxy-7β-[(4-hydroxybenzoyl)oxy]-21αH-24-norhopa-4(23),22(29)-diene (**1**)*

Off white solid; UV (MeOH) λ_{max} nm: 253; $\Delta\varepsilon_{336}$ +45.7° (*c* 0.54, MeOH); for ^{1}H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 2; HRESIMS *m/z*: 561.3592 $[M-H]^-$ (calc. for $C_{36}H_{49}O_5^-$, 561.7713).

*3.6 3β,6β,11α-trihydroxy-7β-[(4-hydroxybenzoyl)oxy]-21αH-24-norhopa-4(23),22(29)-diene (**2**).* Off white solid; UV (MeOH) λ_{max} nm: 258; $\Delta\varepsilon_{336}$ +30.6 (*c* 0.54, MeOH); for ^{1}H NMR and ^{13}C

NMR spectroscopic data, see Tables 1 and 2; HRESIMS *m/z*: 577.3541 $[M-H]^-$ (calc. for $C_{36}H_{49}O_6^-$, 577.7707).

*3.7 11α-acetoxy-3β,6β-dihydroxy-7β-[(4-hydroxybenzoyl)oxy]-21αH-24-norhopa-4(23),22(29)-diene. (**3**).* Off white solid; UV (MeOH) λ_{max} nm: 258; $\Delta\varepsilon_{336}$ -64.00 (*c* 0.54, MeOH); for ^{1}H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 2; HRESIMS *m/z*: 619.3653 $[M-H]^-$ (calc. for $C_{38}H_{51}O_7^-$, 619.8073).

*3.8 3β,6β-dihydroxy-7β,11α-di[(4-hydroxybenzoyl)oxy]-21αH-24-norhopa-4(23),22(29)-diene (**4**).* Off white solid; UV (MeOH) λ_{max} nm: 258; for ^{1}H NMR, for ^{1}H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 2; HRESIMS *m/z*: 697.3673 $[M-H]^-$ (calc. for $C_{43}H_{53}O_8^-$, 697.8761). Insufficient compound was available to measure optical rotation.

*3.9 3β,6β-dihydroxy-7β-[(4-hydroxybenzoyl)oxy]-24-norhopa-4(23),17(21)-diene (**5**).* Off white solid; UV (MeOH) λ_{max} nm: 256; $\Delta\varepsilon_{336}$ +21.1 (*c* 0.54, MeOH); for ^{1}H NMR and ^{13}C NMR spectroscopic data, see Tables 2 and 3; HRESIMS *m/z*: 561.3588 $[M-H]^-$ (calc. for $C_{36}H_{49}O_5^-$, 561.7713).

*3.10 3β,6β,11α-trihydroxy-7β-[(4-hydroxybenzoyl)oxy]-24-norhopa-4(23),17(21)-diene (**6**).* Off white solid; UV (MeOH) λ_{max} nm: 258; $\Delta\varepsilon_{336}$ +16.9 (*c* 0.54, MeOH); for ^{1}H NMR and ^{13}C NMR spectroscopic data, see Tables 2 and 3; HRESIMS *m/z*: 577.3541 $[M-H]^-$ (calc. for $C_{36}H_{49}O_6^-$, 577.7707).

3.11 6β,11α-dihydroxy-7β-[(4-hydroxybenzoyl)oxy]-3-oxo-24-norhopa-4(23),17(21)-diene (7). Off white solid; UV (MeOH) λ_{max} nm: 255; $\Delta\varepsilon_{336}$ +13.8 (*c* 0.54, MeOH); for ^{1}H NMR and ^{13}C NMR spectroscopic data, see Tables 2 and 3; HRESIMS *m/z*: 575.3386 $[M-H]^-$ (calc. for $C_{38}H_{47}O_6^-$, 575.7548).

Acknowledgements

Biomedical NMR Centre, National Institute for Medical Research, Mill Hill, London, for access to high-field NMR instrumentation and Drs. Tam Bui and Alex Drake, Pharmaceutical Optical & Chiroptical Spectroscopy Centre, Department of Pharmacy, King's College, London, for chiroptical data. Dr. Renée Grayer for comments made on the manuscript. This work was funded from a McKnight Foundation grant to P.C.S. and S.R.B. (Grant No: 13-335) and an EU 9th European Development Fund grant to P.C.S. from the African Caribbean and Pacific Science and Technology Programme (FED/2013/329-272). The contents of this document are the sole responsibility of the authors and can under no circumstances be regarded as reflecting the position of the McKnight Foundation or the European Union.

References

Beentje, H. J., 1994. Kenya Trees, Shrubs and Lianas. National Museums of Kenya, Nairobi.

Belmain, S. R., Amoah, B. A., Nyirenda, S. P., Kamanula, J. F., Stevenson, P. C., 2012. Highly variable insect control efficacy of *Tephrosia vogelii* chemotypes. J. Agric. Food Chem. 60, 10055–10063.

Bruschi, P., Morganti, M., Mancini, M., Signorini, M. A., 2011. Traditional healers and laypeople: A qualitative and quantitative approach to local knowledge on medicinal plants in Muda (Mozambique). J. Ethnopharmacol. 138, 543–563.

Chávez, J. P., David, J. M., Yang, S. W., Cordell, G. A., 1997. 24-norhopene derivatives from *Diatenopteryx sorbifolia*. J. Nat. Prod. 60, 909–911.

Cuellar, M. J., Giner, R. M., Recio, M. C., Just, M. J., Manez, S., Cerda, M., Hostettmann, K., Rios, J. L., 1997a. Zanhasaponins A and B, antiphospholipase A(2) saponins from an antiinflammatory extract of *Zanha africana* root bark. J. Nat. Prod. 60, 1158–1160.

Cuellar, M. J., Giner, R. M., Recio, M. D., Just, M. J., Manez, S., Rios, J. L., Bilia, A. R., Msonthi, J., Hostettmann, K., 1997b. Three new oleanane saponins from *Zanha africana*. J. Nat. Prod. 60, 191–194.

Fabry, W., Okemo, P., Ansorg, R., 1996. Fungistatic and fungicidal activity of East African medicinal plants. Mycoses 39, 67–70.

Grzywacz, D., Stevenson, P. C., Mushobozi, W. L., Belmain, S., Wilson, K., 2014. The use of indigenous ecological resources for pest control in Africa. Food Security 6, 71–86.

Isaka, M., Palasarn, S., Supothina, S., Komwijit, S., Luangsa-ard, J. J., 2011. Bioactive compounds from the scale insect pathogenic fungus *Conoideocrella tenuis* BCC 18627. J. Nat. Prod. 74, 782–789.

Isman, M. B., 2006. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annu. Rev. Entomol, vol. 51. Annual Reviews, Palo Alto, pp. 45–66.

Jain, D. C., Tripathi, A. K., 1991. Insect feeding-deterrent activity of some saponin glycosides. Phytother. Res. 5, 139–141.

Kambizi, L., Afolayan, A. J., 2001. An ethnobotanical study of plants used for the treatment of sexually transmitted diseases (njovhera) in Guruve District, Zimbabwe. J. Ethnopharmacol. 77, 5-9. 123–123

Kapundu, M., Warin, R., Delaude, C., 1992. Structure of zanhagenic acid from *Zanha africana*. B. Soc. Chim. Belg. 101, 283–286.

Kestenholz, C. Stevenson P.C., Belmian, S.R., 2007. Comparative study of field and laboratory evaluations of the ethnobotanical *Cassia sophera* L. (Leguminosae) for bioactivity against the storage pests *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) and *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) J. Stored Prod. Res. 43, 79–86.

Liu C., Liao, Z.X., Liu, S.J., Ji, L.J., Sun, H.F., 2014. Two new 2,3-seco-hopane triterpene derivatives from *Megacodon stylophorus* and their antiproliferative and antimicrobial activities. Planta Med. 80, 936–941.

Lavaud, C., Sayagh, C., Humbert, F., Pouny, I., Delaude, C., 2015. Triterpenoid saponins from root bark of *Zanha golungensis* (Sapindaceae). Carbohydr. Res. 402, 225–231.

Mkoga, Z. J., Shetto, R. M., Mkomwa, S., Mwakimbwala, R., Kabungo, D. A., Ndegeulaya, D., 2004. Efficacy of botanical insecticides on *Sitophilus* species on maize in the Southern Highlands of Tanzania. Tanzania Agricultural Training Newsletter 19, 6–8.

Mongalo, N. I., McGaw, L. J., Finnie, J. F., Van Staden, J., 2015. *Securidaca longipedunculata* Fresen (Polygalaceae): A review of its ethnomedicinal uses, phytochemistry, pharmacological properties and toxicology. J. Ethnopharmacol. 165, 215–226.

Nibret, E., Ashour, M. L., Rubanza, C. D., Wink, M., 2010. Screening of some Tanzanian medicinal plants for their Trypanocidal and cytotoxic activities. Phytother. Res. 24, 945–947.

Nozzolillo, C., Arnason, J. T., Campos, F., Donskov, N., Jurzysta, M., 1997. Alfalfa leaf saponins and insect resistance. J. Chem. Ecol. 23, 995–1002.

Parr, M. J., Tran, B. M. D., Simmonds, M. S. J., Credland, P. F., 1996. Oviposition behaviour of the cowpea seed beetle, *Callosobruchus maculatus*. Physiol. Entomol. 21, 107–117.

Parr, M. J., Tran, B. M. D., Simmonds, M. S. J., Credland, P. F., 1998. Duration of behaviour patterns during oviposition by the bruchid beetle, *Callosobruchus maculatus*. Physiol. Entomol. 23, 150–157.

Prince, R. C., Elmendorf, D. L., Lute, J. R., Hsu, C. S., Haith, C. E., Senius, J. D., Dechert, G. J., Douglas, G. S., Butler, E. L., 1994. 17-α(H),21-β(H)-Hopane as a conserved internal marker for estimating the biodegradation of crude-oil. Environ. Sci. Technol. 28, 142–145.

Recio, M. C., Giner, R. M., Manez, S., Rios, J. L., Marston, A., Hostettmann, K., 1995. Screening of tropical medicinal plants for antiinflammatory activity. Phytother. Res. 9, 571–574.

Shinoda, T., Nagao, T., Nakayama, M., Serizawa, H., Koshioka, M., Okabe, H., Kawai, A., 2002. Identification of a triterpenoid saponin from a crucifer, *Barbarea vulgaris*, as a feeding deterrent to the diamondback moth, *Plutella xylostella*. J. Chem. Ecol. 28, 587–599.

Stevenson, P. C., Dayarathna, T. K., Belmain, S. R., Veitch, N. C., 2009. Bisdesmosidic saponins from *Securidaca longepedunculata* roots: evaluation of deterrency and toxicity to Coleopteran storage pests. J. Agric. Food Chem. 57, 8860–8867.

Stevenson, P. C., Kite, G. C., Lewis, G. P., Forest, F., Nyirenda, S. P., Belmain, S. R., Sileshi, G. W., Veitch, N. C., 2012. Distinct chemotypes of *Tephrosia vogelii* and implications for their use in pest control and soil enrichment. Phytochemistry 78, 135–146.

Swanepoel, W., 2013. Sapindaceae *Zanha africana*, a new distribution record for Namibia. Bothalia 43, 88–89.