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Chemical constituents from the root bark of *Ozoroa insignis*

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1. Subject and source

The roots of *Ozoroa insignis* Del. (Heeria insignis Del.) (Anacardiaceae) are used as a remedy for diarrhea, venereal diseases, tapeworm and hookworm, schistosomiasis, kidney trouble, migraine, and malaria (Liu and Abreu, 2007). In previous biological screening of *O. insignis* extracts, anthelmintic effect, cytotoxic activity, and topoisomerase inhibition were reported (Liu and Abreu, 2006a). The root bark of *O. insignis* were collected in Masaai land, Rift valley province in Kenya, in January 2005 and authenticated by Simon Mathenge, of Nairobi University, Kenya. A voucher specimen (MM/08/04) is deposited in Nairobi University herbarium, Chiromo Campus.

2. Previous work

Early studies regarding the chemical constituents of *O. insignis* revealed the presence of tirucallane triterpenes (Liu and Abreu, 2006a), alk(en)yl phenols (Liu and Abreu, 2006b; Rea et al., 2003), one macrolide (Liu and Abreu, 2007), and 6-pentadecylsalicylic acid (He et al., 2002).

3. Present study

The dried and powdered root barks (1 kg) of *O. insignis* were exhaustively and sequentially extracted with *n*-hexane, CH_2Cl_2, EtOAc, and MeOH. Each extract was concentrated *in vacuo* to obtain *n*-hexane, CH_2Cl_2- and ethyl acetate-soluble fractions. The CH_2Cl_2 soluble extract (80 g) was subjected to VLC on silica gel using petroleum ether, petroleum ether–EtOAc, EtOAc–MeOH and finally, pure MeOH as the mobile phase and yielded 65 fractions (F_{1-65}). Fraction F_{19-27} were further separated by silica gel column chromatography eluting with petroleum ether–EtOAc (9:1) to give white cotton needles of

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β-amyrin (**1**, 20 mg) (Heupel, 2005) and fraction B. Similarly, Sephadex® LH-20 CC of fraction B, which was eluted with MeOH:CH_2Cl_2 (5:5) gave four fractions (C–F). Betulonic acid (**2**, 70 mg) (Kuroyangi et al., 1986) was purified from fraction C by crystallization using petroleum ether–acetone (8:2). Further purification of fraction C [petroleum ether–acetone (8.5:1.5)] afforded magnificol (**3**, 7 mg) (Ulubelen et al., 1989).

The ethyl acetate crude extract (50 g) was similarly subjected to VLC on silica gel and eluted with a gradient of petroleum ether and acetone yielding 55 fractions (F_{1-55}). Repeated column chromatography of F_{33-38} using petroleum ether–acetone (7.5:2.5) afforded betulinic acid (**4**, 8 mg) (Ikuta et al., 1995). Similarly purification of fraction F_{20-25} using a Sephadex® LH-20 column, eluted with a mixture of CH_2Cl_2–MeOH (100:0 to 9:1) furnished 6-tridecyl anacardic acid (**5**, 3.0 mg) (Li et al., 2004) and 6-[8(*Z*)-pentadecenyl]anacardic acid (**6**, 4.5 mg) (Rea et al., 2003). Fraction F_{38-45} on Sephadex® LH-20 column using petroleum ether–acetone (7:3) afforded 6-[10(*Z*)-heptadecenyl]anacardic acid (**7**, 5.5 mg) (Pan et al., 2006) and 6-[nonydecyl]anacardic acid (**8**, 3.5 mg) (Navarrete et al., 1989). Preparative TLC of F_{46-55} eluted with acetone:CH_2Cl_2 (1:9) yielded 5,2′,4′- trihydroxy flavone (**9**, 6.7 mg). The structures of known compounds (**1**–**8**) (Fig. 1) were established conclusively by UV, IR, MS and extensive ^{1}H- and ^{13}C NMR spectra analysis and comparison with literature data.

Ozoranone (**9**) was obtained as a yellow powder and the UV spectrum exhibited absorption maxima at 263 and 328 nm. This was supported by IR bands at 1720 cm^{-1} for carbonyl absorption, a broad signal at 3400 cm^{-1} for non-chelated hydroxyl groups. Analysis of the HREIMS gave a molecular ion at *m*/*z* 270.0519 $[M]^+$, corresponding to the molecular formula $C_{15}H_{10}O_5$, supported by the ^{1}H NMR, ^{13}C NMR and DEPT analysis. The ^{1}H NMR in $CDCl_3$ of ozoranone (**9**) showed signals for seven deshielded protons. From the ^{1}H–^{1}H-COSY, two pairs of three protons were coupling to one another while one proton appeared as a singlet, suggesting the presence of two pairs of ABX spin system of three aromatic protons each. The first ABX spin system of three aromatic protons of ring A appeared at δ 7.59 (d, 2.0 Hz, H-5), 7.36 (dd, $J = 8.0$, 2.0 Hz, H-7), 6.96 (d, $J = 8.0$ Hz, H-8) and the ring B ABX spin system appeared at δ 7.63(d, $J = 8.0$ Hz, H-6′), δ 6.84 (d, $J = 2.0$ Hz, H-3′) and δ 6.80 (dd, $J = 8.0$, 2.0 Hz, H-5′). The ^{1}H NMR spectrum also indicated the presence of a singlet at δ 6.49 and its position at C-3 was confirmed by HMBC correlation to C-2 and C-4. The ^{13}C NMR and DEPT spectra revealed 15 carbon signals, including a carbonyl carbon at δ 181.4 and fourteen aromatic carbons at δ 98.5–168.0. From these data, compound **9** was considered as a 2′,4′,6-oxygenated flavone. The position of the hydroxyl groups at C-2′, C-4′, and C-6 along with assignments of all carbons and hydrogen were confirmed by interpretation of the cross signals in the ^{1}H–^{1}H COSY (Fig. 2), ^{1}H–^{13}C HMQC and ^{1}H–^{13}C HMBC spectra (Fig. 2) combined with the coupling constants of the signals in the ^{1}H NMR spectrum. Therefore, based on the above data, the structure of compound **9** was established as 2-(2,4-dihydroxyphenyl)-6-hydroxy-4*H*-chromen-4-one (Fig. 1).

The flavonoid (**9**) is new as a natural product but has been reported as a synthetic compound in a Japanese patent (Kyogoku et al., 1979).

Ozoranone (**9**): Yellow powder. ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.49 (1H, s, H-3), 6.80 (1H, dd, $J = 8.0$, 2.0 Hz, H-5′), 6.84 (1H, d, $J = 2.0$ Hz, H-3′), 6.96 (1H, d, $J = 8.0$ Hz, H-8), 7.36 (1H, dd, $J = 8.0$, 2.0 Hz, H-7), 7.59 (1H, d, 2.0 Hz, H-5), 7.63 (1H, d, $J = 8.0$ Hz,

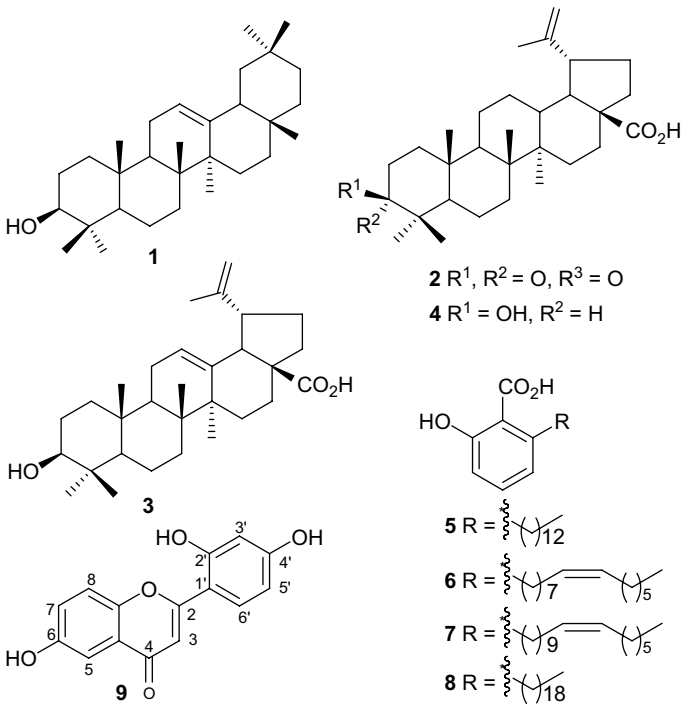

Fig. 1. Structures of Compounds **1–9** isolated from *Ozoroa insignis*.

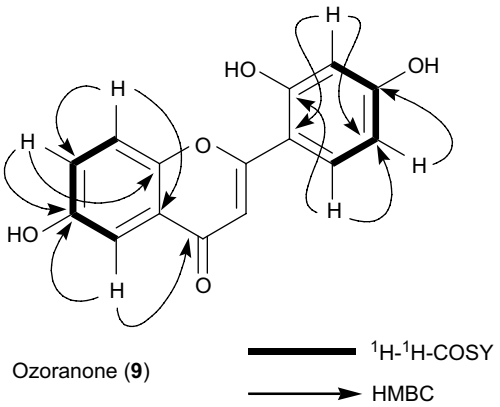

Fig. 2. Selected ^{1}H–^{1}H COSY and HMBC correlations for compounds **9**.

H-6′). ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 98.5$ (C-3′), 111.3 (C-3), 112.1 (C-5′), 114.1 (C-1′), 115.6 (C-8), 117.7 (C-5), 119.9 (C-10), 124.7 (C-7), 125.6 (C-6′), 145.2 (C-9), 146.4 (C-6), 147.3 (C-4′), 165.7 (C-2′), 168.0 (C-2), 181.4 (C-4). IR-ν_{max} ($CHCl_3$): 3400, 1720, 1590, 710 cm^{-1}. UV ($CHCl_3$) λ_{max}: 263 (3.10), 328 (4.10). HREIMS: m/z 270.0519 (Calcd. 270.0527 for $C_{15}H_{10}O_5$).

4. Chemotaxonomic significance

Alkyl phenols, alkylhexenones, tannins, triterpenes, and flavones are widely distributed in the Anacardiaceae-family (Kapche et al., 2007). The present study reports the isolation of one oleaneane(**1**)and three lupane type triterpenoids (**2**–**4**), four anacardic acid[alk(en)yl-phenol] derivatives (**5**–**8**), and one flavone (**9**) for the first time from the root bark of *O. insignis*. Interestingly, compounds **1, 2** and **4** were characterized for the first time from the genus *Ozoroa* and have been isolated from the genus *Rhus* of the same family (Franke et al., 2001; Gu et al., 2007; Lee et al., 2005). This finding confirms that the genera *Ozoroa* and *Rhus* are closely related taxonomically. On the other hand, compound **3** was characterized for the first time from the Anacardiaceae-family, and thus isolation of compounds **1**–**4** in the present investigation is a major contribution to chemotaxonomic studies of the Anacardiaceae-family. Many alk(en)yl phenol derivatives were obtained from anacardiaceous plants (Kapche et al., 2007) and recently Liu and Abreu reported 41 alk(en)yl phenols from *O. insignis* (Liu and Abreu, 2006b). The carbon lengths of side chains in previously known alkenylphenols and alkenylsalicylic acids from Anacardiaceae have usually been C15 or C17 (Masuda et al., 2002). Therefore compound **8** with alkyl chain of C19 is less common in the Anacardiaceae-family (Liu and Abreu, 2006b). Compound **6** has been reported from *O. insignis* (Rea et al., 2003) but this is the first report of compounds **5, 7** and **8** in the genus *Ozoroa* as well as in the Anacardiaceae-family. Interestingly, compounds **5**–**7** have been isolated from the genus *Ginkgo* of the Ginkgoaceae family (Li et al., 2004; Ni and Wu, 2006; Pan et al., 2006). Thus the isolation of the compounds **5**–**7** in the family Anacardiaceae is particularly interesting since this strengthens the chemotaxonomic relationship of Anacardiaceae and Ginkgoaceae. This is the first report of a flavone, ozoranone (**9**), from the genus *Ozoroa*, although flavones have been reported from other species within the family Anacardiaceae (Matsuda, 1966).

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