Klodorone A and klodorol A: new triterpenes from Kleinia odora

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(Received 14 March 2014; final version received 14 April 2014)

Re-investigation of the EtOH extract of the aerial parts of Kleinia odora led to the isolation of two new triterpenes; klodorone A (3) and klodorol A (4), together with two known compounds: β-amyrin (1) and germanicol (2), which were reported from this plant for the first time. Their structures were determined by using extensive 1D (1H, 13C and DEPT) and 2D (1H–1H COSY, HMQC and HMBC) NMR and mass spectral measurements in addition to comparison of their data with the literature.

Keywords: Kleinia odora; Asteraceae; triterpenes

1. Introduction

The genus Kleinia (Asteraceae) comprises 40 species of which several species are known to be rich sources of oxygenated sesquiterpenoids and triterpenoids (Al Musayeib et al. 2013). Oleanane- and ursane-type triterpenoids had been extensively explored for their biological and pharmacological activities. These compounds have been found to exhibit a variety of properties. These activities include antitumour, anti-viral, anti-inflammatory, hepatoprotective, gastro-protective, antimicrobial, anti-diabetic and anti-protozoal activities (Sun et al. 2006; Dominguez-Carmona et al. 2010; Al Musayeib et al. 2013). Previous chemical investigation of Kleinia odora growing in Saudi Arabia resulted in the isolation and characterisation of lupeol, luponone, epilupeol, lupeol acetate, ursolic acid, 3β,11α-dihydroxy 12-ene, brein and ursolic acid lactone (Al Musayeib et al. 2013). Reviewing the current literature, there are minor reports concerning the chemical investigations for the constituents of K. odora growing in Saudi Arabia. Therefore, a phytochemical study of the plant was performed aiming to identify more of its constituents. This article reports the isolation and characterisation of two new triterpenes: klodorone A (3) and klodorol A (4), together with two known compounds: β-amyrin (1) (Mahato and Kundu 1994) and germanicol (2) (Gonzalez et al. 1981), which are reported for the first time from the aerial parts of K. odora (Figure 1).

2. Results and discussions

Compound 3 was obtained as colourless needles. It gave a positive Liebermann–Burchard reaction, indicating its triterpenoidal nature (Reinhold 1935). Based on its HR-EI-MS and NMR data, its molecular formula was deduced to be C30H50O2. Six degrees of unsaturation was

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evident from the molecular formula, five of which were accounted for by the pentacyclic rings and one by a ketone group. The $^1$H NMR spectrum of 3 indicated five methine multiplets at $\delta_H$ 1.35, 1.45, 1.48, 1.15 and 1.42 which were assigned to H-5, H-9, H-18, H-19 and H-20, respectively. They correlated with the carbon signals resonating at $\delta_C$ 54.8 (C-5), 46.9 (C-9), 49.4 (C-18), 41.3 (C-19) and 40.9 (C-20) in the HMQC spectrum (Supplementary Figure S4). The doublet signal at $\delta_H$ 1.48 ($J = 7.1$ Hz) was ascribed to the H-18α proton (Ibrahim et al. 2012). Moreover, the $^1$H NMR spectrum revealed signals for eight methyl groups, including six singlets at $\delta_H$ 1.08 (H3-23), 1.03 (H3-24), 1.00 (H3-25), 1.11 (H3-26), 1.24 (H3-27) and 1.03 (H3-28) and two doublets at $\delta_H$ 0.94 ($J = 6.8$ Hz, H3-29) and 0.93 ($J = 6.8$ Hz, H3-30), supporting the ursane-type carbon framework of 3 (Mahato & Kundu 1994; Begum et al. 1997; Ahmad et al. 2007) (Supplementary Figure S1). $^{13}$C NMR spectrum of 3 indicated resonances for 30 carbons: 8 methyls, 10 methylenes, 5 methines and 7 quaternary carbons including a ketone carbonyl at $\delta_C$ 217.8 (C-3) and an oxygen-bonded carbon at $\delta_C$ 75.1 (C-13). The presence of hydroxyl and ketone groups was confirmed by the IR absorption bands at 3410 and 1725 cm$^{-1}$, respectively. The ketone moiety was positioned at C-3 based on the HMBC cross-peaks of H-1, H-2, H3-23 and H3-24 to C-3. The HMBC correlations of H-11, H-15, H-18 and H3-27 to C-13 confirmed the connectivity of hydroxyl group at C-13 (Supplementary Figures S5 and S13). In NOESY spectrum, H-5 displayed cross-peaks to H-9, H-18, H-19 and H3-27 to H3-30. Furthermore, H3-25 and H3-26 correlated with H3-24, H3-28 and H3-29, H-20 to H-28, as well as H3-23 to H-5 and H3-27 (Supplementary Figure S6). On the basis of these findings and by comparing with the literature (Mahato & Kundu 1994; Begum et al. 1997;
Ahmad et al. (2007), the structure of 3 was assigned as ursa-18αH-13β-hydroxy-3-one and a trivial name klodorone A was given to it.

Compound 4 was obtained as colourless needles. It gave a positive Liebermann–Burchard reaction, indicating its triterpenoidal nature (Reinhold, 1935). The HR-EI-MS revealed a molecular ion peak at m/z 442.3815 [M]+, which is consistent with the molecular formula C₃₀H₅₀O₂, requiring six degrees of unsaturation. The IR spectrum indicated absorption bands at 3462 (OH) and 1070 (C-O) cm⁻¹. The ¹H and ¹³C NMR spectra exhibited signals for 8 methyl groups at δH/δC 1.16 (H₃-23)/28.4 (C-23), 1.18 (H₃-24)/25.4 (C-24), 0.99 (H₃-25)/16.6 (C-25), 0.95 (H₃-26)/16.8 (C-26), 1.06 (H₃-27)/15.6 (C-27), 1.16 (H₃-28)/23.6 (C-28), 1.10 (H₃-29)/27.4 (C-29) and 1.06 (H₃-30)/21.4 (C-30), 11 methylenes, 3 methines including an oxymethine at δH 3.35 (H-3)/δC 84.8 (C-3) and 8 quaternary carbons, 2 of them are oxygen bearing at δC 72.8 (C-5) and 87.8 (C-10) (Supplementary Figures S7 and S8). The NMR data of 4 were similar to those of dendropanoxide (a hexacyclic triterpene oxide with a D:Bfriedooleanane skeleton isolated from Dendropanax trifidus) except the presence of an additional oxygenated carbon at δC 72.8 (C-5) (Tori et al., 1988). Its position at C-5 was confirmed by the observed J HMBC correlations of H-3, H₃-23 and H₃-24 to C-5 (δC 72.8) (Supplementary Figures S11 and S13). The location of the heterocycle in ring A between C-3 and C-10 was confirmed by means of the HMBC experiment which revealed the correlations H-2, H-8 and H-25 to C-10 and H-2, H₃-23 and H₃-24 to C-3. The correlations of H₃-25 to C-8, C-9, C-10 and C-11 were consistent with the location of a methyl group at C-9. Furthermore, HMBC correlations from H₃-27 to C-18 and C-13 and from H₃-28 to C-17 and C-18 were observed. The relative configuration of 4 was determined by comparing the ¹H and ¹³C NMR chemical shifts as well as coupling constant with those of related compounds in the literature (Tori et al., 1988; Estrada et al., 2002). On the basis of these findings and by comparing the NMR data of 4 with those of structurally related compounds (Tori et al., 1988; Estrada et al., 2002), 4 was identified as dendropanoxide with additional hydroxyl group at C-5 (5α-hydroxy dendropanoxide). To the best of our knowledge, this is a new compound isolated for the first time from K. odora for which we propose the name klodorol A.

The known compounds 1 and 2 were identified by analysing their spectroscopic data (1D, 2D NMR and MS) and by comparing their data with those in the literature as: β-amyrin (Mahato and Kundu, 1994) and germanicol (Gonzalez et al., 1981). Compounds 1 and 2 were isolated for the first time from the plant.

3. Experimental
3.1. General experimental procedures
Optical rotations were measured on a Perkin-Elmer Model 341 LC polarimeter (Perkin-Elmer, Waltham, MA, USA). Melting points were determined using an Electrothermal 9100 Digital Melting Point apparatus (Electrothermal Engineering Ltd, Essex, England). The IR spectra were measured on a Shimadzu Infrared-400 spectrophotometer (Kyoto, Japan). HR-EI-MS was measured on JEOL JMS-HX 110 mass spectrometer (Joel, Peabody, MA, USA). EI-MS was recorded on JEOL JMS-SX/SX 102A mass spectrometer (Joel, Peabody, MA, USA). The 1D and 2D NMR spectra were recorded on a Bruker AMX-400 spectrometer (Bruker, Rheinstetten, Germany) with tetramethylsilane as an internal standard. NMR spectra were referenced to the solvent signals (CDCl₃: 7.26 ppm for ¹H and 77.0 ppm for ¹³C). Solvents were distilled prior to use, and spectral grade solvents were used for spectroscopic measurements. Column chromatographic separations were performed on silica gel 60 (0.04–0.063 mm, Merck, Darmstadt, Germany) and RP-18 (0.04–0.063 mm, Merck). Thin-layer chromatography (TLC) was performed on pre-coated TLC plates with silica gel 60 F₂₅₄ (0.2 mm, Merck). The solvent systems used for TLC analyses were n-hexane–EtOAc (95:5, S1) and n-hexane–EtOAc (90:10, S2). The compounds were detected by UV absorption at λmax 255 and 366 nm followed by spraying with anisaldehyde/H₂SO₄ reagent and heating at 110°C for 1–2 min. All the chemicals were purchased from Sigma Chemical Company (St Louis, MO, USA).
3.2. Plant materials

The plant, *K. odora*, was collected from the south of Saudi Arabia in February 2011. The plant was kindly identified by taxonomist at the Pharmacognosy Department, College of Pharmacy, King Saud University. A voucher specimen has been deposited at the Pharmacognosy Department, College of Pharmacy, King Saud University under the registration number P-15129.

3.3. Extraction and isolation

The air-dried powdered aerial parts of *K. odora* (500 g) were extracted by maceration with 70% EtOH (4 × 2 L) at room temperature. The combined ethanolic extract was concentrated under reduced pressure to afford a dark greenish brown residue (20.4 g). The latter was suspended in distilled water (200 mL) and then partitioned between petroleum ether (3 × 500 mL), CHCl₃ (3 × 500 mL) and *n*-BuOH (3 × 500 mL), successively. Each fraction was concentrated under reduced pressure to give petroleum ether (6.0 g), CHCl₃ (4.2 g), *n*-BuOH (3.6 g) and aqueous (5.8 g) fractions.

The petroleum ether fraction (6.0 g) was subjected to silica gel column chromatography (200 g × 100 cm × 3 cm) using *n*-hexane:EtOAc gradients to obtain nine subfractions (KH-1–KH-9). Subfraction KH-1 was previously investigated by the authors (Al Musayeib et al. 2013). Subfraction KH-4 was chromatographed on an RP-18 column (MeOH–H₂O, 90:10) to yield compounds 1 (8 mg) and 2 (10 mg). The CHCl₃ fraction (4.2 g) was subjected to silica gel column chromatography (150 g × 100 cm × 3 cm) using CH₂Cl₂–EtOAc (90:10) to 100% EtOAc to obtain seven subfractions (KC-1–KC-7). Subfractions KC-1, KC-2, KC-5 and KC-7 were previously investigated (Al Musayeib et al. 2013). Subfraction KC-3 was chromatographed over an RP-18 column using MeOH–H₂O (90:10) to afford compounds 3 (10.2 mg) and 4 (6 mg). The other fractions were stored for further investigation.

3.4. Spectral data

Klodorone A (3): Colourless needles; m.p. 188–190°C; [α]₂⁵° +16.2 (c = 0.1, CHCl₃); IR γmax (KBr): 3410, 2945, 1725, 1635, 1020 cm⁻¹. ¹H NMR (CDCl₃, 500 MHz): δH 1.98 (2H, t, J = 7.3 Hz, H-1), 2.53 (1H, m, H-2A), 2.40 (1H, m, H-2B), 1.35 (1H, m, H-5), 1.58 (1H, m, H-6A), 1.45 (1H, m, H-6B), 1.42 (1H, m, H-7A), 1.01 (1H, m, H-7B), 1.44 (1H, m, H-9), 1.18 (2H, m, H-11), 1.49 (2H, m, H-12), 1.73 (2H, m, H-15), 1.91 (1H, m, H-16A), 1.43 (1H, m, H-16B), 1.48 (1H, d, J = 7.1 Hz, H-18), 1.15 (1H, m, H-19), 1.42 (1H, m, H-20), 1.88 (1H, m, H-21A), 1.21 (1H, m, H-21B), 1.76 (1H, m, H-22A), 1.43 (1H, m, H-22B), 1.08 (3H, s, H-23), 1.03 (3H, s, H-24), 1.00 (3H, s, H-25), 1.11 (3H, s, H-26), 1.24 (3H, s, H-27), 1.03 (3H, s, H-28), 0.94 (3H, d, J = 6.8 Hz, H-29), 0.93 (3H, d, J = 6.8 Hz, H-30). ¹³C NMR (CDCl₃, 125 MHz): δC 39.5 (C-1), 34.1 (C-2), 217.8 (C-3), 47.3 (C-4), 54.8 (C-5), 19.6 (C-6), 35.6 (C-7), 36.0 (C-8), 46.9 (C-9), 36.7 (C-10), 21.9 (C-11), 33.5 (C-12), 75.1 (C-13), 43.2 (C-14), 26.2 (C-15), 35.7 (C-16), 42.6 (C-17), 49.4 (C-18), 41.3 (C-19), 40.9 (C-20), 28.0 (C-21), 40.9 (C-22), 26.7 (C-23), 14.7 (C-24), 15.0 (C-25), 15.8 (C-26), 24.7 (C-27), 21.1 (C-28), 16.0 (C-29), 19.9 (C-30). HR-EI-MS: m/z 442.3821 [M]+ (calc 442.3811, C₃₀H₅₀O₂).

Klodorol A (4): Colourless needles; m.p. 99–100°C; [α]₂⁵° +24.3 (c = 0.1, CHCl₃); IR γmax (KBr): 3462, 2925, 1614, 1070 cm⁻¹. ¹H NMR (CD₃OD, 500 MHz): δH 2.52 (2H, q, J = 7.5 Hz, H-1), 1.64 (1H, m, H-2A), 1.54 (1H, m, H-2B), 3.35 (1H, dd, J = 6.5, 3.5 Hz, H-3), 1.63 (1H, m, H-6A), 1.45 (1H, m, H-6B), 1.29 (1H, m, H-7A), 1.01 (1H, m, H-7B), 1.48 (1H, m, H-8), 1.65 (2H, m, H-11), 1.61 (1H, m, H-12A), 1.52 (1H, m, H-12B), 1.54 (1H, m, H-15A), 1.03 (1H, m, H-15B), 1.49 (1H, m, H-16A), 1.25 (1H, m, H-16B), 1.89 (1H, d, J = 6.8 Hz, H-18), 1.90 (1H, m, H-19A), 1.56 (1H, m, H-19B), 1.74 (1H, m, H-21A), 1.32 (1H, m, H-21B), 1.16 (3H, s, H-23), 1.18 (3H, s, H-24), 0.99 (3H, s, H-25), 0.95 (3H, s, H-26), 0.96 (3H, s, H-27), 1.16 (3H, s, H-28), 1.10 (3H, s, H-29), 1.06 (3H, s, H-30). ¹³C NMR
(CD$_3$OD, 125 MHz): $\delta_C$ 32.5 (C-1), 26.8 (C-2), 84.8 (C-3), 51.1 (C-4), 72.8 (C-5), 20.7 (C-6), 23.2 (C-7), 56.5 (C-8), 44.3 (C-9), 87.8 (C-10), 35.8 (C-11), 26.3 (C-12), 41.0 (C-13), 41.0 (C-14), 35.0 (C-15), 36.5 (C-16), 41.5 (C-17), 51.4 (C-18), 38.0 (C-19), 27.3 (C-20), 32.5 (C-21), 38.0 (C-22), 28.4 (C-23), 25.4 (C-24), 16.6 (C-25), 16.8 (C-26), 15.6 (C-27), 23.6 (C-28), 27.4 (C-29), 21.4 (C-30). HR-EI-MS: $m/z$ 442.3815 [M]$^+$ (calc 442.3811, C$_{30}$H$_{50}$O$_2$).

4. Conclusion

In conclusion, investigation of the aerial parts of *K. odora* afforded two new and two known compounds. Their structures were established by different spectroscopic analyses.

**Supplementary material**

Supplementary material relating to this article is available online, alongside Figures S1–S13.

**Acknowledgements**

This research was supported by a grant from the ‘Research Center of the Female Scientific and Medical Colleges’, Deanship of Scientific Research, King Saud University.

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