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The major diterpenoids of the genus *Arctopus* (Apiaceae) with notes on their chemotaxonomic and medicinal significance

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ABSTRACT

The main diterpenoids in the roots of *Arctopus* (Apiaceae, tribe Saniculeae) have been identified for the first time. The major compounds are manool, *ent*-trachyloban-19-oic acid and a kauren-19-oic acid, while methyl-16β-hydroxy-*ent*-kaur-11-en-19-oate is a minor diterpene. Comparative studies of non-polar extracts by means of LC–MS showed that the isolated main compounds are present in all three species of *Arctopus*. The study also revealed that the diterpenoid pattern in *Arctopus* is very similar to that of the genus *Alepidea*. The tuberous roots of *Arctopus* and the rhizomes and roots of *Alepidea* are important traditional medicines in South Africa, used mainly for infections and respiratory ailments. Their efficacy is ascribed to antimicrobial and anti-inflammatory activity. The known biological activities of the isolated diterpenes provide a scientific rationale for the traditional uses. The chemical similarity in diterpenoids also supports the idea that the two genera are closely related, despite their conspicuous morphological differences.

1. Introduction

Tubers of the three species of *Arctopus* L. (*sieketroost* or *platdoorn* in Dutch) are well known as important Khoi-San and Cape Dutch traditional medicines and general tonics, used for a wide range of ailments that includes venereal diseases and respiratory ailments. The recorded medicinal properties of *Arctopus* (Van Wyk and Gericke, 2000; Magee et al., 2007; Van Wyk et al., 2009) include antimicrobial and anti-inflammatory activity against infections such as gonorrhoea, syphilis, lepra and elephantiasis. It is also used against tuberculosis and bladder ailments and is considered demulcent and diuretic. Topically, the resin is used as treatment for ringworm, while decoctions are used as washes and dressings for ulcers and blotches. Furthermore, decoctions of the root are reportedly used to treat epilepsy and have sedative properties (Stafford et al., 2005).

Species of *Alepidea* F. Delaroche are popular traditional medicines in South Africa (De Castro and Van Wyk, 1994; Stafford et al., 2005; Van Wyk et al., 2009). Rhizomes of *Alepidea amatymbica* Eckl. & Zeyh., *Alepidia cordifolia* B.-E.van Wyk (both known as *ikhathazo* in Zulu) and other species are widely used to treat respiratory ailments during the winter period and are commonly sold at traditional medicine markets (De Castro and Van Wyk, 1994; Hutchings et al., 1996). Bioassays of the roots of *Alepidea* revealed antihypertensive, antimicrobial and diuretic properties (Hutchings, 1989; Somova et al., 2001).

Molecular systematic studies have shown that *Arctopus* and *Alepidea* are related and are now formally classified in the tribe Saniculeae of the Apiaceae (Calviño and Downie, 2007; Magee et al., 2010). This relationship was confirmed by the discovery of phenolic acids [(*R*)-3′-*O*-β-D-glucopyranosylrosmarinic acid and its aglycone, rosmarinic acid, together with caffeic acid] in *Arctopus* and *Alepidea* species (Olivier et al., 2008), several species of *Eryngium* L. and *Sanicula europaea* L. (Le Claire et al., 2005).

The presence of diterpenes in *Alepidea* was reported by Rustaiyan and Sadjadi (1987), Holtzapfel et al. (1995) and Somova et al. (2001). Comparative TLC and GC–MS studies showed that the non-polar terpenoids of *Alepidea* species were almost identical to that of the genus *Arctopus* (Van Wyk et al., 2009), but the identities of the *Arctopus* compounds have not yet been determined by spectroscopic methods.

In view of the putative close relation between the two genera and the fact that both are important in traditional medicine, the aim of this paper is to report on the isolation and identification of the main diterpenes of *Arctopus* and to compare the diterpenoid pattern in this genus with that of *Alepidea*.

2. Materials and methods

2.1. General procedures

TLC analysis of the non-polar fractions was performed with silica gel $60F_{254}$ plates (Merck) and detected with 5% ethanolic sulfuric acid and 1% ethanolic vanillin (heated to 100 °C). Kieselgel GF_{254} (15 μm, Merck)

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was used for flash chromatography. Chemicals used for extraction, TLC and isolation were obtained from Merck as EtOH (96%), sulphuric acid (AR), vanillin (AR), MeOH (GR), $CHCl_3$ (CP), CH_2Cl_2 (CP), hexane (CP) and diethyl ether (CP). CP grade solvents and water were distilled before use.

LC–MS quantitative analyses were conducted with a Waters API Quatro Micro instrument. A Waters SunFire column (C18 3.5 μm, 4.6×150 mm, with SunFire C18 3.5 μm, 4.6×20 mm guard cartridge) was used for the separation of the components by using gradient elution: 70–100% of solvent B (solvent A is 2% formic acid, 10 mM ammonium formate; solvent B is acetonitrile) over a period of 50 min at a flow rate of 1 mL/min at 35 °C. An APCI MS source was used in positive mode (settings included Corona voltage: 7 V; cone voltage: 20; RFI: 40; source: 120 °C; desolvation temperature: 450 °C; desolvation gas: 55 L/h; cone gas: 50 L/h). The formic acid (98–100% pure, Suprapur®), water (Ultrapur®) and acetonitrile (hypergrade for liquid chromatography, LiChrosolv ®) used was obtained from Merck, and the ammonium formate (97% pure) from Sigma–Aldrich.

Accurate masses of the isolated compounds were obtained by means of a Waters SYNAPT HDMS system with a QTOF design equipped with a lockspray interface to improve mass accuracy. A Waters Acquity BEH C18 UPLC column (C18 1.7 μm, 2.1×100 mm) was used in all experiments. The column was kept at 40 °C. The chromatographic procedure entailed 0.1 min of 60% MeOH in water (v/v), followed by a 6 min linear gradient to 90% MeOH in water (v/v), kept for 1 min before a 1 min linear gradient back to the starting conditions (60% MeOH in water) followed by 2 min equilibration. The flow rate was kept constant at 0.3 mL/min and the total analysis time was 10 min. A PDA detector was placed in tandem before the mass detector and was programmed to scan 200–500 nm with a scan rate of 20 spectra per second and a resolution of 1.2 nm. Ionisation of all the compounds was achieved with chemical ionisation in positive mode with the following source settings: capillary voltage of 2.5 kV, sample cone voltage of 20 V, extraction cone voltage of 4 V and carona pin voltage to 20 V. The source temperature was set at 120 °C, the desolvation temperature at 350 °C and the desolvation gas flow at 500 L/hr (nitrogen gas). Leucine enkephalin was used as a lockmass calibrant at 50 pg/mL and was continuously infused at 10 μL/min. Sampling of the lockmass signal was done every 30 s. Data collection was done using Waters MassLynx software (version 4.1). All empirical formulae calculations were done using the embedded software tools. The MeOH (for liquid chromatography, LiChrosolv®) were obtained from Merck while the leucine encephalin (≥97%, HPLC grade) was obtained from Sigma-Aldrich.

^{1}H NMR and ^{13}C NMR experiments were performed on a Bruker Avance 300 spectrometer operating at 300 MHz (^{1}H) and 75 MHz (^{13}C) in $CDCl_3$ (Uvasol®, obtained from Merck), with the solvent used as internal standard in both cases. The 2D experiments (COSY, HSQC, HMBC, NOESY and DEPT) were done on the same instrument at 300 MHz for ^{1}H and 75 MHz for ^{13}C spectra using the same solvent.

2.2. Plant material

Samples of tuberous roots (*Arctopus*) or rhizomes with roots (*Alepidea*) were collected by A.R. Magee (ARM), B.-E. Van Wyk (BEVW), J.S. Boatwright (JSB) and P.J.D. Winter (PW) and are listed in Table 1, together with their provenances (all in South Africa) and author citations (not given from here on). Voucher specimens were deposited in the herbarium of the Department of Botany and Plant Biotechnology at the University of Johannesburg (JRAU).

2.3. Diterpenoid variation study by LC–MS and TLC

Dry ground plant material (0.3 g) of all species (samples A, B, C and D) were extracted in 3 ml $CHCl_3$:MeOH (1:1) overnight, where after the mixtures were filtered and the filtrates evaporated to dryness. The dry extracts were reconstituted in 1 mL of MeOH, of which 5 μL was injected into the Waters API Quatro Micro LC–MS. The samples were diluted 10 times if it was found that the initial preparation was too concentrated. The results are given in Table 2.

Table 1
Provenances, voucher specimens and sample numbers of the rhizome and root material of *Arctopus* and *Alepidea* species used for extraction and analysis. The relative yields of non-polar compounds are also given. For abbreviations of collectors see text.

Species and voucher specimens	Sample reference number	Locality or provenance	% yield (g/g dry weight)
Arctopus echinatus L. – PW & BEVW 170	A	Du Toit's Kloof Pass	11.8
Arctopus monacanthus Sond. – BEVW 4141(a)	B	Citrusdal, Elandskloof	21.6
Arctopus dregei Sond. – ARM & JSB 31	C	Malmesbury	8.9
Alepidea cordifolia B.-E.Van Wyk – PW 252	D	Mphendle	21.6

The presence of the diterpenoids were confirmed by TLC in hexane/ether (2:3) followed by visualization with vanillin/EtOH and H_2SO_4/EtOH.

2.4. Isolation and identification of compounds **1**, **2** and **3**

Compounds **1** (55.2 mg) and **2** (151.9 mg) (Fig. 1) were isolated by extracting 24.6 g of dry ground root material of *A. monacanthus* (sample B) overnight in 250 mL MeOH. The mixture was filtered and the volume of the filtrate reduced under vacuum to yield a viscous oily mixture. A phase separation was done by rinsing the extract with 50% MeOH/H_2O to dissolve and consequently remove the polar compounds leaving only the viscous non-polar mixture containing the diterpenes. Flash column chromatography with gradient elution [100% hexane, increasing the diethyl ether component incrementally with addition of 5% diethyl ether until 100% diether ether was reached, each increment being 100 mL of mixed solvent] was applied for separation. Fractions of 20 mL were collected. Fraction 5 of the first separation yielded a pure sample of compound **1**. Repeated flash column chromatography of fraction 6 with a hexane:diethyl ether mixture (3:2) afforded a purified sample of compound **2**. Both compounds **1** and **2** are major compounds in this non-polar fraction and co-eluted with a third major compound (18.4 mg) which could not be isolated successfully, i.e. TLC analysis shows these three compounds as poorly resolved blue and violet zones with similar R_f-values. Later preliminary LC–MS and NMR spectroscopic analyses showed that the impure compound could be similar to *ent*-kaur-16-en-19-oic acid (compound **4** in Fig. 1). Compound **3** was obtained from fraction 8 of the first separation and purified by means of preparative TLC in hexane:diethyl ether (3:2).

The pure compounds **1**, **2** and **3**, as well as the impure major compound mentioned above, were subjected to 1D and 2D ^{1}H NMR and ^{13}C NMR spectroscopic experiments including COSY, DEPT, NOESY, HMBC and HSQC, in order to elucidate the (3D) structures. The relevant spectroscopic data of compounds **1**, **2** and **3** are summarized in the results section below and NMR data for all three compounds is available as electronic supplementary material. The accurate masses of compounds **1**, **2** and **3** were determined by means of the Waters SYNAPT HDMS system. These isolated compounds together with the unidentified kauren-19-oic acid were used for quantification during LC–MS analyses of the three *Arctopus* species and *Alepidea cordifolia* using a Waters API Quatro Micro instrument (Table 2).

3. Results

3.1. Variation, isolation and identification of diterpenoids

All four species studied yielded relatively large amounts of non-polar compounds (Table 1) with compounds **1** and **2** being two of the major

Table 2
A quantitative comparison of isolated major compounds in all three species of *Arctopus* and *Alepidea cordifolia* as performed by LC–MS (without derivatisation, single measurements) using known amounts of isolated compounds, calculated as % (g/g dry extract × 100).

Compound	LC–MS Rt (min)[a]	*Arctopus echinatus*	*Arctopus monacanthus*	*Arctopus dregei*	*Alepidea cordifolia*
		Sample A (%)	Sample B (%)	Sample C (%)	Sample D (%)
Manool (**1**)	34.13	–	2.74	16.27	1.38
Kauren-19-oic acid	16.55	0.96	7.74	2.66	4.47
Ent-trachyloban-19-oic acid (**2**)	21.77	3.13	–	24.27	29.57
Methyl-16β-hydroxy-*ent*-kaur-11-en-19-oate (**3**)	9.19	0.06	0.63	0.77	–

[a] See Section 3.1 for ionization products and mass data.

compounds. A few minor diterpenes such as compound **3** were also detected (through TLC and LC–MS studies), indicating similarity amongst *Arctopus* species but marked differences from the *Alepidea* sample.

The three major diterpenes reported here were identified as manool (**1**), *ent*-trachyloban-19-oic acid (**2**) and a kauren-19-oic acid (similar to compound **4**, Fig. 1) through 1D and 2D NMR spectroscopical analyses and accurate mass determination by HR–APCI–UPLC–MS, while methyl-16β-hydroxy-*ent*-kaur-11-en-19-oate (**3**) is a minor kaurenoic acid ester derivative.

Manool (**1**) is a well-known labdane-type diterpene distinguished from its enantiomer, 13-epimanool, by NMR: the signal of H-17a shows a shift at δ_H 4.46 for manool, and at δ_H 4.50 for 13-epimanool (Barrero et al., 1993). The value obtained from our analysis showed a shift at δ_H 4.44 for H-17a, indicating that we probably isolated manool. The other ^{1}H and ^{13}C NMR data of **1** is in agreement with those in literature for manool (Rowe and Scroggins, 1964; Barrero et al., 1993; Lu et al., 1995). HR–APCI–UPLC–MS analysis produced a molecular ion peak at *m/z* 273.2571 suggesting a molecular formula of $C_{20}H_{33}$, indicative of the loss of the hydroxyl group, but confirming unsaturation for the compound. The spectral data obtained for compound **2** confirmed its identity and corresponded well with those reported for *ent*-trachyloban-19-oic acid (Mitscher et al., 1983; Leong and Harrison, 1997; Takahashi et al., 2001; Morris et al., 2005). An HMBC experiment of the third unknown major diterpene revealed 20 carbons, three being tertiary methyl carbons, with other significant features such as a carbonyl carbon (C-19) and several sp^2-hybridized carbons, mostly corresponding with that of a kauren-19-oic acid such as *ent*-kaur-16-en-19-oic acid (**4**) (Mitscher et al., 1983; Holtzapfel et al., 1995; Morris et al., 2005; Batista et al., 2007). Compound **3** is an ester derivative showing the presence of 21 carbons, where the carbonyl carbon (C-19) showed a long-range coupling in the HMBC with the methyl protons of C-18 and the methoxy-protons on C-21. The DEPT spectrum for **3** showed two monoprotonated sp^2 carbons (δ_C 127.0 and 132.5), with coupling constants ($\delta_{H\text{-}11}$ 5.50, *J* 8.8 and $\delta_{H\text{-}12}$ 5.88, *J* 8.9) indicating a *cis*-orientation. A long-range HMBC coupling was also observed between C-9 and H-11, with further COSY correlation between H-9 and H-11, H-11 and H-12 and between H-12 and H-13 clarifying the position of the double bond. C-16 was deshielded (δ_C 83.8), indicative of oxygenation. The signal for C-17 (25.8 ppm) corresponds to the literature value for that of a β-epimer (Croft et al., 1974; St. Pyrek, 1984). The identity of **3** could thus be established as methyl-16β-hydroxy-*ent*-kaur-11-en-19-oate (Mitscher et al., 1983; Leong and Harrison, 1997; Takahashi et al., 2001; Morris et al., 2005; Batista et al., 2007).

The LC–MS study (Table 2) was done to quantify the isolated compounds in the three *Arctopus* species and *A. cordifolia and* to compare the two genera chemotaxonomically. Large quantities of manool (**1**), *ent*-trachyloban-19-oic acid (**2**) and the kaurene-19-oic acid were detected, but may not necessarily be present in all the *Arctopus* species simultaneously. The kauren-19-oic acid was present in all four samples. Furthermore, the results showed that all three *Arctopus* species contain small amounts of the minor kaurene ester derivative (**3**), while it is absent in *A. cordifolia*.

3.1.1. Manool (**1**)

Colourless crystalline solid; ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$): see electronic supplementary material. Positive APCI–VTOF–MS: $m/z = 273.2571$ $[M+H^+-H_2O]^+$ (calcd for $C_{20}H_{33}$: 273.2582).

3.1.2. Ent-trachyloban-19-oic acid (**2**)

Colourless crystalline solid; ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$): see electronic supplementary material. Positive APCI–VTOF–MS: $m/z = 303.2299$ $[M+H]^+$ (calcd for $C_{20}H_{31}O_2$: 303.2324).

3.1.3. Impure kauren-19-oic acid

Colourless crystalline solid; positive APCI–VTOF–MS: $m/z = 301.2121$ $[M+H]^+$ (calcd for $C_{20}H_{29}O_2$: 301.2168).

3.1.4. Methyl-16β-hydroxy-ent-kaur-11-en-19-oate (**3**)

Colourless crystalline solid; ^{1}H NMR ($CDCl_3$) δ: 0.70 (3H, s), 0.99 (2H, m), 1.07 (1H, br d, *J* = 8.2), 1.15 (3H, s), 1.29 (3H, s), 1.39 (1H, d, *J* = 3.0), 1.41 (1H, m), 1.47 (3H, m), 1.58 (1H, dd, *J* = 12.2, 5.8), 1.73 (3H, m), 1.82 (1H, m), 1.83 (1H, dd, *J* = 5.8, 3.0), 1.85 (1H, dd, *J* = 12.4, 3.0), 2.15 (1H, m), 2.18 (1H, dd, *J* 6.1, 2.9), 3.60 (3H, s), 5.50 (1H, dd, *J* = 8.8, 2.9), 5.88 (1H, t, *J* = 8.9). ^{13}C NMR ($CDCl_3$) δ: 15.3 (q), 19.0 (t), 21.8 (t), 25.8 (q), 28.7 (q), 34.4 (t), 38.0 (t), 38.6 (s), 40.0 (t), 41.3 (t), 43.2 (s),43.9 (s),50.1 (d), 51.2 (q), 55.8 (d), 58.6 (t), 61.2 (d), 83.8 (s), 127.0 (d), 132.5 (d), 177.9 (s). Positive APCI-VTOF-MS: $m/z = 315.2317$ $[M+H^+-H_2O]^+$ (calcd for $C_{21}H_{31}O_2$: 315.2324).

4. Discussion

4.1. Chemosystematic significance of diterpenes identified

In previous studies it was suggested that (*R*)-3′-*O*-β-D-glucopyranosylrosmarinic acid may be a chemosystematic marker

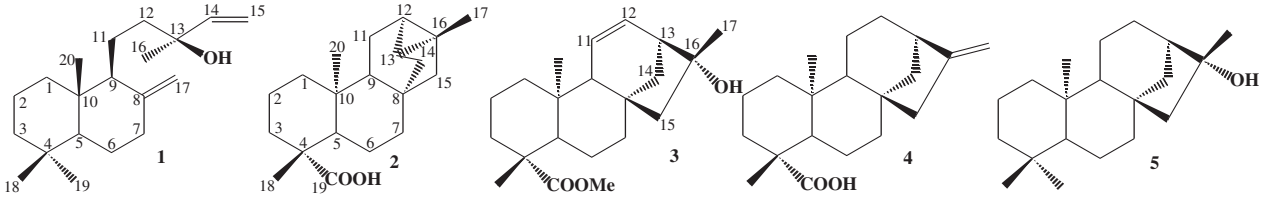

Fig. 1. Structures of isolated diterpenes: **1**, manool; **2**, *ent*-trachyloban-19-oic acid; **3**, methyl-16β-hydroxy-*ent*-kaur-11-en-19-oate. Important structures mentioned in the text: **4**, *ent*-kaur-16-en-19-oic acid; **5**, *ent*-kauran-16-ol.

compound for the tribe Saniculeae of the Apiaceae (including *Arctopus*, *Alepidea*, *Eryngium* L. and *Sanicula* L.) (Le Claire et al., 2005; Olivier et al., 2008). The pattern of main diterpenoids shown in Table 2 indicates that *Arctopus* and *Alepidea*, despite their obvious morphological differences, are indeed chemically closely related. Labdane-type (i.e. manool, **1**) and pimarane-type diterpenes, (i.e. compounds **2**, **3** and the kauranes) (Otto and Wilde, 2001) are widely distributed in the Plant Kingdom [including the Apiaceae, e.g. *Elaeoselinum* W.D.J.Koch ex DC. – see Mongelli et al. (2002)], but have not yet been reported in the Saniculeae except for *Alepidea* and *Arctopus* (Rustaiyan and Sadjadi, 1987; Holtzapfel et al., 1995; Somova et al., 2001). A wider survey of other taxa is therefore desirable, especially since *Alepidea* and *Arctopus* are successively sister (in a cladistic sense) to the rest of the tribe, which includes *Actinolema* Fenzl, *Astrantia* L., *Hacquetia* Neck. ex DC., *Sanicula*, *Eryngium* and *Petagnaea* Caruel (Magee et al., 2010). Some *Eryngium* species, i.e. *E. bourgatii* Gouan and *E. glaciale* Boiss. contain phyllocladene-type tetracyclic diterpenes (Palá-Paúl et al., 2005a,b) but these are derived from isopimaranes (Otto and Wilde, 2001).

4.2. Medicinal significance of diterpenoids

Manool (**1**) is a known resinous substance (Patricio et al., 2002) and has been found to exhibit antibacterial activity against *Staphylococcus aureus* and *Stenotrophomonas maltophilia,* as well as antifungal activity against *Candida albicans* (Ulubelen et al., 1994; Topçu and Gören, 2007; Ugur et al., 2010). Furthermore manool showed potential anti-inflammatory activity (Li et al., 2009; Yang et al., 2010) as well as cytostatic activities against human malignant cell strains (Pratsinis et al., 2010).

The antimicrobial activity of *ent*-trachyloban-19-oic acid (**2**) against *S. aureus* and *Mycobacterium smegmatis* is comparable to that of *ent*-kaur-16-en-19-oic acid (compound **4** in Fig. 1, similar to the unidentified major kauren-19-oic acid in *Arctopus*), another major diterpene identified in *Alepidea* species (Holtzapfel et al., 1995). It would thus be worthwhile to assess both these compounds as anti-tubercular drugs (Mitscher et al., 1983; Zgoda-Pols et al., 2002). Compound **2** also displayed *in vivo* anti-inflammatory activity (Días-Viciedo et al., 2008). *Ent*-trachyloban-19-oic acid (**2**), *ent*-kaur-16-en-19-oic acid (**4**) and kauran-16-ol (**5**) [a hydroxylated kaurene from *Alepidea* (Holtzapfel et al., 1995), see Fig. 1] exhibit trypanomicidal activity (Batista et al., 2007). *Alepidea amatymbica* extracts and the diterpenes isolated from it [including *ent*-trachyloban-19-oic acid (**2**) and *ent*-kaur-16-en-19-oic acid (**4**)] display substantial hypotensive effects, decreased heart rate and showed diuretic activity (Somova et al., 2001).

Ent-kaur-16-en-19-oic acid (**4**) has anti-inflammatory, antibacterial (against *S. aureus*, *M. smegmatis, Bacillus subtilis* and *Escherichia coli*), antifungal (against *Saccharomyces cerevisiae, Cladosporium herbarum* and *C. albicans*) and molluscicidal properties (Ghisalberti, 1997; García et al., 2007; Ambrosio et al., 2008). It also has analgesic properties, is able to induce hyperthermia and inhibits vascular smooth muscle contractility (Ambrosio et al., 2006). The C-19 carboxyl group is reportedly responsible for the inhibitory ability and methylation of this group reduces but does not abolish the antispasmodic activity (Ambrosio et al., 2004). Lastly, this compound displays anti-proliferative action in tumor cells (human breast cancer, human colon cancer and leukemia cancer) (Fatope and Audu, 1996; Ambrosio et al., 2006).

The phenolic acids of *Arctopus* and *Alepidea* (rosmarinic acid and its glucoside) contribute to antioxidant, antiphlogistic, astringent, anti-inflammatory, antimutagenic, antibacterial and antiviral activities (Olivier et al., 2008). It is now clear that the diterpenes also contribute to the medicinal value of the two traditional medicines. The diversity of biological activities demonstrated for the main compounds in *Arctopus* and *Alepidea* supports the traditional notion that the two genera are important tonic plants (Van Wyk and Gericke, 2000).

5. Conclusions

The discovery of several classes of diterpenes in *Arctopus* (and *Alepidea*) adds a new dimension to our understanding of the medicinal value of these important traditional medicines. *Arctopus* has remained chemically poorly known despite its importance in traditional medicine. The medicinal uses of *Arctopus* in Cape Herbal Medicine are in agreement with those of *Alepidea* in Sotho and Zulu traditional medicine, and these now appear to be due to the same main chemical compounds.

The similar patterns of major diterpenes in *Arctopus* and *Alepidea* support the idea of a close relationship, despite marked morphological differences. The systematic position of *Arctopus* within the Apiaceae has remained uncertain for a long time due to several of its morphological features being unusual (Pimenov and Leonov, 1993; Magee et al., 2008). The chemical similarity reported here supports the findings of recent molecular studies (e.g. Magee et al., 2008, 2010 and references cited therein) and the genus is now firmly placed within a monophyletic Saniculeae. Since *Arctopus* and *Alepidea* are not monophyletic in a cladistic sense but successively sister group to the tribe Saniculeae (Magee et al., 2010), a wider survey of other genera (and indeed other tribes of Apiaceae) will undoubtedly yield interesting results. From a wider quantitative variation study where more samples of *Arctopus* and *Alepidea* species are included, it could also be established whether the major diterpenes may be applicable as phytochemical characters on a species level.

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