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A Chemotaxonomic Survey of Kaurene Derivatives in the Genus *Alepidea* (Apiaceae)

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Key Word Index—*Alepidea*; Apiaceae; diterpenoids; kaurene derivatives; kaurenoic lactones; chemotaxonomy.

Abstract—Lipophilic extracts from the roots of 16 species of *Alepidea* were studied by GC–MS for the presence of kaurenoic acids, dehydrokaurenoic acids, kaurenic lactones, hydroxykaurenoic acids and other kaurene derivatives. Various mixtures of these compounds are present in yields of up to 11.8% dry wt. in the rhizomes and roots. Two different isomers of kaurenoic acid occur in *Alepidea*, the one as a major compound in all the species, the other as a major compound only in some of them. The first isomer is clearly a useful chemical marker for the genus *Alepidea*, but the apparent chemical dichotomy is not obviously correlated with morphological patterns. *Alepidea amatymbica*, however, is part of a morphologically isolated group within the genus and also differs from other species in its chemical complexity and in the unique presence of kaurenoic lactones.

Introduction

Alepidea (Apiaceae, subfamily Saniculoideae) is a genus of *ca* 26 species of herbaceous geophytes endemic to grassland areas of eastern and southern Africa (Cannon, 1978; Townsend, 1989; Burtt, 1991). The rhizomes and roots of *A. amatymbica* and other species are used in some parts of South Africa as a traditional medicine (known as Ikhathazo), for the treatment of colds and chest complaints (Watt and Breyer-Brandwijk, 1962). A bioassay revealed antihypertensive, antimicrobial and diuretic properties (Hutchings, 1989). Several kaurene derivatives have been identified from the above-ground parts of *A. amatymbica* (as *A. amatynsia*) (Rustaiyan and Sadjadi, 1987), but the actual active constituents have never been isolated and tested. The distribution and chemosystematic value of kaurene derivatives in the genus *Alepidea* are reported here for the first time, and we give a preliminary evaluation of these compounds as potential taxonomic characters.

Materials and Methods

Plant materials. Samples of roots and fruits were collected *in situ* or from cultivated plants (voucher specimens are all in JRAU; AdC=*A. de Castro*): *A. amatymbica* Eckl. & Zeyh.: sample 1, *AdC 143*; sample 2, *AdC 185*; *A. capensis* (Berg.) R. A. Dyer: *AdC 251*; *A. comosa* Dümmer: sample 1, *AdC 178*; sample 2, *AdC 192*; *A. galpinii* Dümmer: sample 1, *AdC 210*; sample 2, *AdC 224*; *A. insculpta* Hilliard & Burtt: *AdC 203*; *A. longifolia* Dümmer: *AdC 239*; *A. natalensis* Wood & Evans: *AdC 186*; *A. serrata* Eckl. & Zeyh.: sample 1, *AdC 261*; sample 2, *AdC 265*; *A. setifera* N. E. Br.: *AdC 142*; *A. thodei* Dümmer: *AdC 211*; *A. woodii* Oliv. in Hook.: sample 1, *AdC 206*; sample 2, *AdC 225*.

Procedures. Fresh roots were rapidly air-dried, ground to a fine powder and extracted with CH_2Cl_2 (2 × 50 ml). The extracts were filtered through celite and the solvent evaporated under reduced pressure. The extracts were derivatized by treatment with diazomethane and were analysed both before and after esterification. Samples were dissolved in a fixed volume of $CHCl_3$ and studied by GC–MS. Mass spectra (mass

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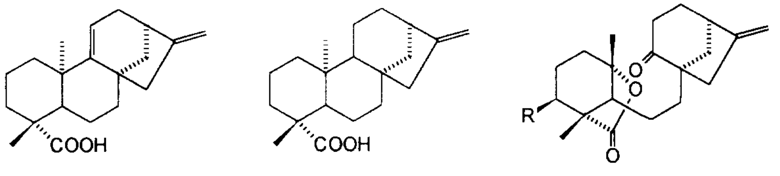

1a

2a

3, R = H

4, R = OCOMe

FIG. 1. STRUCTURES OF THE KAURENE DERIVATIVES 1–4 AS DETAILED BELOW.

values higher than 60) were recorded on a Saturn 4 mass spectrometer. Retention times (R_t) given in mins. We used a DB-1 fused silica capillary column (30 m × 0.25 mm i.d.; He as carrier gas at 1 ml min^{-1}; column temperature 200° to 300° at 7° min^{-1}, 0.8 min isotherm; injector 150°C; EI and CI detection at 150°C; transfer line 260°C; filament emission was 5 μA for both EI and CI; ionization voltage 50 eV; split ratio 10:1; injection volume 0.5 + 0.5 μl solvent plug). All the major compounds were identified by their GC retention times (R_t) as well as a comparison of their EI and CI mass spectra. The structural types of unknown compounds were tentatively assigned on the basis of their mass spectra (molecular weights and fragmentation patterns). EI mass spectral data of all the major kaurene derivatives detected in *Alepidea* are recorded below.

Dehydrokaurenoic acids (as methyl esters)—**1a** (R_t 7.53), *m/z* 314 (18), 299 (100), 282 (4), 267 (7), 255 (28), 239 (55); **1b** (R_t 7.71), *m/z* 314 (100), 299 (94), 282 (12), 267 (71), 255 (34), 239 (38); **1c** (R_t 8.05), *m/z* 314 (100), 299 (30), 282 (36), 267 (71), 255 (40), 239 (45); **1d** (R_t 8.28), *m/z* 314 (100), 299 (26), 282 (10), 267 (5), 255 (35), 239 (20). **Kaurenoic acids** (as methyl esters)—**2a** (R_t 7.98), *m/z* 316 (100), 301 (35), 284 (8), 273 (13), 257 (51), 241 (21); **2b** (R_t 8.45), *m/z* 316 (100), 301 (33), 284 (6), 273 (63), 257 (100), 241 (18); **2c** (R_t 7.45), *m/z* 316 (100), 301 (15), 284 (9), 273 (12), 257 (24), 241 (16); **2d** (R_t 8.37), *m/z* 316 (37), 301 (80), 284 (41), 273 (12), 257 (100), 241 (48). **Lactone 3**—(R_t 10.82), *m/z* 316 (18), 298 (38), 271 (44), 270 (42), 254 (100), 239 (15). **Acetoxy lactone 4**—(R_t 10.85), *m/z* 374 (4), 314 (100), 299 (40), 296 (44), 270 (32). **Hydroxykaurenoic acids** (as methyl esters)—**5a** (R_t 9.90), *m/z* 332 (5), 314 (100), 299 (40), 282 (61), 255 (42), 239 (17); **5b** (R_t 10.14), *m/z* 332 (14), 314 (100), 300 (14), 299 (13), 282 (18), 255 (55), 239 (20). **Kaurene hydrate 6**—(R_t 7.85), *m/z* 290 (5), 275 (6), 272 (10), 257 (100), 244 (7), 229 (10). **Kaurenes**—**7a** (R_t 5.90), 272 (38), 257 (100), 244 (76), 229 (12); **7b** (R_t 6.28), 272 (7), 257 (100), 244 (4), 229 (7).

Results

Lipophilic extracts of the powdered dried rhizomes of *A. amatymbica* collected in different localities were analysed. This confirmed the presence of *ent*-9,(11)-dehydro-16-kauren-19-oic acid (**1a**), *ent*-16-kauren-19-oic acid (**2a**), wedelia *seco*-kaurenolide (**3**) and (**4**), the 3β-acetoxy derivative of **3**, previously reported as constituents of the roots and aerial parts of *A. amatymbica* (Rustaiyan and Sadjadi, 1987). The extracts contained additional kaurene derivatives not previously reported.

A comparative study of dichloromethane extracts of the roots of several *Alepidea* species showed the presence of kaurene derivatives in every case. The distribution of all the major compounds found in 11 species of *Alepidea* is summarised in Table 1.

Discussion

The overall pattern summarised in Table 1 is consistent with the following generalizations:

(1) the roots of all the species examined contained one or more kaurene derivatives. These diterpenoids are common in the plant kingdom, and have been detected in more than 26 families (Buckingham, 1994), including the Araliaceae (*Aralia*) and indeed also in two other genera of Apiaceae (*Distichoselinum* and *Elaeoselinum*, both of the Apioideae–Laserpitieae).

(2) Some of the species contained variable (possibly as a result of high volatility) amounts of two kaurene isomers [molecular weight (MW) = 272].

TABLE 1. DISTRIBUTION OF KAURENE DERIVATIVES IN 16 ROOT EXTRACTS FROM 11 SPECIES OF *ALEPIDEA*. The distribution of the compounds are given as percentages of total yield, as estimated from GC–MS results using peak area (total ion current). (— = not detected; + = present in small amounts only, i.e. 1% or less of total yield). Authorities for names and voucher specimens are given under Materials and Methods

	Dehydrokaurenoic acids				Kaurenoic acids				Lactones		Hydroxykaurenoic acids		Kaurene hydrate
Major compounds*:	**1a**	**1b**	**1c**	**1d**	**2a**	**2b**	**2c**	**2d**	**3**	**4**	**5a**	**5b**	**6**
A. amatymbica													
sample 1	11	—	—	3	46	23	—	—	2	11	—	4	—
sample 2	8	10	8	8	36	13	—	—	5	9	—	3	—
A. capensis	17	—	—	—	—	52	29	—	—	2	—	—	—
A. comosa													
sample 1	24	—	—	—	—	44	24	—	—	—	8	—	—
sample 2	18	—	—	—	6	29	32	—	—	—	15	—	—
A. galpinii													
sample 1	20	—	—	—	—	80	—	—	—	—	—	—	—
sample 2	+	—	—	—	13	79	—	—	—	—	—	—	8
A. insculpta	+	—	—	—	47	23	—	7	—	—	—	—	23
A. longifolia	+	—	5	6	53	24	10	—	—	+	2	—	—
A. natalensis	4	—	—	—	62	16	—	—	—	+	—	—	18
A. serrata													
sample 1	33	—	—	—	16	38	13	—	—	—	—	—	—
sample 2	30	—	—	—	—	53	17	—	—	+	—	—	—
A. setifera	16	—	—	—	—	49	35	—	—	—	—	—	—
A. thodei	—	—	—	—	87	13	—	—	—	—	—	—	—
A. woodii													
sample 1	—	—	—	—	—	100	—	—	—	—	—	—	—
sample 2	—	—	—	—	—	100	—	—	—	—	—	—	—

*Variable amounts of two isomeric kaurenes (**7a** and **7b**) were detected in most of the samples (see Materials and Methods).

(3) The differences in the composition of extracts from different localities of the same species were consistently smaller than that between species. All of the extracts contained either kaurenoic acids and/or dehydrokaurenoic acids. These derivatives of kaurene are relatively rare in nature and are known only from Fabaceae (*Copaifera* and *Trachylobium*), Cucurbitaceae (*Cucurbita*) and a few genera of Asteraceae (Bohlman, 1981; Buckingham, 1994). Within the family Apiaceae, these acids are clearly useful as chemical markers for the genus *Alepidea*.

(4) In addition to **1a**, the collection of species produced three compounds (MW = 300, MW of corresponding methyl ester = 314), which probably represent structural and/or stereoisomers of **1a**.

(5) In addition to **2a**, the collection of species produced three compounds (MW = 302, MW of corresponding methyl ester = 316) which probably represent structural and/or stereoisomers of **2a**.

(6) Some species produced compounds, the mass spectra of which correspond to hydrated kaurenes (MW = 250).

(7) Some species produced compounds, the mass spectra of which correspond to hydroxy-kaurenoic acids (MW = 302, MW of corresponding methyl ester = 332).

(8) The lactones 3 and 4 were detected only in the rhizomes and roots of *A. amatymbica*. Apart from this species, the lactone **3** is thus far known only from *Wedelia* (Bohlman, 1981) and its 15-hydroxy derivative from *Gnaphalium* (Bohlman, 1981). Compound **4**, the acetoxy lactone, appears to be uniquely associated with *A. amatymbica* and is a good marker for this species. We have also detected **4** as the major kaurene derivative in the fruits of *A. amatymbica*, but this compound was absent from the fruits of five other species. It is interesting to note that the total amount of kaurene derivatives extracted from *A. amatymbica* is markedly higher (up to 11.8% dry wt.) than in any of the other species investigated. Burtt (1982) proposed that only the section *Leiocarpae* Weimarck (smooth-fruited species, which includes *A. amatymbica*) should be given formal recognition at the sectional level. This suggestion seems to be supported by the distinctive kaurenes of *A. amatymbica*, but the other smooth-fruited species, *A. macowanii* Dümmer and *A. multisecta* B. L. Burtt, should be investigated to see if they are chemically similar to *A. amatymbica*.

The data confirms that each of the species produces at least one kaurene derivative. More specifically one compound, which appears to be an as yet structurally undefined kaurenoic acid, is present in every species. This kaurenoic acid is the main constituent in ten of the species. The known kaurenoic acid **1a** is the major constituent of six species. This apparent chemical dichotomy is not obviously correlated with presumed relationships based on morphological evidence such as inflorescence structure, leaf shape and the type and position of marginal setae. Some of the closely related species (e.g. *A. insculpta* and *A. natalensis*) have similar chemical profiles (even in minor compounds) but the taxonomic value of these patterns will be explored in future studies. In addition, we are presently engaged in the structure determination of the kaurenoic acids and dehydrokaurenoic acids not previously described in the literature and in establishing the biogenetic relationship between the various kaurene derivatives.

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