Terpenoids and Phenylethyl Esters from the Exudate of the Australian Plant *Ozothamnus ledifolius* (Asteraceae)

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The leaf exudate of *Ozothamnus ledifolius*, previously reported to contain a series of flavonoid aglycones, mostly consists of a mixture of terpenoids. From this resinous material we now identified four sesquiterpenes, a diterpenediol and two pentacyclic triterpene acids. In the phenolic portion we found three phenylethyl esters.

Introduction

In a previous publication, we dealt with rare flavonoids occurring in the leaf and stem exudates of 2 *Odixia* and 11 *Ozothamnus* species from Australia (Wollenweber *et al.*, 1997). We have now further analyzed the non-flavonoid major portion of the exudate of *Ozothamnus ledifolius*, a species that is exceptional in that its exudate contains two chalcones. Four sesquiterpenes, a diterpenediol and two triterpene acids as well as three isolated aromatics were identified by spectroscopic methods.

Material and Methods

Branches of *Ozothamnus ledifolius* were collected from two sites in Tasmania (collection data and voucher numbers see Wollenweber *et al.*, 1997). Air-dried plant material was briefly rinsed with acetone to dissolve the lipophilic exudates. Concentrated solutions were defatted and passed over Sephadex LH-20 to separate terpenoids from flavonoid aglcyones. Fractions **1–11** of coll.# Puttock 1305 were combined (2.1 g) and subjected to repeated column chromatography on "flash" Si-gel using binary mixtures of increasing polarity (from Hex-AcOEt 2:1 to CH_2Cl_2-MeOH 10:1). We thus obtained **1** (30 mg), **2** (13 mg), **3** (115 mg), **4** (95 mg), **5** (28 mg), **6** (34 mg), **7** (32 mg) and **8** (1.21 g).

Mass spectra were measured on a VG Autospec at 70 eV *via* solid probe. GC-MS was performed on a HP 5890. NMR spectra were recorded on a Bruker AC-300 (300/75.4 MHz) in $CDCl_3$ and Py-d_5 solutions. Multiplicities were assigned through DEPT experiments.

Bisabol-1-one (**2**): Colourless oil; MS and ^1H-NMR as in (Bohlmann *et al.,* 1976).

12-Hydroxybisabol-1-one (**3**): Colourless oil; MS and ^1H-NMR as in (Bohlmann *et al.,* 1981; Bohlmann *et al.,* 1983).

12-Acetoxybisabol-1-one (**4**): Colourless oil; MS and ^1H-NMR as in (Bohlmann *et al.,* 1981).

Betulinic acid (**5**): White powder, mp. 315–317 °C. EI-MS *m/z* (rel int.): 456 (M^+, 47%), 438 (12) 248 (12), 212 (53)203 (31), 189(100) and 175 (30). ^1H-NMR and ^{13}C-NMR as in (Lehn and Ourisson, 1962; Sholichin *et al.,* 1980).

Morolic acid (**6**): White powder, mp. 271–273 °C. EI-MS *m/z* (rel int.): 456 (M^+, 38%), 438 (5), 249 (22), 248 (94), 236 (42), 235 (36), 221 (17), 220 (19), 219 (19), 208 (21), 207 (100), 203 (69), 191 (25), 190 (60) and 189 (67). ^1H-NMR as in (Shamma *et al.,* 1962). ^{13}C-NMR ($CDCl_3$): λ ppm 14.92 (C-27), 15.39 (C-25), 16.00 (C-26), 16.64 (C-24), 18.20 (C-6), 20.88 (C-11), 25.99 (C-12), 27.32 (C-2), 27.93 (C-23), 29.05 (C-30), 29.39 (C-15), 30.36 (C-29), 32.04 (C-20), 33.35, 33.40, 33.55 (C-16, C-21, C-22), 34.54 (C-7), 37.17 (C-10), 38.86 (C-1 and C-4), 40.62 (C-8), 41.32 (C-13), 42.53 (C-14), 47.95 (C-17), 51.13 (C-9), 55.45 (C-5), 78.98 (C-3), 133.07 (C-19), 136.75 (C-18) and 182.14 (C-28).

(-)-Kauran-16α,19-diol (**7**): White powder, mp. 200–202 °C. MS as in (Serebryakov *et al.,* 1970), ^1H-NMR and ^{13}C-NMR as in (Satake *et al.,* 1983).

3-Oxo-4,11(13)-eudesmadien-12-oic acid (**8**): Yellowish needles, mp 154–155 °C. MS and ^1H NMR as in (Bohlmann *et al.,* 1977).

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Results and Discussion

The terpenoid portion of the resinous exudate of *Ozothamnus ledifolius*, previously obtained by CC on Sephadex (Wollenweber *et al.*, 1997), was flash-chromatographed on Si gel to yield 8 fractions or products. ^{1}H-NMR analysis revealed that the first fraction (**1**), appering as a single spot on TLC, is a 9:1 mixture of the *t*- and *c*- isomers of 2-phenylethyl cinnamate which contains traces of its dihydro derivative, 2-phenylethyl 3-phenylpropanoate. These compounds were confirmed by GC-MS.

Next, three bisabolane sesquiterpenoids were eluted. Bisabol-1-one (**2**), 12-hydroxybisabol-1-one (**3**), and 12-acetoxybisabol-1-one (**4**) have been previously isolated from the genus *Stevia* (Bohlmann *et al.*, 1976) (**2**), from *Vernonia neocorymbosa* (Bohlmann *et al.*, 1983) and *Ptilostemon niveus* (Menichini *et al.*, 1986) (**3**), and from *Senecio smithii* (Bohlmann *et al.*, 1981) (**4**), all in the Asteraceae.

Betulinic acid (**5**) is a rather widespread triterpene, first reported from birch bark. The triterpene morolic acid (**6**), on the other hand, is an unusual compound. It was first identified after acidic hydrolysis of the heartwood extract of *Mora excelsa* (Barton and Brooks, 1951). In the same year it was independently found as a natural product in *Aguaria salicifolia* and named aguariolic acid (Dussy and Sosa, 1951). Later, it has also been reported from *Pyracantha coccinea* (Ryabinin and Belous, 1963). Complete identification of our product was accomplished through its ^{13}C-NMR data by comparison with those of its acetyl and methyl ester derivatives (Gonzalez *et al.*, 1981; Majunder *et al.*, 1979). Morolic acid has also been identified in the exudate of *Ozothamnus rosmarinifolius*, where it occurs along with betulin, betulinic acid, and ursolic acid (Siems, unpublished).

(-)-Kauran-16-α,19-diol (**7**), was previously isolated as a natural product from *Xylopia aethiopica* (Ekong *et al.*, 1969). Several years before it had been obtained by chemical transformation from a related diterpene isolated from *Ricinocarpus stylosus* (Henrick and Jefferies, 1964). This is thus only the second report on the natural occurrence of this diterpenediol. The closely related diterpene (-) *ent*-16α-kauranol has recently been reported from the exudates of *Ozothamnus hookeri* and *O. scutellifolius* (Wollenweber *et al.*, 1997).

The major component of the mixture under study is a rare sesquiterpene acid, 3-oxo-4,11(13)-eudesmadien-12-oic acid (**8**). This compound was previously reported from *Ageratina glabrata* (Bohlmann *et al.*, 1977) and from *Xanthium pungens* (Ahmed *et al.*, 1990).

The two samples of *O. ledifolius* studied here yielded the same compounds. The exudate flavonoid patterns of both samples were also found to be identical (Wollenweber *et al.*, 1997). The production of terpenoids as well as of flavonoids thus appears to be species specific.

No correlation is observed between the terpenoids and phenylethyl esters we find in *O. ledifolius* and those compounds identified from the only previously studied species of this genus, *Ozothamnus obcordatus* (Zdero *et al.*, 1991). The latter exhibits five prenylated *p*-hydroxybenzoic acids. This result parallels the existence of completely different flavonoid patterns we recently reported (Wollenweber *et al.*, 1997). The two species produce 22 flavonoid aglycones altogether, but have not a single one in common.

In a recent thesis on Cassiniinae (Reid, 1995), terpenoids have not been considered. Analyses of secondary metabolites, terpenoids as well as flavonoids and other phenolics, from further species of this genus are in progress.

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