Unexpected discovery of 7-Methyljuglone (Ramentaceone) in several Australian sundews

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Introduction

Naphthoquinones are characteristic constituents that have been detected in numerous plant families. There are at least four fundamentally different biosynthetic routes that lead to the naphthoquinone skeleton (Durand & Zenk 1974). Some naphthoquinones are formed via the acetate-polymalonate (= polyketide) pathway, and plant families notoriously known for containing such acetogenins are Iridaceae, Ebenaceae, Plumbaginaceae, Droseraceae, Nepenthaceae, Drosophyllaceae, Dioncophyllaceae, and Ancistrocladaceae. While all these families include species that form plumbagin (= 2-methyljuglone, Fig. 1), its regioisomer 7-methyljuglone (= ramentaceone, Fig. 1) has so far only been detected in Ebenaceae, Nepenthaceae, and Droseraceae (Schlauer *et al*. 2005).

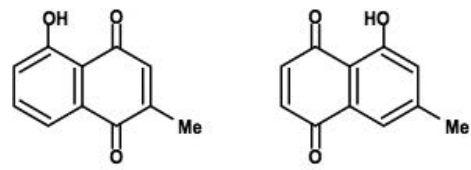

Figure 1: Chemical structures of naphthoquinones detected in different *Drosera* species; plumbagin and 7-methyljuglone.

Since the classical investigations (Zenk *et al*. 1969; Durand & Zenk 1974; Culham & Gornall 1994) it has been known that the different naphthoquinone isomers are characteristic for several sundew (*Drosera* L.) species (or species groups), and can thus be used for chemotaxonomic delimitation and distinction (Schlauer & Fleischmann 2016). The most striking parallels between chemism and systematics are found in the so-called "Australian clade" (*Drosera* subgenus *Ergaleium* (DC.) Drude) that contains all pygmy (*D*. section *Bryastrum* Planch.) and tuberous (*D*. sections *Ergaleium* DC., *Erythrorhiza* (Planch.) Diels, and *Stolonifera* (Planch.) DeBuhr) species together with their relatives (e.g., the fork-leaved sundew, *D. binata* Labill., *D.* section *Phycopsis* Planch., Rivadavia *et al*. 2003). While the pygmy species and their immediate relatives, the *D*. *petiolaris* RBr. ex DC. -group (*D*. sect. *Lasiocephala* Planch.) apparently all lack acetogenic naphthoquinones whatsoever, the tuberous species together with the fork-leaved sundews (*D*. sect. *Phycopsis* Planch.) typically contain plumbagin and not 7-methyljuglone. *Drosera glanduligera* Lehm., an endemic of southwestern Australia and a supposed, isolated member of *D*. subgen. *Ergaleium* (sole member of *D*. sect. *Coelophylla* Planch.), has not been investigated for its naphthoquinones so far, probably because it used to be rare in cultivation. We have now embarked to close this gap, as the species has become a working horse of biomechanics for its rapid tentacle movement (Poppinga *et al*. 2012), and methods have been developed to cultivate this intriguing species (Hartmeyer *et al.* 2013).

Another group of species consists of relatives of the palaeotropical species *D*. *indica* L. (*D*. sect. *Arachnopus* Planch.) in the other large *D.* subgenus *Drosera*. Recently it has been recognized that a

considerable diversity exists among Australian representatives of section *Arachnopus*, and numerous of the taxa occurring there have been described as separate species (Schlauer 2001; Barrett & Lowrie 2013; Lowrie 2014). In previous phytochemical investigations in *D*. sect. *Arachnopus* different naphthoquinones (plumbagin or 7-methyljuglone, respectively) have been reported by different researchers (Zenk *et al*. 1969; Culham & Gornall 1994) who have, however, labelled all these diverse plants as a single collective species "*D. indica*", and as the exact provenance and identity of these plants cannot be reconstructed with certainty it was decided to re-investigate the section, now with appropriately identified plant material.

Materials and methods

All plants used in the present study are annual or short-lived and have been grown from seed. Fresh leaves of *D. glanduligera* Lehm., *D. aquatica* Lowrie*, D. cucullata* Lowrie, *D. finlaysoniana* Wall. ex Planch., *D. fragrans* Lowrie, *D. hartmeyerorum* Schlauer, and *D. serpens* Planch. were detached from rosettes or stems of mature (flowering size) plants and (ca. 50 mg fresh weight, each) extracted within the next few days in 100 µl of hydrotreated light naphtha (petroleum, boiling point range 60-95°C, PZN 7284064 from O. Fischar GmbH, Saarbrücken, Germany, CAS No. 64742-49-0) overnight at 20°C. Extraction supernatants (20 µl each) were spotted to a silica-gel aluminum-backed TLC plate (SIL G/UV254, 0.20 mm, 4 × 8 cm, REF 818 131, Macherey-Nagel, Düren, Germany), focused for spot sharpening with methanol (anhydrous, 99.8%, Sigma-Aldrich, Steinheim, Germany, CAS No. 67-56-1) and developed with toluene (anhydrous, 99.8%, Sigma-Aldrich, Steinheim, Germany, CAS No. 108-88-3). Detection of quinones was enhanced by reacting the dried plates with ammonia gas (Borntraeger reaction). Quinones were identified by comparison to co-chromatographed extracts of *D. intermedia* (used as standard for plumbagin, RF = 0.54) and *D. rotundifolia* (standard for 7-methyljuglone, RF = 0.46) that had been analyzed and confirmed by GC-MS previously (Schlauer *et al*. 2005).

Results

Naphthoquinones were detected in all investigated samples as summarized in Table 1.

Table 1. Species investigated and naphthoquinones detected.

Section	Species	Naphthoquinone
Drosera subgen. *Ergaleium* sect. *Coelophylla*	*D. glanduligera* Lehm.	7-methyljuglone
Drosera subgen. *Drosera* sect. *Arachnopus*	*D. aquatica* Lowrie	7-methyljuglone
	D. cucullata Lowrie	plumbagin
	D. finlaysoniana Wall. ex Planch.	plumbagin
	D. fragrans Lowrie	plumbagin
	D. hartmeyerorum Schlauer	7-methyljuglone
	D. serpens Planch.	plumbagin

Discussion

The detection of 7-methyljuglone in *D. glanduligera* elucidates the naphthoqinone chemism of the last section of *Drosera* hitherto unexplored in that respect and it is at the same time a great surprise, as *D*. subgenus *Ergaleium* was hitherto characterized by either total absence of any acetogenic naphthoquinone (in pygmy species) or by the almost exclusive presence of plumbagin only (in tuberous and fork-leaved species). This confirms the eccentric position of *D*. section *Coelophylla* in subgenus *Ergaleium*.

The naphthoquinones detected in the investigated species of *D*. section *Arachnopus* confirm the chemical diversity that has previously been described for "*D. indica*" s. lat. and now for the first time attribute the different isomers to distinct identified species. Apparently the formation of 7-methyljuglone is limited to a minority of species within *D*. sect. *Arachnopus* (so far only detected in *D. hartmeyerorum* and *D. aquatica*) and among Australian sundew species in general. In the same subgenus *Drosera*, the presence of both isomers (usually in different species) has parallels in *D.* section *Prolifera* that is endemic to Queensland and in section *Drosera* that is most speciose outside Australia (Culham & Gornall 1994).

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