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INFLORESCENCE ODOUR OF *SENECIO ARTICULATUS*: TEMPORAL VARIATION IN ISOVALERIC ACID LEVELS

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Key Word Index—*Senecio articulatus*; Compositae; candle plant; headspace analysis; floral odour; isovaleric acid.

Abstract—Isovaleric acid (3-methylbutanoic acid) was found to be a major constituent of the inflorescence odour of *Senecio articulatus* and contributes to its fetid smell. Other important constituents were linalool and its oxides. Production of isovaleric acid from a single capitulum was found to be greater during the day, when new florets opened, than during the night, whereas the terpenoids showed less temporal variation. The odour is distinct from that of sapromyophilous plants whose odours mimic dung and carrion but, in producing isovaleric acid, shows similarities to other potentially fly pollinated flowers with unpleasant odours. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Senecio articulatus Sch. Bip. (Compositae), popularly known as the candle plant because of the resemblance of the greyish-green, sausage-shaped stems to candles, is native to South Africa and has become a commonly cultivated succulent. The inflorescences, consisting of small discoid capitula of cream-coloured florets, produce a rather repugnant, fetid odour which is more noticeable in the morning. As the inflorescences are relatively inconspicuous, it seems likely that the odour has a role in attracting pollinators.

Foul and fetid floral odours are generally associated with the pollination syndrome of sapromyophily; that is, pollination by insects that breed or feed on decaying matter or fungi [1]. Pollination of *S. articulatus* has not been observed, but various flies, including bluebottles (*Calliphora vomitoria*) and pollen-eating beetles, have been seen to visit the capitula [2].

As part of our interest in the floral odour chemistry of sapromyophilous plants, we have analysed the floral odour of *S. articulatus* to determine whether any of the constituents are common with other species having this pollination syndrome. The odour of *S. articulatus* is also of interest since work on the floral odours of Compositae is very scarce [3], even though the family accounts for about 10% of all flowering plants.

RESULTS AND DISCUSSION

A total of 22 compounds were identified in the inflorescence odour of *S. articulatus* (Table 1). Isovaleric acid was the major component, accounting for 39% of the trapped volatiles, and its pungent, rancid odour was probably responsible for the unpleasant smell of the flowers. Other compounds present were mainly various esters and terpenoids (in particular linalool and its various oxides), most of which have more pleasant fruity or fragrant smells. Some of these other compounds were also found to be produced by the leaves and therefore may have arisen from the vegetative tissue of the inflorescence. When the headspace above a single capitulum was analysed, only isovaleric acid, limonene, linalool and its oxides could be detected (Table 1).

The levels of isovaleric acid in the odour were found to show large temporal fluctuations. A single capitulum produced much more isovaleric acid during the day than during the night (Fig. 1). This appeared to be correlated with the opening of the florets, which occurred during the early morning. Differences in the daytime and night-time levels of the other compounds present were not so pronounced (Table 1); for example, levels of *cis*-linalool oxide (pyranoid form), the other major component of the capitulum odour, tended to gradually increase as more florets opened (Fig. 1).

The timing of floret opening and the release of isovaleric acid suggests that the pollinators are active during the day, supporting the assumption that flies such as *Calliphora vomitoria* may be pollinators [2].

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Table 1. Chemical composition (ppt) of the odour from an inflorescence and a single capitulum of *Senecio articulatus*. The inflorescence had eight capitula at various stages of development and analysis was performed during the day. Compounds marked with an asterisk were also present in the headspace from vegetative tissue. Capitulum data are the mean daytime and night-time values from analyses performed over a four day period (day 2–night 5 in Fig. 1). Also given for this period are the mean day to following night ratios of the rates of production for each compound

ID†	Retention time (min:sec)	Compound	Inf day (ppt)	Capitulum day (ppt)	Capitulum night (ppt)	Capitulum ratio (dy/nt)
a	8:49	l-butyl acetate*	8			
a	9:52	isovaleric acid	387	552	181	7.3
a	10:20	isoamyl acetate	2			
a	13:12	benzaldehyde	62			
a	13:36	myrcene	89			
b	14:11	α-phellandrene*	31			
b	14:22	l-hexyl acetate	7			
b	14:48	*o*-cymene	10			
a	14:54	limonene*	11	4	8	1.3
b	15:03	*cis*-ocimene*	6			
b	15:23	*trans*-ocimene*	3			
b	16:21	*cis*-linalool oxide (furanoid)	14	14	28	1.5
c	16:44	cresol	13			
b	17:16	linalool	46	4	8	1.4
c	17:29	linaloone oxide (pyranoid)‡	19	15	36	1.5
b	19:24	benzyl acetate	13			
c	19:36	*cis*-linalool oxide (pyranoid)§	48	280	616	0.8
c	19:43	*trans*-linalool oxide (pyranoid)	22	119	111	1.8
b	20:14	2-methoxy-*p*-cresol	9			
b	22:50	linalool oxide acetate (pyranoid)¶	183	11	11	1.0
a	26:49	β-caryophyllene	5			
b	29:07	δ-decalactone	11			

† Compound identification criteria: a, comparison of MS and relative retention time with purchased standard; b, comparison of MS and relative retention time with published data; c, comparison of MS with published data.

‡ 3-Oxo-2,2,6-trimethyl-6-vinyltetrahydropyran.

§ 3-Hydroxy-2,2,6-trimethyl-6-vinyltetrahydropyran.

¶ 3-Acetoxy-2,2,6-trimethyl-6-vinyltetrahydropyran.

Field observations are necessary to confirm the nature of the pollinators involved, and experiments to test their response to isovaleric acid would be beneficial. Temporal fluctuations of isovaleric acid levels have also been detected from changes in the perceived odour of the foul-smelling orchid *Masdevallia striatella* [4]. The odour chemistry of this species shows certain similarities with that of *S. articulatus* in containing isovaleric acid together with linalool and its oxides, and it was noted that the odour of the isovaleric acid subsided at night to leave the more pleasant odours of the terpenoids.

Isovaleric acid is an uncommon constituent of floral odours [3]. Apart from its occurrence in some *Masdevallia* species [4], isovaleric acid has only been reported in *Theophrasta americana* and *Deherainia smaragdina* ssp. *smaragdina* of the Theophrastaceae [5], and *Leontopodium alpinum* (the edelweiss) of the Compositae [6]. Levels (% composition) of isovaleric acid in these species were not reported to be as high as in *S. articulatus* and other compounds dominated their odours: 3-methyl-l-butanol in *T. americana*, butyl acetate in *D. smaragdina* and 3-hexenyl acetate in *L. alpinum*. All of these species have unpleasant floral odours and flies have been implicated in their pollination [5], although this has only been shown conclusively in *L. alpinum* where species of *Thricops*, *Coenosia* and *Phaonia* (Muscidae) were found to be the major pollinators [6].

The odour of *S. articulatus* is different from that of sapromyophilous flowers in which the odour clearly resembles dung or rotting meat. Indole, *p*-cresol, 2-heptanone and various terpenoids are characteristic of the dung odour of *Arum maculatum* [7] while dimethyl oligosulphides dominate the carrion odours of *Hydnora africana* [8] and many *Amorphophallus* species (Kite and Hetterscheid, unpublished data). This may reflect a distinction between plants, such as *A. maculatum*, whose odour mimics the pollinators' food and oviposition sites and the pollinators are deceived (and often trapped), and plants such as *S. articulatus* and *L. alpinum* which produce an odour that pollinators

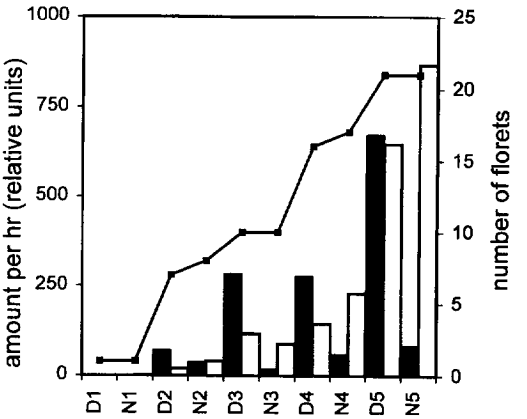

Fig. 1. Relative rates of production of isovaleric acid (solid bars) and *cis*-linaool oxide (pyranoid form) (open bars) from a single capitulum of *Senecio articulatus* compared to the number of florets open (■–■) at the end of the sampling period. Data were recorded at the end of each day and night over a five day period. The single florets which opened during night 2 and night 4 did not have dehiscent anthers and are therefore assumed to have opened just prior to the day period.

associate with a reward; *L. alpinum* has been shown to produce nectar rich in amino acids (which is said to be preferred by flies such as *Sarcophaga bullata* [9]) and we found that the nectar of *S. articulatus* was rich in glucose (amino acids could not be detected). Some pollinator species may be attracted to both types of odour. In *Calliphora vicina*, for example, the odours of decaying meat and flowers are perceived through different antennal receptors, each responding to a different class of compounds [10]. Thus, fly pollinated plants can potentially exploit different aspects of the pollinators' behaviour and are likely, therefore, to produce odours conforming to various chemical types.

EXPERIMENTAL

Odour analysis. A specimen of *Senecio articulatus* Sch. Bip. growing at the Royal Botanic Gardens, Kew (Kew accession number 1984-4094), was used for the odour analyses. A cut inflorescence was covered with a round-bottomed flask into which was inserted a trap (100 mm long × 3 mm diameter; SGE Ltd) packed with 100 mg of 35/60 Tenax TA. Air was sucked through the trap at a rate of 25 ml min^{-1} using a portable pump for a period of 5 hr during the day. The compounds collected on the trap were analysed by direct thermal desorption-GC-MS (thermal desorption injector, SGE Ltd; GC, Perkin–Elmer Model 8500; MS, Finnigan-MAT ion trap detector). The trap was desorbed for 4 min in a flow of helium carrier gas at 250° onto the end of a 25 m × 0.22 mm i.d. × 0.25 μm BPX5 (SGE Ltd) capillary column which was being cooled by means of a liquid CO_2 cold trap (SGE Ltd). After desorption, the cold trap was turned off and chromatography then proceeded using an oven temp. programme of 40–240° at 4° min^{-1}. The effluent from the column was split between a FID, to record peak areas, and the ion trap detector, to record EI-MS (scan rate 1 s^{-1}; *m/z* scanned 38–400, automatic gain control set at 38 a.m.u.). Compounds were identified by comparison of R_t and MS with published data [11, 12] or with purchased standards. Compounds in the headspace above 10 detached leaves were recorded in a similar manner.

Time course study. In the time course experiment the production of volatiles was monitored from a single capitulum of 21 budded florets still left attached to the plant (other capitula in the inflorescence were removed). The capitulum was covered by a small Quickfit adaptor into which a trap was screwed. The headspace above the capitulum was sampled during each day (09:30–16:30) and night (17:30–08:30) for a period of 5 days after which time all the florets had opened. After each collection the trap was analysed (taking 1 hr) and reused for the next collection. The number of florets open was also recorded at the end of each sampling period. The temp. was kept constant (22°) throughout, and the room lights were switched on at 08:30 and off at 17:30; there was no natural light.

Nectar analysis. Nectar was removed from florets using a micropipette and subjected to sugar analysis by standard paper chromatographic techniques [13] and screened for amino acids by GC-MS [14] after removal of sugars by ion exchange chromatography.

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