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S-Alk(en)yl-L-cysteine sulfoxides, alliinase and aroma in *Leucocoryne*

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Abstract

Levels of *S*-alk(en)yl-L-cysteine sulfoxides, alliinase and enzymatically generated pyruvic acid were determined in the bulb, leaf and scape of five species and a natural hybrid of *Leucocoryne* (Liliaceae), a genus of ornamental geophytes indigenous to Chile. (+)-*S*-Methyl-L-cysteine sulfoxide (MCSO) was present in all plant parts of all species at levels between 0.09 and 1.41 mg g^{-1} fr. wt. *Trans*-(+)-*S*-(1-propenyl)-L-cysteine sulfoxide (PRENCSO) was present in plant parts of three species only (*L. angustipetala*, *L. odorata* and *L. purpurea*) at levels between 0.12 and 1.82 mg g^{-1} fr. wt. No other *S*-alk(en)yl-L-cysteine sulfoxides were detected. Alliinase (EC 4.4.1.4) was detected in the leaf, bulb and scape of *L. angustipetala* and *L. purpurea*, only in the leaves of *L. coquimbensis* and *L. purpurea* x *L. coquimbensis*, and only in the bulb of *L. odorata*. Enzymatically generated pyruvic acid was detected in all plant parts of all species at levels between trace amounts and 5.33 μmol g^{-1} fr. wt. As PRENCSO is produced only in *Leucocoryne* species exhibiting a strong and unpleasant onion-like aroma, it is probable that the enzymatic degradation of PRENCSO is the main cause of that aroma. Consequently, *Leucocoryne* cultivars should be selected in species and hybrids that lack the ability to synthesise PRENCSO.

Keywords: *Leucocoryne*; Liliaceae; Aroma characterisation; *S*-Alk(en)yl-L-cysteine sulfoxides; Alliinase; Pyruvic acid

1. Introduction

Leucocoryne (Liliaceae) is a genus of ornamental geophytes indigenous to Chile, comprised of 12 species (Zoellner, 1972). *Leucocoryne* have flowers in shades of blue, violet and white and as they are relatively long lasting, there is interest in this plant as a floricultural crop. Although some species have sweetly scented flowers, the scapes (stems of inflorescences) of other species emit an aroma, characteristic of onions, when cut or bruised. This could limit the further development of those species as cut flowers. There is, however, variation between species in the intensity of this undesirable aroma. The five species and natural hybrid selected for analysis reflect this variation in that *L. angustipetala, L. odorata,* and *L. purpurea* possess a strong and unpleasant aroma and *L. coquimbensis, L. ixioides* and the *L. purpurea* × *L. coquimbensis* hybrid possess a sweet aroma (D. Brundell, personal communication).

The aroma of onion (*Allium cepa*, Liliaceae) results from the enzymatic hydrolysis of the *S*-alk(en)yl-L-cysteine sulfoxides (ACSOs) by alliinase (EC 4.4.1.4) to produce volatile S compounds and the by-products pyruvic acid and NH_3 (Lancaster and Boland, 1990). Although pyruvic acid itself does not contribute to the aroma, it has been widely used as an assay of volatile S aroma because of its high correlation with aroma and its ease of quantitation (Wall and Corgan, 1992; Randle and Bussard, 1993).

Four ACSOs have been identified in members of the genus *Allium*: *trans*-(+)-*S*-(1-propenyl)-L-cysteine sulfoxide (PRENCSO), (+)-*S*-(2-propenyl)-L-cysteine sulfoxide (ALLYLCSO, commonly known as *S*-allyl cysteine sulfoxide), (+)-*S*-propyl-L-cysteine sulfoxide (PCSO) and (+)-*S*-methyl-L-cysteine sulfoxide (MCSO). Flavour variation among *Allium* species depends on which ACSOs are present and their concentrations (Block, 1992). Onions are characterised by high levels of PRENCSO, lower levels of MCSO and PCSO and an absence of ALLYLCSO. Garlic is characterised by high levels of ALLYLCSO and the absence of PCSO. MCSO is the main constituent of many ornamental *Allium* species.

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PCSO has been detected in another member of the Liliaceae, *Ipheion uniflorum* (Tsuno, 1958).

ACSOs are also found in families other than Liliaceae. MCSO is found in several members of the Brassicaceae (formerly Cruciferae) and in some of the Apiaceae (formerly Umbelliferae), Asteraceae (formerly Compositae) and Fabaceae (formerly Leguminosae), (Fujiwara et al., 1958; Hegnauer, 1963).

Alliinase occurs in most, if not all, *Allium* species (Lancaster et al., 2000). It has also been reported in other genera in the Liliaceae, including *Tulbaghia* (Jacobsen et al., 1968). Alliinase like activity has also been found in *Brassica* species (Brassicaceae, formerly Cruciferae), (Ramirez and Whitaker, 1998) and *Albizzia lophanta* (Fabaceae, formerly Leguminosae), (Schwimmer and Kjaer, 1960).

Leucocoryne species have not previously been characterised for their ACSO and alliinase content despite their onion-like aroma. The results presented here are for an analysis of a range of *Leucocoryne* species and a naturally occurring interspecific hybrid with different aromas and aroma intensities.

2. Results and discussion

2.1. ACSO content in plant parts of Leucocoryne

MCSO was detected in the bulb, leaf and scape of all *Leucocoryne* analysed (Table 1). Concentrations were highest and above 1 mg g^{-1} fr. wt in the bulbs of *L. angustipetala* and *L. purpurea*. Bulbs of the other four species contained MCSO at less than 1 mg g^{-1} fr. wt, with *L. ixioides* the lowest at 0.41 mg g^{-1} fr. wt. For all species, MCSO concentrations were lowest in the leaf compared to the bulb and the scape. Leaf concentrations ranged from 0.09 to 0.31 mg g^{-1} fr. wt. The scape contained the widest range of MCSO concentrations. Concentrations were lowest in *L. purpurea* x *L. coquimbensis* and *L. ixioides* and several fold higher in the other species.

PRENCSO was detected only in *L. angustipetala, L. odorata* and *L. purpurea* and was present in bulbs, leaves and scapes. *L. angustipetala* contained the highest levels of PRENCSO in the plant parts analysed.

There was no PCSO or ALLYLCSO detected in any of the *Leucocoryne* analysed.

Table 1
Concentrations of (+)-*S*-methyl-L-cysteine sulfoxide (MCSO), *trans*-(+)-*S*-propenyl-L-cysteine sulfoxide (PRENCSO), enzymatically generated pyruvic acid and alliinase specific activities in bulbs, leaves and scapes of analysed *Leucocoryne*

Plant material	*S*-Alk(en)yl-L-cysteine sulfoxides (mg g^{-1} fr. wt)			Alliinase (nkat mg^{-1} protein)	Pyruvic acid (μmol g^{-1} fr. wt)
	MCSO	PRENCSO	Total		
L. angustipetala					
Bulbs	1.3	1.8	3.2	2.3	3.9 (0.07)[a]
Leaves	0.31	0.41	0.73	2.1	–[b]
Scapes	0.95	0.51	1.5	1.4	1.6 (0.06)
L. coquimbensis					
Bulbs	0.81	n.d.[c]	0.81	n.d.	2.6 (0.04)
Leaves	0.13	n.d.	0.13	0.62	0.76 (0.06)
Scapes	0.75	n.d.	0.75	n.d.	1.7 (0.05)
L. ixioides					
Bulbs	0.41	n.d.	0.41	n.d.	0.36 (0.04)
Leaves	0.12	n.d.	0.12	n.d.	0.77 (0.09)
Scapes	0.36	n.d.	0.36	n.d.	1.1 (0.12)
L. odorata					
Bulbs	0.66	1.2	1.9	1.4	5.3 (0.07)
Leaves	0.18	0.41	0.59	n.d.	2.7 (0.10)
Scapes	1.3	0.39	1.7	n.d.	2.5 (0.10)
L. purpurea					
Bulbs	1.4	1.1	2.50	0.80	2.5 (0.07)
Leaves	0.17	0.12	0.29	1.5	0.63 (0.09)
Scapes	0.95	0.36	1.3	0.83	3.0 (0.04)
L. purpurea x *L. coquimbensis*					
Bulbs	0.52	n.d.	0.52	n.d.	0.70 (0.03)
Leaves	0.09	n.d	0.09	0.48	1.6 (0.22)
Scapes	0.14	n.d	0.14	n.d.	1.0 (0.07)

[a] SEM presented in brackets, $n = 3$.
[b] Background absorbance greater than sample.
[c] n.d. = not detected.

The levels of MCSO reported here for *Leucocoryne* are generally lower than those reported for onion of 0.86–3.09 mg g^{-1} fr. wt (Randle et al., 1995). Higher levels of MCSO have also been reported for many of the ornamental *Allium* species, although by a different analytical method (Bernhard, 1970). The levels of PRENCSO reported above for *Leucocoryne* are comparable to those for onions growing under conditions that lead to mild aromas (Randle et al., 1995).

2.2. Alliinase activity in plant parts of Leucocoryne

Alliinase activity was detected only in some of the partially purified extracts from the *Leucocoryne* analysed. Specifically, alliinase activity was detected in bulbs, leaves and scapes of *L. angustipetala* and *L. purpurea*, only the leaves of *L. coquimbensis* and *L. purpurea* x *L. coquimbensis* and only the bulbs of *L. odorata*. Further purification and concentration of the alliinase protein would be required to measure alliinase activity in the other extracts.

The alliinase activity reported here for *Leucocoryne* is much less than the levels reported for onion bulbs (79 nkat mg^{-1} protein) and roots (31 nkat mg^{-1} protein), using the same method (Clark et al., 1998; Lancaster et al., 2000). Onions and garlic, however, are well recognised for their very high levels of alliinase, estimated to be up to 6% by weight of the soluble protein (Nock and Mazelis, 1987). Further, low levels of alliinase activity per se have not been shown limit the conversion of ACSOs to pyruvic acid (J. E. Lancaster and M. L. Shaw, unpublished results).

2.3. Pyruvic acid in plant parts of Leucocoryne

Enzymatically generated pyruvic acid was detected in all parts of the *Leucocoryne* analysed, at levels between 0.63 and 5.33 μmol g^{-1} fr. wt. There were significant differences in pyruvic acid levels between plant parts in different *Leucocoryne*. High levels of pyruvic acid were detected in bulbs of *L. angustipetala* and *L. odorata* and low levels in the bulbs of *L. ixioides* and *L. purpurea* x *L. coquimbensis*. Levels of enzymatic generated pyruvic acid tended to be lower in leaves than scapes.

The higher levels (2–5 μmol g^{-1} fr. wt) of pyruvic acid reported here are comparable to those detected in crushed onion bulbs. Pyruvic acid values of 9–11 μmol g^{-1} fr. wt have been reported for a pungent onion cultivar (Hanum et al., 1995) and 2–5 μmol ml^{-1} juice for mild onion cultivars (Randle et al., 1999). Values as low as 0.8 μmol g^{-1} fr. wt have been reported but these values were for mild cultivars growing under conditions with low sulfur supply (Randle et al., 1995), conditions which reduce onion aroma. However, the levels of pyruvic acid detected in *L. ixioides* and *L. purpurea* x *L. coquimbensis* are much lower than those reported even for very mild onions (Randle et al., 1995).

2.4. Relationship of chemical compounds to perceived aroma

All *Leucocoryne* analysed produced MCSO, but only *L. angustipetala, L. odorata,* and *L. purpurea* produced PRENCSO, those species that possess the strong and undesirable aroma of onions when cut or bruised (D. Brundell, personal communication). As PRENCSO is the compound responsible for pungency in onions (Block, 1992), it is probable that the enzymatic degradation of PRENCSO is the main cause of the onion aroma in *Leucocoryne*. Consequently, *Leucocoryne* cultivars should be selected from species that do not synthesise PRENCSO so that they do have an unpleasant aroma. However, as the interspecific hybrid between *L. purpurea* and *L. coquimbensis* (a PRENCSO producer and a non-PRENCSO producer, respectively) does not synthesise PRENCSO, this offers the possibility to select against the ability to synthesise PRENCSO in a breeding programme.

3. Experimental

3.1. Plant material

At least 20 plants from each of five species (*L. angustipetala* Gay, *L. coquimbensis* Phil., *L. ixioides* (Hook.) Lind., *L. odorata* Lindl., *L. purpurea* Gay) and one natural hybrid (*L. purpurea* Gay x *L. coquimbensis* Phil.) were collected from a nursery at Glenbrook, New Zealand. Plants were transferred to the laboratory and separated into bulbs, leaves and scapes (stems only, no flowers) for analysis.

3.2. Determination of ACSOs by HPLC

Bulb, leaf and scape material from ten plants of each species and the hybrid was combined to give samples between 15 and 50 g g^{-1} fr. wt and weighed. ACSOs from each sample were extracted in MeOH–$CHCl_3$–H_2O (12:5:3 v/v/v) and ethanol (80%) according to Lancaster and Kelly, 1983. *S*-Butyl-L-cysteine sulfoxide was synthesised (Lancaster and Kelly, 1983) and added as an internal standard at 1 mg g^{-1} fr. wt to determine the percentage recovery through the extraction, derivatisation and analysis. The aqueous extracts (1 g fr. wt equivalents) were lyophilised at −36° in vacuo and then redissolved in 1 ml deionised water. Sample fractionation was performed by ion-exchange chromatography with Dowex 1×8 (200–400 mesh, 10 x 40 mm column) resin in the OAc form. A 0.5 ml sample of extract was loaded onto the column and ACSOs eluted with 0.1 M HOAc. Samples were lyophilised and derivatised with phenyl isothiocyanate and analysed by RP-HPLC (Randle et al., 1995). Authentic standards and *Allium*

samples of known ACSO composition were used to identify peak components (Randle et al., 1995).

3.3. Measurement of alliinase activity

Bulb, leaf and scape material of each species and the hybrid was combined to give samples of approximately 10, 3 and 5 g, respectively. Tissues were finely ground on ice in an equivalent volume (w/v) of buffer [50 mM Tris–HCl (pH 7.5), 0.5 M NaCl, 30% (v/v) ethylene glycol, 2.5 μg ml^{-1} pyridoxal-5-phosphate]. Samples (75 μl) were spun through a Micro Bio-Spin 30 column (Bio-Rad, Hercules, California) previously equilibrated in the above buffer. The spin column both desalted samples and removed background pyruvic acid prior to assay.

Alliinase activity was measured by a coupled NADH/LDH assay in 0.2 M Tricine–KOH (pH 8.0), 0.1 mM NADH, 12.5 units LDH, 5.0% (v/v) alliinase preparation, 0.01 M PCSO substrate at 20°C (Schwimmer and Mazelis, 1963). Activity was calculated from the conversion of NADH to NAD^+ as measured by the decrease in A_{340} (Schwimmer and Mazelis, 1963) and reported as nkat. The protein concentration of the eluant was determined using a refinement of the Bradford dye binding assay (Spector, 1978).

3.4. Determination of enzymatically produced pyruvic acid

Triplicate samples (0.5 ml) of tissue ground in buffer, from above, were incubated for 20 min at room temperature. Pyruvic acid was determined spectrophotometrically by the method of Randle and Bussard (1993). Levels of endogenous pyruvic acid were determined by grinding equivalent tissue samples in 5% TCA and assaying for pyruvic acid. Background levels were subtracted from the sample levels to give enzymatically generated pyruvic acid.

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