PHYTOCHEMICAL ANALYSIS
Phytochem. Anal. **14**, 360–365 (2003)
Published online in Wiley InterScience (www.interscience.wiley.com). **DOI**: 10.1002.pca.728

Quantification of Sideroxylonals in *Eucalyptus* Foliage by High-performance Liquid Chromatography

Ian R. Wallis,[1]* Anthony J. Herlt,[2] Bart M. Eschler,[1,2]† Midori Takasaki[1]‡ and William J. Foley[1]

[1]School of Botany and Zoology, Australian National University, Canberra ACT 0200, Australia
[2]Research School of Chemistry, Australian National University, Canberra ACT 0200, Australia

This paper describes the extraction and quantification of sideroxylonals, a group of formylated phloroglucinol compounds found in the foliage of some eucalypt species. Samples of dry, ground foliage were Soxhlet-extracted with light petroleum spirit:acetone (4:1) and the resultant extract analysed (in the presence of internal standard) by reversed-phase HPLC without further purification. The yield of sideroxylonals was exponential with time and showed an inflection at ca. 4 h of extraction. It is recommended that samples be extracted for 6 h, giving a 92% recovery of the sideroxylonals. The title compounds deteriorate under various conditions, e.g. 10% are lost when foliage is oven-dried at 40°C compared to freeze-drying. Storing samples in mobile phase led to a slow deterioration of sideroxylonals with a 7% loss after 4 days, while 22% of these compounds were lost from dry, ground eucalypt leaf stored at room temperature for 20 months. Copyright © 2003 John Wiley & Sons, Ltd.

Keywords: Formylated phloroglucinol compound; eucalypt; folivorous; marsupial.

INTRODUCTION

Sideroxylonals (Fig. 1) are a group of formylated phloroglucinol compounds found in the leaves and flower buds of some species of the genus *Eucalyptus*. These compounds have a wide range of biological effects (Ghisalberti, 1996), e.g. they suppress *Staphylococcus aureus* and *Bacillus subtilis* (Satoh *et al*., 1992), they are strong inhibitors of human plasminogen activation (Neve *et al*., 1999), they are the most potent antifouling agents known (Singh *et al*., 1996), and they also limit the consumption of *Eucalyptus* leaves by koalas and other folivorous marsupials and insects (Lawler *et al*., 1998, Lawler and Foley, 2002). Three isomers of sideroxylonal have been described, namely, sideroxylonal A (**1**), sideroxylonal B (**2**) and sideroxylonal C (**3**) (Eschler and Foley, 1999), as well as a structural variant known as grandinal which is not considered in this paper.

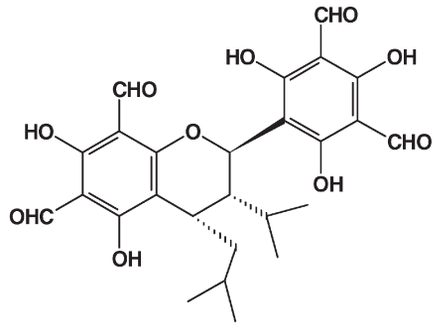
The sideroxylonals occur patchily in the genus *Eucalyptus* and their occurrence appears restricted to some species of the dominant grouping of eucalypts, the informal subgenus *Symphyomyrtus* (Eschler *et al*., 2000). In addition, there is considerable intra-specific variation in the concentration of these compounds. Thus Lawler *et al*. (2000) reported that in *Eucalyptus polyanthemos* the foliar concentration of the sideroxylonals varied from 0 to 13 mg/g of dry matter, but we have since found trees with concentrations as high as 26 mg/g of dry matter (Wallis, unpublished results). Foliage from *E. melliodora* contains even more sideroxylonals with levels ranging from 0 to 52 mg/g of dry matter (Wallis, unpublished data). Of the three isomers, **1** occurs in the highest concentrations, followed by **3**, with **2** being rarely detectable. The ratio of the concentrations of **3** to **1** tends to be fairly constant, thus in 13 trees of *E. melliodora* with total sideroxylonal concentrations ranging from 4 to 45 mg/g of dry matter, the ratio of **3** to **1** was 0.39 (SD = 0.037). The equivalent ratio for 17 trees of *E. polyanthemos* with total sideroxylonal concentrations ranging from 2 to 26 mg/g of dry matter was 0.31 (SD = 0.044) (Wallis, unpublished data).

Sideroxylonals are a potentially valuable natural product and, although **2** has been prepared synthetically (Tatsuta *et al*., 1999), the synthesis is inefficient. In contrast, the high concentrations of **1** and, to a lesser extent **3**, found in some *Eucalyptus* leaves suggests that extraction of these compounds from natural sources is the most economical method for obtaining sufficient material for further testing. This, together with the important ecological role played by sideroxylonals in Australian forests, led us to develop chromatographic methods to quantify foliar sideroxylonals in samples of *Eucalyptus*.

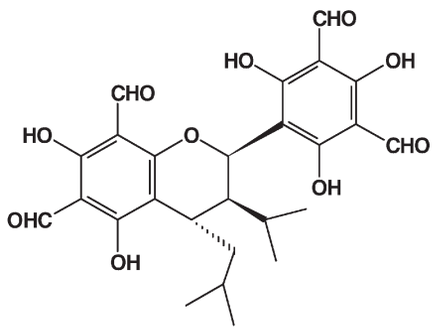
In this paper we report the extraction and quantification of sideroxylonals from *Eucalyptus* foliage. We also provide information on the recovery of sideroxylonals during extraction, the losses that occur when drying *Eucalyptus* leaves under different conditions, the deterioration of sideroxylonals when sideroxylonal-rich extracts are stored in the mobile phase, and the deterioration of the compounds with respect to time in dried ground plant material and in leaf extracts.

* Correspondence to: I. R. Wallis, School of Botany and Zoology, Australian National University, Canberra ACT 0200, Australia.
Email: ian.wallis@anu.edu.au
† Present address: John Curtin School of Medical Research, Australian National University, Canberra ACT 0200, Australia.
‡ Present address: Kyoto Pharmaceutical University, Yamashima Ku, Kyoto 6078414, Japan.
Contract/grant sponsor: Australian Research Council.

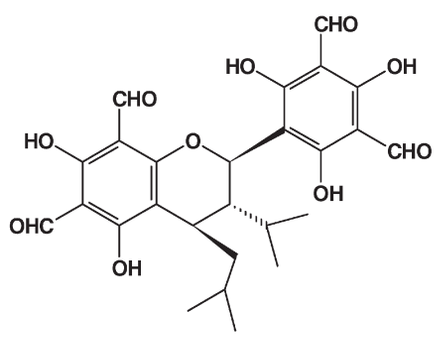
Received 25 March 2002
Revised 27 May 2002
Accepted 1 June 2002



Sideroxylonal A (1)



Sideroxylonal B (2)



Sideroxylonal C (3)

Figure 1. The structures of sideroxylonals A, B and C.

EXPERIMENTAL

Chemicals. Petroleum spirit (boiling point 40–60°C), acetone, dichloromethane, methanol, phloroglucinol, trifluoroacetic acid, 3,4-dimethylphenol and 2-ethylphenol were all of analytical grade. HPLC-grade acetonitrile and methanol were used for sample preparation and in the mobile phases.

Plant materials. Small branches were cut from the mid-canopy of mature eucalypts (10–30 m tall), and leaves (ca. 75 ***g***) were either stripped and placed in plastic bags stored on ice, or the entire branch was placed in a plastic bag and transported to the laboratory where it was stored in a cool room (ca. 5°C) with the stems of the branches standing in water. When stored in this manner, foliage remained fresh for about a week. Leaves that were sampled from these branches were stored at –20°C prior to drying.

Sample extraction. Unless stated otherwise, leaf samples were freeze-dried and ground to pass through a 1 mm sieve in a Cyclotec 1093 Mill (Tecator, Hoganas, Sweden). The resulting powder was stored in the dark in 50 mL clear plastic specimen containers. In order to obtain a sideroxylonal-rich extract, dried ground leaf material (1.5500 ± 0.0500 ***g***) was weighed into a cellulose extraction thimble (80 × 20 mm i.d.; Whatman, Maidstone, UK) and refluxed with 100 mL of light petroleum spirit:acetone (4:1) in a Soxhlet extractor (40 mL siphoning volume) connected to a 250 mL round-bottom flask which was heated on a water bath (85°C) for 4 h. After reflux had ceased, any solvent remaining in the Soxhlet extractor was combined with that in the flask and the total solvent was removed by rotary evaporation at 50°C. The resulting crude extract was transferred quantitatively into a pre-weighed 20 mL glass vial with five aliquots (3 mL each) of dichloromethane:methanol(4:1). The vial was dried under a stream of air for 24 h and then left exposed to the air in a fume cupboard for a further 48 h before being reweighed. At this stage the crude extract was a dark brown viscous material which, for further manipulation, needed to be scraped from the walls and bottom of the vial. The vial was capped and stored in the dark before further analysis. The typical yield of crude extract depended on the species of *Eucalyptus* and the concentration of sideroxylonals (Table 1). The relationship between the yield of sideroxylonals and of crude extract, after subtracting the yield of sideroxylonals from that of crude extract to avoid spurious correlations due to part–whole correlations (Christians, 1999), was examined by regression analysis.

Chromatographic analysis. The chromatographic separations were carried out on an SGE (Ringwood, Australia) GL Wakosil II 3C18RS column (250 × 4.0 mm i.d.; 3 μm) connected to a Waters Alliance (Milford, MA, USA) HPLC system consisting of a model 2690 separation module with an on-board autosampler fitted with a 250 μL syringe and a 100 μL sample loop, and a model 996 photodiode array detector. Optimal separation was obtained by isocratic elution with acetonitrile:water containing 0.1% trifluoroacetic acid (93:7). The flow rate was 0.75 mL/min with a column temperature of 40°C and a run time of 15 min. The typical operating pressure was 90 bar with a pressure ripple of 1 bar. Waters Millennium (version 3.2) software was used for both data collection and integration. Compounds **1**, **2** and **3** were detected at 275 nm and eluted at 8.92, 9.35 and 12.34 min, respectively. Quantitative determination was made by calibration with standards prepared in our laboratory (Eschler and Foley, 1999).

Internal standard. Phloroglucinol, 3,4-dimethylphenol and 2-ethylphenol were evaluated as potential internal standards for HPLC analysis of the sideroxylonals. Phloroglucinol was discarded because it eluted near the void volume under the chromatographic conditions used and its peak tended to coincide with other early-eluting material. 2-Ethylphenol was preferred to 3,4-dimethylphenol because it eluted later (at 3.60 min) and tended not to interfere with other components. The

Table 1. The yields, and their relationships, of crude extracts and of sideroxylonals from leaves of various *Eucalyptus* species

Species	*n*	Crude extract (mg/g dry leaf)[a]	Sideroxylonals (mg/g dry leaf)[a]	Regression: sideroxylonals *vs* corrected crude extract[b]
E. melliodora	120	75–200; 135; 23.2	0.21–55; 25.7; 11.8	$r^2 = 30.6$; $F_{1,113} = 51.2$; $p < 0.001$; SE = 8.51
E. microcorys	124	86–172; 134; 18.1	10.4–45; 23.7; 7.1	$r^2 = 25.8$; $F_{1,117} = 42.0$; $p < 0.001$; SE = 5.45
E. polyanthemos	37	68–156; 100; 24.8	0.21–26.3; 8.8; 6.2	$r^2 = 26.2$; $F_{1,31} = 12.4$; $p < 0.001$; SE = 3.50
E. nitens (mixed ages, Tasmania)	32	42–88; 58; 9.0	0.90–5.54; 2.38; 1.07	$F_{1,30} = 0.63$; $p = 0.44$
E. nitens seedlings (low CO_2)	5	83–92; 87; 4.1	14.0–21.6; 17.0; 2.8	Small sample size
E. nitens seedlings (high CO_2)	5	54–71; 61; 6.8	3.8–14.5; 10.2; 4.1	Small sample size
E. cosmophylla	40	96–222; 138; 24	4.8–48; 19.1; 10.4	$r^2 = 59.3$; $F_{1,38} = 57.8$; $p < 0.001$; SE = 6.07

[a] Results show the range, mean and standard deviation, respectively.
[b] Corrected crude extract = crude extract (mg/g leaf dry matter) minus sideroxylonals (mg/g leaf dry matter).

response factors of 2-ethylphenol over a wide range of absorbance values were determined by injecting 2 to 25 μL of three standard solutions of concentrations 100, 200 and 300 mg/L.

Quantitative determination. A known mass (ca. 8 ***g***) of 20% methanol in acetonitrile (both HPLC-grade) containing 0.300 mg/mL of 2-ethylphenol as internal standard was added to a known amount of plant extract (10 ± 1 mg). This mixture was placed in an ultrasonic bath until the extract dissolved (ca. 2 min) and the resulting solution filtered (0.45 μm) into an autosampler vial (2 mL). An aliquot (15 μL) of this solution was injected onto the column for each chromatographic run. Samples were prepared in batches so that they would be analysed within 5 h of preparation.

Recovery of sideroxylonals. The recovery of sideroxylonals was measured by extracting these compounds from samples prepared by mixing various amounts (14.9–76.8 mg) of sideroxylonal-rich powder with freeze-dried, ground foliage (1.545 ± 0.002 ***g***) of *E. rossii*, a species that does not contain sideroxylonals. The samples were analysed by HPLC and the recovery calculated using three independent standard curves prepared from the same sideroxylonal-rich powder.

The effect of drying conditions on the yield of sideroxylonals. Variation attributable to the method of drying leaves was examined in a randomised block design where 'trees' were regarded as blocks and the 'drying regimen' as the treatment. Samples of foliage from 16 trees of *E. melliodora* of differing sideroxylonal content were collected, frozen, and divided into four portions. One portion was freeze-dried whilst the others were dried to constant mass in a forced draught oven at 40, 60 or 80°C. The drying times of leaves in the four treatments were 96, 48, 24 and 16 h, respectively. The samples were then ground, extracted for 4 h and prepared for HPLC. The sample vials were randomised immediately before analysis.

The effect of extraction time on the yield of sideroxylonals from *Eucalyptus* leaf. Determining the appropriate extraction time for the complete recovery of crude extract and of the sideroxylonals was performed in two stages. Initially, seven samples of foliage of *E. melliodora* were freeze-dried and extracted in duplicate for either 4 or 13 h using the procedure described above. The solid remaining from the 13 h extraction was extracted again for 13 h, which confirmed that no sideroxylonal remained. This comparison (paired *t*-test) suggested that the longer extraction time the more sideroxylonals could be recovered, thus the experiment was repeated using samples from fourteen trees and extraction times of 2, 4, 6, 12 and 16 h in a factorial design.

Foliage of *E. melliodora* was chosen because it varies widely in content of sideroxylonals and thus permitted the selection of a concentration range (14–47 mg sideroxylonals/g dry matter) which mimics that found in natural stands of *E. melliodora* (Wallis *et al*., 2002). However, in this part of the study, trees with very low concentrations of sideroxylonals (<10 mg/g dry matter) were avoided. It was assumed that all of the sideroxylonal would be extracted in 16 h, thus results for the other extraction times are expressed as a percentage of the 16 h value.

The deterioration of sideroxylonals in crude extracts stored in mobile phase. Twenty-four samples derived from the study of leaf drying methods were retained in their autosampler vials, stored in the dark at room temperature and analysed again by HPLC after 4, 10 and 15 days in order to measure any changes in sideroxylonal concentration. It was assumed that minimal deterioration had occurred before the samples were initially analysed (i.e. day 0). The fate of decomposed sideroxylonals was not further investigated.

The effect on the yield of sideroxylonals of long term storage of dry, ground *Eucalyptus* foliage. The fourteen samples of *Eucalyptus* foliage used for the extraction vs time experiment were stored in clear plastic vials, with minimal exposure to fluorescent light, at room temperature (21°C) for 20 months. After this time the samples were re-analysed using identical extraction and HPLC methods and the results were evaluated using a paired *t*-test.

Statistical analysis. Data were analysed using Genstat 5 software (Numerical Alqorithms Group, Oxford, UK; For the analysis of variance, the full model always

included interaction terms. Non-significant terms were dropped from the full model to give the final reduced model. All models were tested to ensure that residual values were normally distributed and randomly distributed against fitted values. With regression models, values with high residual variation (>2.5) were excluded in the process of model testing.

RESULTS AND DISCUSSION

HPLC analysis

The optimal separation of the sideroxylonals was obtained using the isocratic conditions described with USP tailing factors of less than 2.0 for the peaks of interest (Fig. 2). The resolution of **1**–**3** could not be improved using different mobile phases (such as methanol, and different concentrations of trifluoroacetic acid), or by using gradient elution with acetonitrile:water containing 0.1% trifluoroacetic acid, e.g. starting from 60:40 and progressing to 60:10 in 60 min. Likewise, altering the column temperature had little effect on the resolution. The longer, narrower-bore Wakosil column (250 × 4 mm i.d.) gave better separation than shorter, wider columns (e.g. 150 × 4.6 mm i.d.) of the same stationary phase.

Internal standard

Injecting 2–25 µL aliquots of three standards (100, 200 and 300 mg/L) of 2-ethylphenol gave absorbance values ranging from 0.03 to 1.0 which readily covered the typical range (0.2–0.6) determined in the analysis of sideroxylonals. A log–log regression analysis identified three data points with high residual variation (>2.5). In all cases, they were from 2 µL injections suggesting, as expected, that the autosampler on the HPLC was most variable at very low injection volumes. These points were deleted from the final regression model as in:

$$\log(\text{area}) = 13.99866\ (\text{SE} = 0.00234) + 1.00421\ (\text{SE} = 0.00183) * \log(\text{2-ethylphenol injected})$$

which accounted for 100% of the variation ($r^2 = 100$; $F_{1,34} = 301{,}414$; $p < 0.0001$) and satisfied the requirements of linear regression.

Recovery of sideroxylonal

A 6 h Soxhlet extraction recovered 92.2% (SD = 3.9%) of the sideroxylonals which had been added to leaf material of *E. rossii*. There was no relationship between the amount of sideroxylonal added to the *Eucalyptus* leaf and the amount recovered ($p > 0.10$). Clearly, this method of determining recovery is not ideal because it effectively measures the recovery of free sideroxylonals. This differs from the normal situation where the sideroxylonals are presumably contained within the plant cell, although freeze-drying and grinding may rupture the cell wall and make the sideroxylonals more extractable. Using the described method, the within-sample coefficient of variation for sideroxylonals was less than 3% over a wide range of sideroxylonal concentrations. This suggests that a constant proportion, if not all, of the sideroxylonal is extracted and any that remains is not extractable with petroleum spirit:acetone (4:1).

The effect of drying conditions on the recovery of sideroxylonals

Drying conditions significantly affected the yield of crude extract ($F_{3,108} = 6.77$; $p < 0.001$) and the recovery of both sideroxylonal A ($F_{3,108} = 86.0$; $p < 0.001$) and sideroxylonal C ($F_{3,108} = 76.4$; $p < 0.001$; Fig. 3). Freeze-drying gave a greater yield of crude extract than did oven-drying, but there was no difference in yield of crude extract between the three oven temperatures employed. Likewise, oven-drying yielded significantly less sideroxylonal: relative to freeze-drying, oven-drying at 40 or 60°C caused losses of roughly 10% in sideroxylonal content, whilst increasing the oven temperature to 80°C caused a further 10% loss.

The relationship between crude extract and sideroxylonal concentration in various eucalypts

The sideroxylonals represent only a small proportion of the crude extract (10–20%; Table 1). Nevertheless, for *E. cosmophylla*, *E. melliodora*, *E. microcorys* and *E. polyanthemos*, there was a strong positive relationship between the yield of sideroxylonals and that of crude

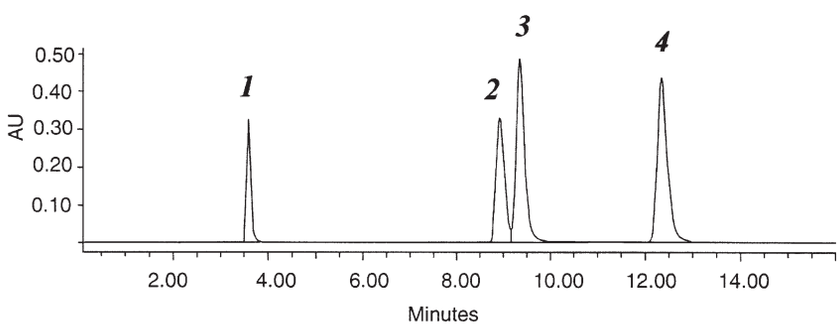


Figure 2. HPLC separation of the sideroxylonals. Key to peak identities: **1**, 2-ethylphenol (internal standard; 3.60 min); **2**, sideroxylonal A (8.92 min); **3**, sideroxylonal B (9.35 min); and **4**, sideroxylonal C (12.34 min). (For details of the chromatographic protocols see Experimental section.)

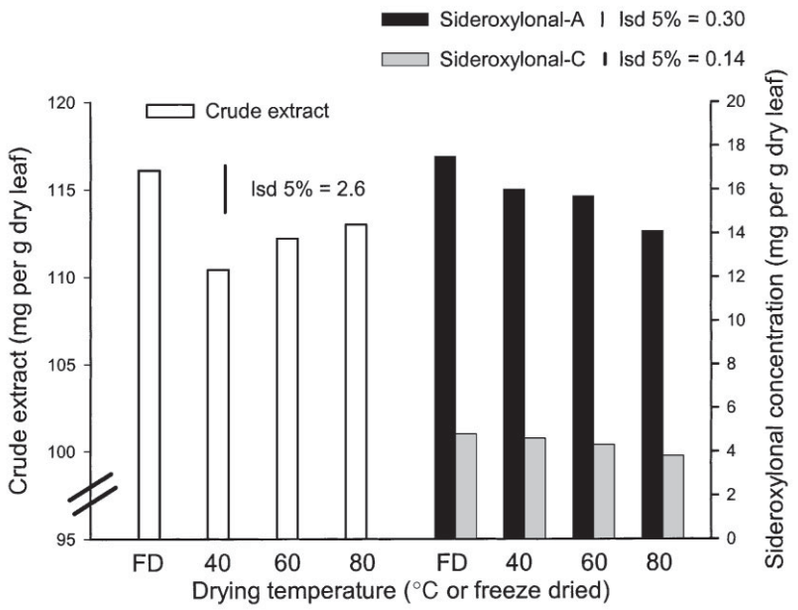


Figure 3. The mean yield of crude extract (open columns) and of sideroxylonals (shaded columns) when foliage from various *Eucalyptus melliodora* was freeze-dried (FD) or dried in a forced-draught oven at temperatures of 40, 60 or 80°C. Bars are least significant differences (lsd) with a probability of $p < 0.05$.

extract minus sideroxylonals ($r^2 = 25$–59%; $p < 0.001$ for the four species). There was no relationship between these variables for a relatively large sample of *E. nitens* ($n = 32$) but, unlike the other eucalypts, these were plantation trees with a low content of sideroxylonal. The relationship between yield of sideroxylonals and crude extract indicates that trees which produce high concentrations of sideroxylonals also produce higher concentrations of other lipophilic substances which may be important in plant defence.

The effect of extraction time on the yield of sideroxylonals from *Eucalyptus* leaf

Soxhlet extraction of dry, ground *Eucalyptus* leaf for 13 h rather than for 4 h yielded roughly 12% more crude extract (means = 120, 133; $F_{1,13} = 25.76$; $p < 0.001$) and 14% more sideroxylonal (means = 35.6, 40.5; $F_{1,13} = 20.04$; $p < 0.001$). The yield of sideroxylonals was closely related to the yield of crude extract (linear regression $r^2 = 0.92$; $F_{1,12} = 149$; $p < 0.001$; SE of observations = 3.10). The fact that an extra 9 h of extraction yielded 10 to 15% more of each constituent prompted us to repeat the study as a factorial design. In this study, increasing the extraction time from 2 to 4 h significantly increased the yield of sideroxylonals but further increases in extraction time had no effect (Fig. 4). The data suggest that the relationship is exponential with an inflection point near 4 h. Thus slight changes in extraction conditions, e.g. the temperature of the extraction, could markedly alter the yield of sideroxylonals and this is the most likely explanation for the discrepancy between the two extraction-time studies. It is important to note that no decline in sideroxylonal yield with increasing extraction time was observed indicating that the compound was stable under the extraction conditions. The recommended time for extraction of sideroxylonals using the described method is thus 6 h (see Fig. 4).

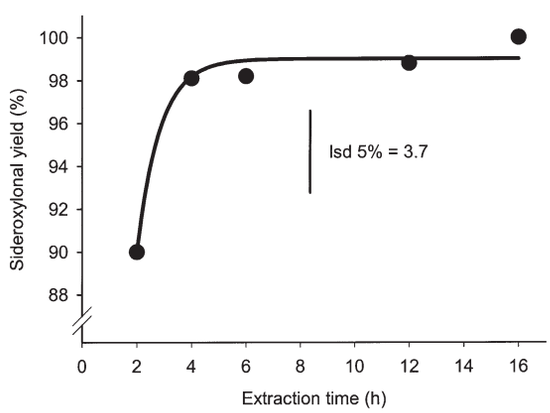


Figure 4. The effect on the yield of sideroxylonal of extracting dry, ground *Eucalyptus melliodora* foliage for 2, 4, 6, 12 or 16 h. Results are expressed as the yield of sideroxylonals as a percentage of the yield after extracting for 16 h.

The deterioration of sideroxylonals in crude extracts stored in mobile phase

Sideroxylonals decompose when they are stored in HPLC mobile phase (Fig. 5). Although the effect of storage time is highly significant ($p < 0.001$), with significantly less sideroxylonals present at each consecutive measurement ($p < 0.01$), the deterioration is slow. After 4, 10 and 15 days of storage there were 7, 15 and 21% less sideroxylonals, respectively, compared to the original samples. These data suggest that it is acceptable to analyse samples within a few hours of preparation.

The effect on the yield of sideroxylonals of long term storage of dry, ground *Eucalyptus* foliage

Storing dry, ground *E. melliodora* leaf at room temperature for 20 months resulted in losses of sideroxylonals

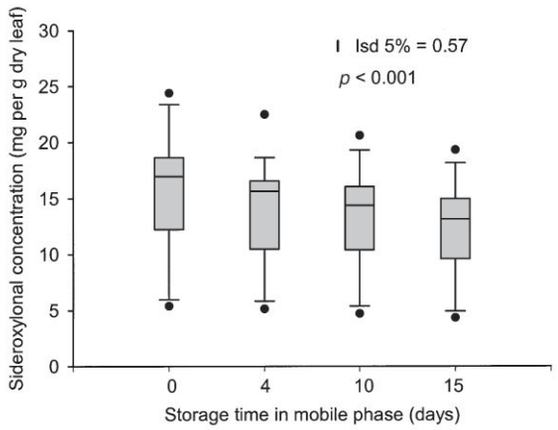


Figure 5. The effect on yield of sideroxylonal of storing samples in mobile phase for 0, 4, 10 and 15 days. Data are represented as box plots where the box incorporates the 25–75% quartiles and the median, the error bars the 10 and 90% quartiles, and the dots are the 5 and 95% quartiles. The bar is the least significant difference ($p < 0.05$).

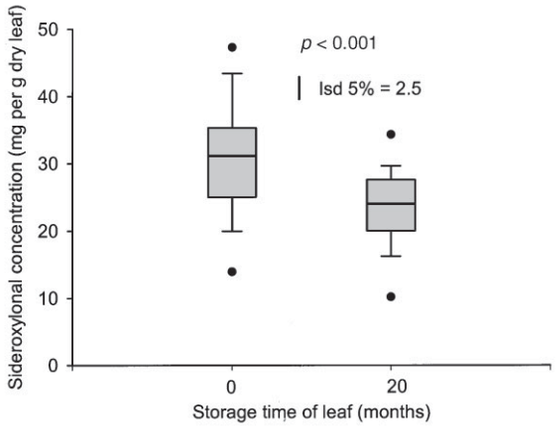


Figure 6. The effect on sideroxylonal yield of storing dry, ground *Eucalyptus melliodora* foliage for 20 months. Data are presented as box plots where the box incorporates the 25–75% quartiles and the median, the error bars the 5 and 95% quartiles and the dots are the minima and maxima. The bar is the least significant difference ($p < 0.05$).

ranging from 4 to 42% (mean = 22%; SD = 8.2%; $p < 0.001$; Fig. 6). The magnitude of the proportional loss from ground leaf was unrelated to the initial concentration of sideroxylonals. In contrast, there was little loss of sideroxylonals when crude extracts were stored for long periods. Thus, crude extracts from twelve trees, which varied in sideroxylonal content from 0.5 to 52 mg/g dry matter, were stored for 22 months prior to re-analysis and a paired *t*-test showed that the stored samples contained slightly less sideroxylonals (means = 23.7, 23.1), but the result was insignificant ($t_{11} = 1.66$; $p = 0.066$). No attempt was made to model the loss of sideroxylonals from ground leaf material over time or to identify the degradation products or, indeed, to minimise the loss of sideroxylonals. Storing samples at room temperature for 20 months is perhaps extreme, but the fact that sideroxylonals are lost suggests a need for understanding the timing of decomposition. Regardless of whether it is important to know the exact concentrations of sideroxylonals in foliage or the relative concentrations between trees, samples need to be extracted soon after the foliage is dried, assuming that no losses occur when freshly picked leaves are stored frozen. The results suggest that if samples are extracted and stored in the dark, then losses of sideroxylonals occur very slowly.

The procedures described in this paper for the extraction and determination of sideroxylonals provide a simple and accurate method of analysis with an average coefficient of variation between samples of less than 3%. However, the analysis is slow and could probably be improved by modifying the drying step after transfer of the crude extract to the glass vial.

Acknowledgments

This work was supported by research grants from the Australian Research Council to Dr. W. J. Foley and together with Dr. H Etoh (Shizuoka University, Japan). The authors wish to thank Stuart Dennis for help with the preparation of the figures, and Professor T. Konoshima (Kyoto Pharmaceutical University, Japan) for assisting with the study visit of M.T. to Australia.

REFERENCES

Christians JK. 1999. Controlling for body mass effects: is part–whole correlation important? *Phys Biochem Zool* **72:** 250–253.

Eschler BM, Foley WJ. 1999. A new sideroxylonal from *Eucalyptus melliodora* foliage. *Aust J Chem* **52:** 157–158.

Eschler BM, Pass DM, Willis R, Foley WJ. 2000. Distribution of foliar formylated phloroglucinol derivatives amongst *Eucalyptus* species. *Biochem System Ecol* **28:** 813–824.

Ghisalberti EL. 1996. Bioactive acylphloroglucinol derivatives from *Eucalyptus* species. *Phytochemistry* **41:** 7–22.

Lawler IR, Foley WJ. 2002. Chemical ecology of *Eucalyptus* essential oils in plant-herbivore interactions. In *Medicinal and Aromatic Plants—Industrial Profiles, Vol. 22—Eucalyptus*, Coppen JJW (ed.). Taylor and Francis: London; 324–344.

Lawler IR, Foley WJ, Eschler B, Pass DM, Handasyde K. 1998. Intraspecific variation in secondary metabolites determines food intake by folivorous marsupials. *Oecologia* **116:** 160–169.

Lawler IR, Foley WJ, Eschler BM. 2000. Foliar concentration of a single toxin creates habitat patchiness for a marsupial folivore. *Ecology* **81:** 1327–1338.

Neve J, de Almeida Leone P, Carroll AR, Moni RW, Paczkowski NJ, Pierens G, Björquist P, Deinum J, Ehnebom J, Inghardt T, Guymer G, Grimshaw P, Quinn RJ. 1999. Sideroxylonal C, a new inhibitor of human plasminogen activator inhibitor type-1, from the flowers of *Eucalyptus albens J Nat Prod* **62:** 324–326.

Satoh H, Etoh H, Watanabe N, Kawagishi H, Arai K, Ina K. 1992. Structures of sideroxylonals from *Eucalyptus sideroxylon. Chem Lett* 1917–1920.

Singh IP, Takahashi K, Etoh H. 1996. Potent attachment-inhibiting and -promoting substances for the blue mussel, *Mytilus edulis galloprovincialis*, from two species of *Eucalyptus. Biosci Biotechnol Biochem* **60:** 1522–1523.

Tatsuta K, Tamura T, Mase T. 1999. The first total synthesis of sideroxylonal-B. *Tetrahedron Lett* **40:** 1925–1928.

Wallis IR, Watson ML, Foley WJ. 2002. Secondary metabolites in *Eucalyptus melliodora*: field distribution and laboratory feeding choices by a generalist herbivore, the common brushtail possum. *Aust J Zool* **50:** 507–519.