PERGAMON

Biochemical Systematics and Ecology 28 (2000) 813–824

biochemical systematics and ecology

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# Distribution of foliar formylated phloroglucinol derivatives amongst *Eucalyptus* species

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Received 25 August 1999; accepted 15 November 1999

**Abstract**

Formylated phloroglucinol compounds (FPCs) in *Eucalyptus* leaves are important determinants of feeding in marsupial folivores and have a wide range of other biological actions. We conducted a survey of the occurrence of formylated phloroglucinol compounds (euglobals, macrocarpals and sideroxylonals) in acetone–petrol extracts of 41 species of *Eucalyptus* from among seven informal subgenera growing on the East Coast of Australia. We used electrospray ionisation, Fourier transform ion cyclotron resonance mass spectrometry (ESI-FTMS) to search crude extracts of eucalypt leaves for molecular weights characteristic of FPC compounds. We found masses characteristic of reported FPCs in 27 of the 41 species examined. The most frequently identified group of compounds was the sideroxylonals. Notable was the lack of known FPCs in the informal subgenus *Monocalyptus*. 

*Keywords:* *Eucalyptus*; Phloroglucinol; Aldehyde; Euglobal; Macrocarpal; Sideroxylonal; Poymorphism; Marsupial

## 1. Introduction

*Eucalyptus* (L'Hérit) is widely known as a source of terpenoids (Boland et al., 1991) and polyphenolic compounds (Hillis, 1966) used in chemosystematic studies (Hillis,

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0305-1978/00/$ - see front matter 
PII: S0305-1978(99)00123-4

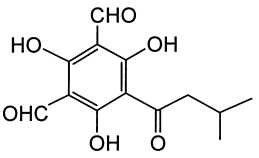
1966; Li et al., 1996, 1997; Brophy et al., 1998; Dunlop et al., 1997) some of which are biologically active. The relative ease of extraction, separation and identification of terpenoids from *Eucalyptus* foliage has meant that many studies of biological activity in *Eucalyptus* have focused solely on this group of compounds (Coppen, 2000). More recently, Japanese researchers have isolated a new group of compounds in *Eucalyptus* with wide-ranging biological activity (e.g. Kozuka et al., 1982a,b). These compounds are mono to tetra-formylated phloroglucinol based derivatives with an attached terpene moiety (isoprene, a monoterpene or a sesquiterpene). To date, all except one example (jensenone from *Choriocarpa subargentea*: Myrtaceae: Brophy and Goldsack, 1994) have been isolated from eucalypts. Our interest in these compounds was sparked by our finding that macrocarpal G was a highly effective antifeedant for at least one marsupial herbivore, the common ringtail possum (*Pseudocheirus peregrinus*) (Pass et al., 1998; Lawler et al., 1998).

Previous work had reported the occurrence of these formylated phloroglucinol compounds (FPCs) in 13 species of *Eucalyptus* representing a limited number of groupings in the genus (Table 1). There are about 800 species of eucalypts and these have been grouped into eight informal subgenera (Pryor and Johnson, 1971) two of which (*Corymbia* and *Blakella*) have recently been described as a new genus *Corymbia* (Hill and Johnson, 1995). All previous studies of the FPCs have been made of members of the informal subgenus *Symphyomyrtus*. We hypothesised that the antifeedant effects of macrocarpal G were more widespread within *Eucalyptus* and so we wanted to get a rapid overview of the occurrence of FPCs in a range of *Eucalyptus* species covering most of the currently recognised sub-genera in the genus. In particular, we wanted to know whether FPCs were widespread in species that are important foods for koalas and other folivorous marsupials and whether particular structural types such as the euglobals had a restricted distribution within *Eucalyptus*.

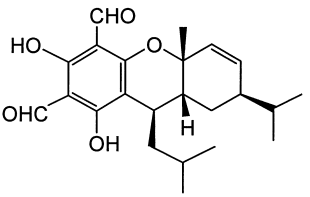
To date seven different general structural groupings of FPCs (Fig. 1) (and many isomers) have been reported largely from eucalypts grown in Japan (Table 1 and Ghisalberti, 1996). Since the separation of all these different compounds chromatographically is demanding (Akano et al., 1981; Eschler and Foley, 1999) we employed electrospray ionisation, Fourier transform ion cyclotron resonance mass spectrometry (ESI-FTMS) to search crude extracts of eucalypt leaves for molecular weights characteristic of FPC compounds. In addition, we used an NMR procedure to estimate approximate concentrations of FPC present in each species surveyed. This was calculated in terms of μmol of aldehyde present per gram of dry leaf. We believe that this semi-quantitative approach is an essential first step to identifying broad patterns of variation in such a large genus so that more detailed chromatographic studies can be better targeted.

## 2. Methods

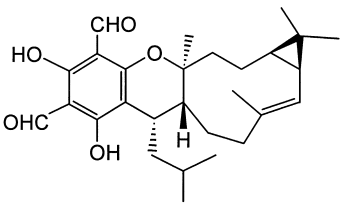
We selected 41 species that included seven of the eight informal subgenera of *Eucalyptus* identified by Pryor and Johnson (1971). Most species were chosen because we knew that they were regularly browsed by folivorous marsupials or else were



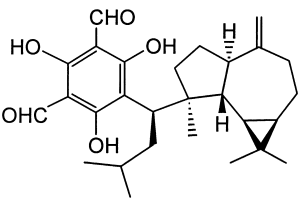

(1) Jensenone (MW 266.07902)



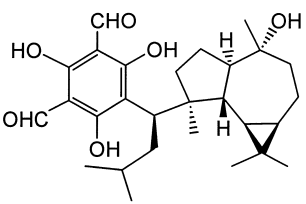

(2) Euglobal Ia$_1$ (MW 386.20989)



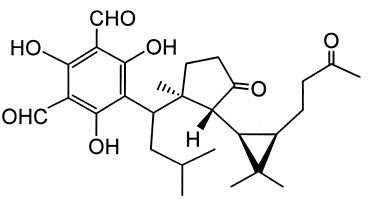

(4) Euglobal III (MW 454.27189)



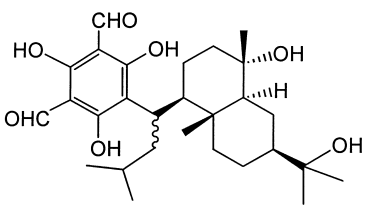

(4) Macrocarpal C (MW 454.27189)



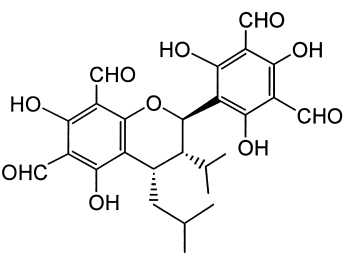

(5) Macrocarpal A (MW 472.28245)




(6) Eucalyptone (MW 486.26171)




(7) Macrocarpal I (MW 490.29301)




(8) Sideroxylonal A (MW 500.16822)

Fig. 1. Structures of some known formylated phloroglucinol compounds isolated from *Eucalyptus*. Numbering of each structure corresponds to the columns in Tables 1 and 2.

dominant species within the habitats of folivorous marsupials on the East Coast of Australia. The remainder were chosen to ensure that all informal subgenera were represented (e.g. *E. cloeziana* in the monospecific informal subgenus *Idiogenes*). Most trees were growing in their natural distribution. Eight samples came from cultivated trees, but all these were growing in unwatered, unfertilised areas removed from

Table 1
Reported occurrence of formylated phloroglucinol compounds in *Eucalyptus* (all species examined are from informal subgenus *Symphomyrtus*)

| Grouping ≫<br>Species | 1<br>Simple FPCs | 2<br>Non-oxygenated monoterpene euglobals | 3<br>Mono or di-oxygenated monoterpene euglobals | 4<br>Non-oxygenated sesquiterpene euglobals and macrocarpals | 5<br>Mono-oxygenated sesquiterpene macrocarpals | 6<br>Eucalyptone | 7<br>Di-oxygenated sesquiterpene macrocarpals | 8<br>Sid-eroxylonals |
|---|---|---|---|---|---|---|---|---|
| *jensenii* | Jensenone[a] | | | | | | | |
| *sideroxylon* | | | | | | | | A,B[b] |
| *macrocarpa* | | | | G( = M-C)[c] | A,[d] B,C,D,E,F[c] | | | |
| *incrassata* | | | | III, V, In-1[e] In-2, In-3[f] | | | | |
| *amplifolia* | | Am-1, Am-2[f] | | | A,B,E[g] | Am-1[g] | | |
| *blakelyi* | | Bl-1,Ib, Ic,IIa[h] | | | | | | |
| *tereticornis* | | T1,IIc[i] | | | | | | |
| *globulus (euglobals)* | | $Ia_1$,$Ia_2$,Ib,Ic,IIa,IIb, IIc[j,k] | | III,IV,IVb,V,VI,VII, VIII,IX[l,m] | | | | |
| *globulus (macrocarpal)* | | | | M-C( = G)[n,o] | M-A,M-B,M-D, M-E[n], H[o] | Eucalyptone[o,p] | I,J[o] | |
| *pulverulenta* | Grandinol[q] | | | | | | | |
| *viminalis* | | | | | | Euvminal[r] | | |
| *grandis* | Grandinol,[s] Homograndinol[t] | G1,G2,G3, | | | | | | |
| | | G4,G5,[u,v] G6,G7[w] | | | | | | A[x] Grandinal[y] |
| *robusta* | | Robustadial-1,-2[zaa] | | | | | | |

[a]Boland et al. (1992).
[b]Satoh et al. (1992).
[c]Yoneyama et al. (1989).
[d]Murata et al. (1990).
[e]Takasaki et al. (1994b).
[f]Takasaki et al. (1994a).
[g]Singh and Etoh (1995).
[h]Takasaki et al. (1990).
[i]Kokumai et al. (1991).
[j]Kozuka et al. (1982b).
[k]Amano et al. (1981).
[l]Kozuka et al. (1982b).
[m]Sawada et al. (1980).
[n]Nishizawa et al. (1992).
[o]Osawa et al. (1996).
[p]Osawa et al. (1995).
[q]Bolte et al. (1984).
[r]Savina et al. (1992).
[s]Crow et al. (1977).
[t]Takasaki et al. (1995).
[u]Xu et al. (1984).
[v]Takasaki et al. (1994c).
[w]Singh et al. (1998).
[x]Singh et al. (1996).
[y]Singh et al. (1997).
[z]Yamakoshi et al. (1992).
[aa]Cheng and Snyder (1988).

immediate human influences. We did not collect voucher specimens since our aim was to conduct a rapid survey and not to report on new compounds. Details of collection localities can be obtained from WJF.

We collected foliage from four individual trees from within a circle of 100 m diameter. Our previous studies have shown that the concentration of FPC can vary significantly among individuals growing adjacent (Pass et al., 1998; Lawler et al., 2000) and we wanted to avoid the possibility of collecting only a single phenotype.

Approximately 25 g of mature, adult foliage was collected from the mid-canopy of each tree and bulked together with the other samples from that species and stored at $-20^{\circ}C$. A subsample was taken and ground with liquid nitrogen in a mortar and pestle. A further subsample (5 g) of this material was then extracted in a 20 : 80 (v/v) mixture of acetone and light petroleum spirit (40 – 60°C boiling fraction) in a Soxhlet apparatus for 16 h. Subsequent extraction of the residue with methanol and examination of the extract by TLC showed no evidence of FPC compounds when treated with the ketone/aldehyde specific stain, dinitrophenyl hydrazine/phosphoric acid.

For FTMS analysis, about 5 mg of dried crude extract was dissolved in 1 ml MeOH and 10 μl diluted to 1 ml in MeOH. This solution was continually infused at a flow rate of 1 $\mu l\,min^{-1}$ into the external electrospray source (Analytica of Bradford, Bradford, CT) of a Bruker BioApex 47e Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (FTMS) operating in negative ion mode with broadband (low resolution (6–10 k FWHM at m/z 500)) detection. Typically, the signal was averaged over 16 transients prior to Fourier transformation, requiring a data acquisition time of about 1 min, and the consumption of about 1 μg of the crude extract. Since the nature of the compounds we expected to detect was well known from previous investigations, no attempt was made for precise calibration or accurate mass measurement, measured mass within 0.05 Da being considered an acceptable confirmation of the presence of the compounds of interest.

We used a semi-quantitative NMR procedure to estimate the concentration of total FPCs in each extract, as μmol of aldehyde present per gram of dry leaf. We compared the integration of the total area of the aldehyde proton peak to the peak area for an internal standard (m-dinitrobenzene). This gives an indirect measure of the number of moles of FPC present but depends on the number of formyl groups in each compound. For example, 1 mol of the sideroxylonals would give twice the area of 1 mol of the euglobals/macrocarpals and four times the area of 1 mol of the G-euglobals. This is because the sideroxylonals have four aldehydes per molecule, the euglobals/macrocarpals two and the G-euglobals one. Although we recognise the limitations of this method, we feel that it is a useful first approximation in a wide-ranging survey of a relatively new group of metabolites. As a check on the validity of the method, we collected five samples of foliage from both *E. sideroxylonal* and *E. polyanthemos* that contained the dimeric FPCs, the sideroxylonals, as the major constituent. We chose the particular samples trees to span a wide range of concentration. We extracted FPCs as described above and estimated the concentration of two isomers of sideroxylonal (A and B) by HPLC. The HPLC method involved eluting standards and samples isocratically in methanol : water : trifluoroacetic acid (95 : 4.9 : 0.1) on a reverse phase column (Waters Novapak C18, $3.9 \times 150$ mm) at 1.0 ml/min with

ultra-violet detection (275 nm). The calibration curve was generated over 2–3 order of magnitude in concentration.

## 3. Results and discussion

It is clear from our results that compounds having identical masses to known FPCs are widely distributed within *Eucalyptus* (Table 2). In some instances isolation of the compounds is evidence that our inference of the presence of the compound from observations of the accurate molecular mass is warranted. For example, we have isolated Macrocarpal G from the *Eucalyptus ovata* sample that was used in these experiments (Pass et al., 1998; Lawler et al., 1998) together with a range of monoterpene (e.g. Euglobal Ia, Ib, IIa, BI–1) and sesquiterpene (Euglobal III) euglobals (Pass et al., 1998). Similarly, we have isolated sideroxylonal A and B from *E. polyanthemos, E. sideroxylonal*, *E. melliodora* and *E. microcorys* samples used for this survey and sideroxylonal C from *E. melliodora* (Eschler and Foley, 1999). There is also some evidence from this work that there could be other, as yet unknown FPCs (column 8 of Table 2). For example, compounds with molecular masses consistent with the molecular formulas ($C_{23}H_{30}O_{5+n}$) could represent "oxidised" monoterpenoid euglobals. Similarly, we observed indications of compounds with molecular formulas that could represent macrocarpals plus two protons ($C_{28}H_{40+2}O_{5-7}$) or "oxidised" sideroxylonals ($C_{26}H_{28}O_{10+n}$).

Compounds with masses identical to known FPCs were present in 27 of the 41 species examined. Most notable was the lack of euglobals, macrocarpals, and sideroxylonals in species belonging to the informal subgenus *Monocalyptus* although we did detect masses that may represent as yet unreported FPCs. Similarly, we observed no masses that could be attributed to reported FPCs in *E. cloeziana*, the sole member of the informal subgenus *Idiogenes*, and several species of the informal subgenus *Corymbia* (e.g. *E. clarksoniana*). However, note that we did not detect any masses characteristic of FPCs in *E. globulus*, a species that provided the source for the first isolation of euglobals. This finding is discussed further below. The most widespread structural grouping was the sideroxylonals. To date three stereoisomers, sideroxylonals A, B and C, and a regiomer, grandinal, have been reported but it appears that sideroxylonal A is the most common isomer of this molecular weight (Eschler and Foley, 1999).

It is important to note that there is evidence that *Eucalyptus* is polymorphic for FPCs and that a negative result here does not exclude the possibility that another specimen of a particular species will contain FPCs. For example in *E. polyanthemos* we measured by HPLC, a range of concentrations of sideroxylonal A between 0 and 1.3% of dry matter whereas in *E. melliodora* the range of concentrations of sideroxylonal A was even greater (0.01% of dry matter to 3.9% of dry matter). Therefore, if during this survey we had sampled trees that contained only traces of the compound, then we might have concluded that sideroxylonals did not occur in either of these species. Similarly, published work (summarised in Table 1) has reported the isolation of a range of euglobals and macrocarpals in *E. globulus*, yet the tissues that we sampled

contained no detectable compounds. Our specimens were from trees grown in a commercial plantation for wood fibre and in view of the common negative correlation between growth rates and plant defence, it is possible that the breeding programme has selected against this group of secondary metabolites in commercial stocks. Given the variability and wide distribution of *E. globulus*, variability should not be surprising. Accordingly, a negative result cannot be taken as definitive evidence that a particular species always lacks FPCs, but this is true of any survey (e.g. Boland et al., 1991) with a polymorphic genus such as *Eucalyptus*. Wider sampling might identify individuals that contain the compounds of interest.

Nonetheless we believe that the consistent lack of euglobals, macrocarpals and sideroxylonals in all species in the informal subgenus *Monocalyptus* that we examined is more likely a real result rather than a reflection of any polymorphism. This position is supported by data collected by Konoshima and Takasaki (2000) who could not find euglobals in any species of the informal subgenus *Monocalyptus* that they examined using LC–MS.

Eucalypts from the informal subgenus *Monocalyptus* differ from those of the other large informal subgenus *Symphyomyrtus* in several ways including a lower susceptibility to insect attack, lower early growth rates and lower root/shoot ratios (Noble, 1989; Stone et al., 1998). If the lack of FPCs in the informal subgenus *Monocalyptus* is confirmed through more detailed chemical studies, future work could profitably examine whether FPCs play any role in promoting insect specialisation on eucalypts of the two informal subgenera and whether there is a different basis of chemical defence between these two important groupings.

Although no formal studies have been carried out, several biosynthetic schemes have been proposed (Ghisalberti, 1996) for the FPCs. For example, Kozuka et al. (1982a,b)) proposed that the euglobals are formed by a Diels-Alder reaction between common leaf terpenes (e.g. bicyclogermacrene) and an *o*-quinone methide intermediate derived from grandinol. Similarly, the sideroxylonals may be formed by a Diels-Alder-type dimerisation of an *o*-quinone methide intermediate and a styrene intermediate both derived from grandinol and the macrocarpals by the addition of the relevant sesquiterpene to a carbocationic species derived from grandinol (Ghisalberti, 1996; Pass et al., 1998). Most species in the informal subgenus *Monocalyptus* contain significant concentrations of foliar terpenes and if simple FPCs also occur (e.g. *E. stellulata*), then the lack of formation of euglobals or macrocarpals may be due to lack of appropriate enzymes to effect the condensation of the two compounds. Other studies (Lawler et al., 2000) have demonstrated a correlation between foliar 1,8 cineole and sideroxylonal concentrations in *E. polyanthemos* suggesting that the biosynthesis of FPCs is linked with the biosynthesis of terpene precursors. Clearly, detailed biosynthetic studies are required to evaluate these options.

The NMR method used to quantify the total FPCs present in each extract depends on assumptions about the main structural types present. The method is based on integrating the signal from the formyl groups that are present on the aromatic ring and then assuming that the aldehyde signal is only due to the predominant FPCs present. A sideroxylonal-rich species will have 1 mol of compound present for every 4 mol of aldehydes present. For macrocarpal/euglobal rich species the ratio is

Table 2
Electrospray ionisation Fourier transform mass spectral analysis of acetone–petrol extracts of *Eucalyptus* foliage[a]

| Subgenus | Grouping≫ Species | 1 Simple FPCs | 2 Mono-terpene euglobals | 3 Non-oxygenated sesquiterpene euglobals and macrocarpals | 4 Mono-oxygenated sesquiterpene macrocarpals | 5 Eucalyptone | 6 Di-oxygenated sesqui-terpene macrocarpals | 7 Sideroxy-lonal | 8 Other FPCs? | 9 Other compounds | 10 μmol 'CHO'/ dry leaf, by NMR |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Blakella* | *tessellaris* | | | | | | | | + | + + | 0 |
| *Corymbia* | *clarksoniana* | | | | | | | | | + + | Trace |
| | *citriodora* | | + | | | | | + | | + + | 1.06 |
| | *maculata* | | + | | | | | | + + | | 0.82 |
| | *peltata* | | | | | | | | + + | | 3.51 |
| | *torelliana* | | + | | | | | | + + | | 30.5 |
| *Eudesmia* | *phoenicea* | | + + | + | + | + | | + | | + | 5.39 |
| *Idiogenes* | *cloeziana* | | | | | | | | + | + + | 0 |
| *Monocalypts* | *acmenoides* | | | | | | | | + | + + | 0 |
| | *delegatensis* | | | | | | | | + | + + | Trace |
| | *haemastoma* | | | | | | | | | + + | 7.85 |
| | *nigra* | | | | | | | | + + | | 0 |
| | *nitida* | | | | | | | | + | + + | 6.41 |
| | *obliqua* | | | | | | | | + | + + | Trace |
| | *pauciflora* | + | | | | | | | + + | | 1.31 |
| | *pilularis* | | | | | | | | | + + | 0 |
| | *regnans* | | | | | | | | | + + | 0 |
| | *stellulata* | + | | | | | | | + | + + | 0 |
| *Symphomyrtus* | *drepanophylla* | | + | + | + + | + | | + | | + | 28.2 |
| | *jensenii* | + | + | | | | | | + + | | 56.5 |
| | *melliodora* | | + | + | + | + | | + + | | + | 92.7 |
| | *sideroxylon* | | | | | | | + + | + | | 120.1 |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *polyanthemos* | | | | | + | | + | | | | + | + | | + | | | | 116.4 |
| *cladocalyx* | + | | + | | + | | + | | | + | | + | | + | | | + | 1.97 |
| *camaldulensis* | | | + | | + | | + | | + | | | + | | + | | | | 58.5 |
| *exserta* | | | + | | + | | + | | + | | | + | | + | | | | 34.7 |
| *tereticornis* | | | + | | + | | + | | + | | | + | | + | | | | 39.9 |
| *acaciaeformis* | + | | + | | | | | | | | | + | | + | | | | 54.1 |
| *dalrympleana* | + | | | | | | | | | | | + | | | | | + | 0 |
| *dunnii* | | | | | + | | + | | + | | | + | | + | | | | 0 |
| *globulus* | | | | | | | | | | | | | | + | | | | 8.84 |
| *nitens* | + | | + | | | | + | | | | | + | | | | | + | 1.83 |
| *ovata* | | | | | + | | + | | + | | | + | | + | | | | 76.6 |
| *rubida* | | | + | | + | | + | | + | | | + | | | | | | 9.79 |
| *microcorys* | | | | | | | + | | | | | + | | | | | | 126.6 |
| *botryoides* | + | | + | | + | | + | | | | | + | | + | | | + | 0 |
| *grandis* | + | | + | | | | | | | | | + | | + | | | | 23.7 |
| *pellita* | | | + | | | | | | | | | + | | | | | + | 0 |
| *punctata* | | | | | | | | | | | | + | | + | | | | 8.71 |
| *resinifera* | | | | | | | | | | | | + | | | | | | 65.9 |

[a] + Evidence of particular compounds being present. + + main compound(s) present (negatively ionisable).

1 Includes jensenone (MW = 266), grandinol (252), homograndinol (252) and other unknown simple FPCs.

2 Euglobals without any oxygens on the monoterpene part of the molecule. Includes all known monoterpene Euglobals (386).

3 Euglobals and macrocarpals without any oxygens on the sesquiterpene part of the molecule. Includes all known sesquiterpene euglobals (454) and Macrocarpal G, M-C(454).

4 Macrocarpals with one oxygen on the sesquiterpene part of the molecule. Includes macrocarpal A, M-A, B, M-B, C, M-C, D, M-D, E, M-E, F, H (472).

5 Eucalyptone (486) with two oxygens on the sesquiterpene part of the molecule.

6 Macrocarpals with two oxygens on the sesquiterpene part of the molecule. Includes macrocarpal I, J (490).

7 Sideroxylonal (500) and its monomeric fragment (250). Includes grandinal.

8 There is evidence of other FPCs (although far from conclusive). Some are two mass unit higher than existing macrocarpal and others of MW greater than 500, and/or euglobals (or macrocarpals) with one or more oxygens on the monoterpene part of the molecule (MW of 402, 404, 418).

9 This group includes long-chain fatty acids, aldehydes, alcohols, and triterpenoid compounds.

10 NMR results calculated as described in the discussion.

assumed to be 1 : 2. Although this reduces the accuracy of the method, it still indicates the order of compounds present. Another source of error is that although the FPCs provide the main source of known aldehydes present in an acetone–petrol extract of *Eucalyptus* foliage, there are other aldehyde-containing compounds present (e.g. *p*-cymene: Boland et al., 1991). Because of this, a small portion of the aldehyde signal could be due to the presence of aldehyde groups from some simple terpenes and medium-to-long chain fatty aldehydes that commonly occur in the foliage but this should be a small source of error given the low concentrations found in foliage (Boland et al., 1991). We used the NMR method in this survey because there is as yet no effective separation and quantification method that can deal with the diversity of FPCs that can occur in a single species. The NMR method does give a reasonable estimate of the quantities of FPCs present as demonstrated by our comparison of the estimated concentration of sideroxylonal in a series of *E. polyanthemos* and *E. sideroxylonal* (Fig. 2). These samples were analysed by both NMR and also by a more precise HPLC method. At least for those species dominated by sideroxylonals, the NMR method was a reasonable approximation of actual concentrations of foliar FPCs. The elevated quantities found by NMR compared with HPLC suggest that the NMR method measures any macrocarpals and euglobals present as well as the sideroxylonals, while the HPLC method though more accurate only measured the sideroxylonals present.

Notwithstanding these caveats there were significant concentrations of aldehydes, largely derived from FPCs in several species. From this limited survey, it appeared that sideroxylonal-rich species such as *E. melliodora, E. polyanthemos*, and *E. microcorys* accumulate significantly greater concentrations of total FPCs compared with euglobal- and macrocarpal-rich species such as *E. phoenicia, E. drepanophylla* and

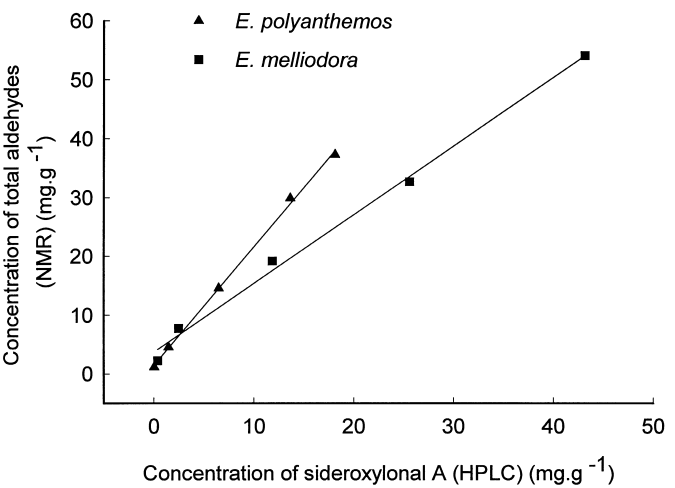



Fig. 2. Relationship between foliar concentrations of sideroxylonal A measured by HPLC and concentration of total aldehydes as determined by semi-quantitative NMR in foliage from five individual trees of *Eucalyptus polyanthemos* and *E. melliodora*.

*E. camaldulensis*. Further work should attempt to correlate concentrations of characterized FPCs with terpene profiles.

In conclusion, FTMS proved extremely useful for conducting a wide-ranging and rapid survey of the presence of a group of poorly known compounds. Our demonstration that the FPCs are widely distributed within the genus suggests that they are likely to play an important ecological role in the ecology of *Eucalyptus*. There is already clear evidence that these compounds have widespread bioactivity (e.g. Ghisalberti, 1996; Pass et al., 1998; Lawler et al., 2000). Accordingly, the study of this group of compounds should be given priority.

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