

Induction of hepatic microsomal oxidative metabolism in mice by essential oil components from some *Eucalyptus* spp. and Queensland fodder trees

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Summary

The essential oil from *Eucalyptus caleyi* as well as the separated monoterpene, sesquiterpene hydrocarbon and sesquiterpene alcohol fractions of the oil, when applied orally (500 mg kg^{-1} body-weight), decreased the pentobarbital sleeping time of mice by about 50% compared with a control group. Treatment with 15 sesquiterpene alcohols, with the essential oils from other *Eucalyptus* spp. or from certain Queensland fodder trees, produced a range of decreases from 13 to almost 70%. Extensive ingestion of drought-fodder plants containing essential oils may similarly be expected to enhance the activity of the liver detoxication enzymes of grazing livestock and thus to increase their susceptibility to mortality from carbon tetrachloride drenches.

1. INTRODUCTION

Fodder trees can provide a cheap and effective means of keeping animals alive during drought (Everist 1969) by virtue of their nutritional value as reported by McDonald and Ternouth (1979) and by others previously. However, a 6% mortality was noted in 1972 when a large flock of sheep, subsisting on tree and shrub foliage in southern Queensland, was drenched with carbon tetrachloride (CCl_4) (Seawright, Steele and Menrath 1972).

Investigating possible causes of the deaths of the sheep, Seawright *et al.* (1972) found that, of the plants most likely to be eaten by the animals in significant amounts, only *Eucalyptus caleyi* foliage elevated the mixed function oxidases of the liver microsomes (MFO) to levels previously achieved with phenobarbitone and DDT treatments. These workers also found an increase in CCl_4 susceptibility, including typical CCl_4 mortality, in one of five test sheep fed *E. caleyi* foliage for 11 days.

Carbon tetrachloride, when used as an anthelmintic for sheep, has occasionally caused unpredictable mortalities (Setchell 1962). Metabolism of the CCl_4 by the MFO is a prerequisite for toxicity to the animal (Garner and McLean 1969).

The rate of metabolism of barbiturates, indicated by the duration of their effects after injection, has been used to estimate the activity of the hepatic MFO *in vivo* (Conney 1967). In particular, pentobarbital and ethanol sleeping times of mice have been employed to study this system after prior dosing with various components of essential oils (Seto and Keup 1969).

The aim of the present work was initially to determine which fractions and/or components of the essential oil of *E. caleyi* could produce elevated levels of MFO in mice; it was later extended to determine if the essential oils from foliage of other *Eucalyptus* spp. and fodder trees were similarly active.

2. MATERIALS AND METHODS

Essential oils from *Eucalyptus* spp. and other fodder trees

Foliage and small branchlets were collected from trees and shrubs during the period 1973 to 1978. For *E. caleyi*, foliage was taken separately from four trees in 1972 and 1974, and a bulk sample from all four trees was also collected. Samples of the *Eucalyptus* spp. and fodder trees were identified and herbarium numbers assigned by the Botany Branch, Department of Primary Industries. The essential oils, steam distilled with cohobation from the foliage, were stored at -20°C . Moisture levels of foliage and yields of essential oils were measured.

The oil samples were characterized by gas liquid chromatography (g.l.c.) on a 50 m support-coated open tubular (SCOT) glass capillary column at 155°C with FFAP as stationary phase. *E. caleyi* oil was divided into four broad boiling point ranges by distillation at reduced pressure in a Nester Faust spinning band still. (See Table 2 for boiling point ranges and percentages by volume.)

Measurement of hepatic microsomal oxidative metabolism in mice

The effect of essential oil fractions from *E. caleyi* and of sesquiterpene alcohols on the hepatic microsomal oxidative metabolism in mice was assessed by the measurement of barbiturate sleeping times (Weisburger, Schmehl and Pai 1965).

Male white mice of the Quackenbush strain (30 to 40 g), bred by the Central Animal Breeding House, University of Queensland, were used in the experiments. The animals (10 per group) were dosed orally, 24 h before the sleeping time determination, with 500 mg kg^{-1} body-weight of either the essential oil, oil fraction, sesquiterpene alcohol or 1,8-cineole diluted to 1 to 9 w/v with arachis oil. A control group, included with each experiment (Fujimoto and Plaa 1961), received arachis oil only. For the measurement of sleeping times (taken as the time elapsed between loss and regaining of righting reflex), mice were injected intraperitoneally with 50 mg kg^{-1} body weight of sodium pentobarbitone. Sleeping times were recorded and results expressed as a percentage of the control treatment. Unpaired 't' tests were used to compare sleeping times of control and treated mice.

3. RESULTS AND DISCUSSION

Yields of essential oils from the *Eucalyptus* spp. and fodder trees investigated are recorded in Table 1. While the moisture content of individual samples varied considerably, the essential oil levels were all substantial except for fuchsia bush. The g.l.c. traces of oils from the four different *E. caleyi* trees showed peaks of the same retention in each, but some marked quantitative differences were evident. The bulk samples of *E. caleyi* oil used in this investigation showed averaged levels of the same components.

The results in Tables 2 and 3 show that the bulk essential oil and the four distillation fractions from it all cause a significant reduction in the barbiturate sleeping time of mice. All are as active as 1,8-cineole, an oxygenated monoterpene and known inducer of the hepatic oxidative enzyme system (Jori, Di Salle and Pescador 1972).

Of the four distillation fractions (F1 to F4) from *E. caleyi*, analysis by g.l.c. showed that F1 consisted mainly of monoterpene hydrocarbons (α -phellandrene 33%, β -phellandrene 16%, *p*-cymene 8%, α -pinene 10%, β -pinene 10%, limonene 7% and terpinolene, α -thujene, γ -terpinene, α -terpinene and myrcene all less than 5%) and about 10% 1,8-cineole. F3 was mainly sesquiterpene hydrocarbons and F4 sesquiterpene alcohols. F2 contained minor amounts of components from F1 and F3 but was predominantly the common terpene alcohols,

Table 1. Moisture levels and essential oil yields from Queensland *Eucalyptus* spp. and fodder trees sampled 1973 to 1978

Sample	Herbarium number	Moisture (% wet weight)	Oil yield (% d.m.)
<i>Eucalyptus caleyi</i>	BRI 258010	49	0.8
<i>Eucalyptus pilligaensis</i>	BRI 242477	14	1.9
<i>Eucalyptus camaldulensis</i>	BRI 242478	25	1.3
<i>Eucalyptus cambageana</i>	BRI 242479	17	1.0
<i>Eucalyptus populnea</i>	BRI 242480	49	2.6
<i>Eucalyptus tessellaris</i>	BRI 250268	..	1.1*
<i>Eromocitrus glauca</i> (limebush)....	BRI 250267	..	1.0*
<i>Geijera parviflora</i> (wilga).....	BRI 242476	53	1.3
<i>Eremophila maculata</i> (fuchsia bush)	61	0.1

*Oil yield as percentage wet weight.

Table 2. Barbiturate sleeping times for mice treated with distillation fractions of the essential oil of *Eucalyptus caleyi*

Treatment	Percentage of total oil	Sleeping time as % of control (mean \pm s.d.)
Control	100 \pm 19
1,8-cineole	56 \pm 15***
F1 (b.p. 35°-70°/10 mm)	28	44 \pm 15***
F2 (50°-80°/2 mm).....	5	48 \pm 11***
F3 (65°-90°/1 mm).....	39	41 \pm 11***
F4 (> 90°/1 mm)	28	44 \pm 11***

Significantly different from control at $P < 0.001$.**Table 3. Barbiturate sleeping times for mice treated with distillation fraction F4 or the total essential oil from *Eucalyptus caleyi

Essential oil of <i>E. caleyi</i> and distillation fraction F4			
Experiment 1		Experiment 2	
Treatment	Sleeping time as % of control (mean \pm s.d.)	Treatment	Sleeping time as % of control (mean \pm s.d.)
Control	100 \pm 10	Control	100 \pm 18
<i>E. caleyi</i> oil	47 \pm 14***	<i>E. caleyi</i> oil	50 \pm 11***
F4	52 \pm 5***	F4	54 \pm 4***
1,8-cineole	67 \pm 10***		

***Significantly different from control at $P < 0.001$.

α -terpineol and terpinen-4-ol. In the present work, the individual sesquiterpene hydrocarbons of F3 were not identified, but capillary g.l.c. examination showed that fraction F4 consisted of about 12 sesquiterpene alcohols of which 6 were major components. Of these, globulol and epiglobulol were separated by preparative thin-layer chromatography and identified by spectroscopy and formation of derivatives.

A number of sesquiterpene alcohols, including some which co-eluted on capillary g.l.c. with peaks in F4, was available to us. Fifteen of these, representing a wide range of structural types, were used as test substances in a sleeping time experiment with the results shown in Table 4. The data indicate that most of the alcohols significantly reduce the barbiturate sleeping time of mice. For cedrol (Table 4) this reduction is similar to that reported by Wade, Holl, Hilliard, Molton and Greene (1968). The activities of several of the alcohols (anymol, cedrol, guaiol, α -santalol) are similar to that recorded for fraction F4 (Table 3). There is no easily discernible relationship between the chemical structures of the alcohols tested and their activities. Of the more active alcohols, anymol (a tertiary alcohol) is monocyclic and unsaturated, α -santalol (primary) and cedrol (tertiary) are tricyclic and saturated, and nerolidol (tertiary) is aliphatic. Of the alcohols tested, ledol, viridiflorol and β -eudesmol, which on g.l.c. evidence are components of the essential oil of *E. caleyi*, are of comparatively low activity. The activity of the sesquiterpene hydrocarbon cedrene is similar to that of the related cedrol (Wade *et al.* 1968), and this shows that inducing activity may be caused by hydrocarbons as well as by alcohols. The activity of fraction F1 confirms this for monoterpene hydrocarbons despite the reported inactivity of α - and β -pinenes in rats (Jori, Bianchetti and Prestini 1969). While the inactive and globular pinenes are similar in shape to the active globular molecule cineole, at least one of the flat molecules of α -terpineol and/or terpinen-4-ol is clearly as active as cineole or more so.

Table 4. Barbiturate sleeping times for mice treated with sesquiterpene alcohols

Treatment	Sleeping time as % of control (mean \pm s.d.)†	Treatment	Sleeping times as % of control (mean \pm s.d.)†
Control	100 \pm 14	β -eudesmol	87 \pm 16 n.s.
Farnesol	84 \pm 18 n.s.	α -cadinol	78 \pm 14*
Nerolidol	65 \pm 9***	β -santalol	62 \pm 16**
Anymol (i)	51 \pm 4***	Cedrol	58 \pm 11***
Anymol (ii)	46 \pm 4***	Ledol	82 \pm 14 n.s.
Bulnesol (i)	85 \pm 7 n.s.	Viridiflorol	78 \pm 18*
Bulnesol (ii)	86 \pm 10 n.s.	β -caryophyllene alcohol ..	85 \pm 13 n.s.
Lanceol	76 \pm 7**	α -santalol	58 \pm 5***
Elemol	80 \pm 16*		
Guaiol	58 \pm 11***		

†Significantly different from control at * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

Consideration of all these results gives no hint of a structure/activity relationship but does clearly indicate that a significant proportion of the volatile terpenoids, which are the major components of most essential oils, are likely to increase MFO activity.

The essential oils of other *Eucalyptus* spp. and of certain fodder trees were therefore prepared and tested. While there is some variation in activity (Table 5) most of these oils showed an activity similar to that observed for *E. caleyi*.

In the present study, essential oils from only a fraction of the potential browse feeds in Queensland have been tested. Emphasis has been placed on terpenoid components but other plant constituents are known to be active also. Wattenberg, Page and Leong (1968), for example, have reported that some flavones are strong inducers of microsomal hydroxylase activity. It is, therefore, probable that components in many other fodder trees and shrubs are capable of modifying the activity of hepatic microsomal detoxifying enzymes. While the significance of this activity for the nutrition and husbandry of animals grazing these feeds

needs further evaluation, it is clear that the drenching with CCl_4 of animals subsisting on drought fodder may be subject to special risk.

Table 5. Barbiturate sleeping times for mice treated with essential oils from *Eucalyptus* spp. and fodder trees

Experiment A*		Experiment B*	
Treatment	Sleeping time as % of control (mean \pm s.d.)	Treatment	Sleeping time as % of control (mean \pm s.d.)
Control	100 \pm 27	Control	100 \pm 13
1,8-cineole	36 \pm 9	1,8-cineole	50 \pm 7
<i>E. † pilligaensis</i>	40 \pm 5	<i>Eromophila maculata</i> (fuchsia)	50 \pm 7
<i>E. camaldulensis</i>	40 \pm 5	<i>Eremocitrus glauca</i> (limebush)	47 \pm 10
<i>E. cambageana</i>	45 \pm 14	<i>E. tessellaris</i>	43 \pm 10
<i>E. populnea</i>	32 \pm 5		
<i>Geijera parviflora</i> (wilga) ..	36 \pm 5		

*All treatments were significantly different from control at $P < 0.001$.

†*E.* indicates *Eucalyptus* spp.

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