Review

25 Years of Natural Product R&D with New South Wales Agriculture

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Abstract: Following recent NSW Government restructuring, the Department of Agriculture now exists in a composite form along with Forestry, Fisheries and Minerals in the new NSW Department of Primary Industries. This paper outlines some of the highlights of secondary metabolite R&D accomplished in the 25 years since the essential oil research unit was transferred from the Museum of Applied Arts & Sciences, Sydney to NSW Agriculture's Wollongbar Agricultural Institute on the NSW north coast.

The essential oil survey was continued, typing the Australian flora as a suitable source of isolates such as myrtenal (*Astartea*), myrtenol (*Agonis*), methyl chavicol (*Ochrosperma*), α-phellandren-8-ol (*Prostanthera*), methyl myrtenate (*Darwinia*), methyl geranate (*Darwinia*), kessane (*Acacia*), cis-dihydroagarofuran (*Prosthanthera*), protoanemonin (*Clematis*), isoamyl isovalerate (*Micromyrtus*), methyl cinnamate (*Eucalyptus*) and bornyl acetate (*Boronia*). Many of these components are used, or have potential use in the fragrance, flavour, medicinal plant or insect attraction fields.

Two weeds toxic to livestock in the Central West of the State are also harvested commercially as medicinal plants. Measurement of hypericin concentrations in the various plant parts of St John's Wort (*Hypericum perforatum*) over two seasons has shown that the weed can be effectively managed by grazing sheep during the winter months when toxin levels are low. Syntheses of β-carbolines tribulusterine and perlolyrine have shown that the former alkaloid was misidentified in the literature and hence not the toxic principle responsible for *Tribulus* staggers in sheep.

Poor quality (high 1,8-cineole – low terpinen-4-ol) oil bearing tea tree (*Melaleuca alternifolia*) plantations have been established to the detriment of many a tea tree farmer. Analytical methods developed to check leaf quality at an early age indicated precursor

sabinene constituents that convert to the active terpinen-4-ol both as the leaf matures or as the precursors are distilled for oil production.

   Tea tree's major insect pest, pyrgo beetle (*Paropsisterna tigrina*), was seen to selectively metabolize only 1,8-cineole from it's monoterpenoid-rich diet. Characterization of these and other metabolites from myrtaceous herbivores showed a species specific production of predominately ring hydroxylated products, some of which were attractive when bioassayed against adult beetles.

Introduction

   The New South Wales (NSW) state government has been investigating natural products available from the Australian flora since the late nineteenth century [1]. After approximately 100 years at the Museum of Applied Arts and Sciences in Ultimo, Sydney, this research unit was relocated, in 1979, to NSW Agriculture, firstly to the Biological and Chemical Research Institute, Rydalmere, Sydney, and then to the Wollongbar Agricultural Institute on the NSW north coast. After 25 years, this research has now become part of the new composite NSW Department of Primary Industries. This paper now follows on from previous reviews [1,2] and summarizes the highlights of investigations into natural products from the Australian flora carried out during the last quarter of a century.

Survey of the Essential Oils of the Australian Flora

   Australian species have continued to be excellent sources of isolates which are of interest for chemical, commercial and chemotaxonomic reasons [3-5]. Investigations in our laboratories have revealed predominantly terpenoid and phenylpropanoid isolates as shown in Table 1. Esters - *iso*-amyl *iso*-valerate (**1**), (+)-bornyl acetate (**2**), methyl geranate (**7**), methyl myrtenate (**8**) - and the hydrocarbon terpinolene (**14**) are important perfumery constituents with potential commercial applications in the fragrance field. Methyl chavicol (**5**), *E*-methyl cinnamate (**6**) and *cis*-sabinene hydrate (**13**) have applications in the flavouring area. *Melaleuca alternifolia* flush growth leaf, rich in **13**, provides a sweet marjoram substitute whereas *Ochrosperma lineare* and *Eucalyptus olida* respectively provide alternatives for the methyl chavicol and methyl cinnamate chemotypes of basil. The latter has been developed, as a result of this investigation, as a small but new essential oil industry for applications requiring natural rather than synthetic methyl cinnamate,. *Cis*-dihydroagarofuran (**3**), kessane (**4**) and α-phellandren-8-ol (**11**) are rare terpenoids with an as yet unexplored potential. Protoanemonin (**12**) has broad ranging medicinal properties [19] and the α-pinene oxidation products (+)-myrtenal (**9**) and myrtenol (**10**) are known for their activity against various insect species [16,23]. The continual demand for flavour and fragrance materials [24] also provides opportunities for the commercial development of plantations as a source of these isolates.

Table 1. List of isolates and their concentrations in volatile oils of Australian native species.

Isolate	Species [a]	Family	Oil Yield (%) [b]	Isolate in oil (%) [c]	Identification [d]	Ref.
iso-amyl *iso*-valerate (**1**)	*Micromyrtus striata*[e]	Myrtaceae	0.6[f,g]	49	gcrt, ms, ir, nmr	6
(+)-bornyl acetate (**2**)	*Boronia latipinna*	Rutaceae	0.9-1.4	61	gcrt, ms, ir, nmr	7
cis-dihydroagarofuran (**3**)	*Prostanthera* sp. aff. *ovalifolia*[e]	Lamiaceae	1.1-3.3[g]	62	gcrt, ms, ir, nmr	8-11
kessane (**4**)	*Acacia nuperrima* ssp. *cassitera*	Mimosaceae	0.9[g]	88	gcrt, ms, ir	12
	Prostanthera sp. aff. *ovalifolia*[e]	Lamiaceae	1.1-3.3[g]	79	gcrt, ms, ir, nmr	9,10
methyl chavicol (**5**)	*Ochrosperma lineare*	Myrtaceae	0.3	82	gcrt, ms	13
E-methyl cinnamate (**6**)	*Eucalyptus olida*[h]	Myrtaceae	1.6-6.1[f,g]	95	gcrt, ms, ir	14
methyl geranate (**7**)	*Darwinia citriodora*	Myrtaceae	0.3[g]	61	gcrt, ms, ir, nmr	8, 15
methyl myrtenate (**8**)	*Darwinia citriodora*	Myrtaceae	0.3[g]	63	gcrt, ms, ir, nmr	15
(+)-myrtenal (**9**)	*Astartea* sp.nov.	Myrtaceae	0.9	26	gcrt, ms, ir	16
myrtenol (**10**)	*Agonis fragrans*	Myrtaceae		20	gcrt, ms	17
α-phellandren-8-ol (**11**)	*Prostanthera staurophylla*	Lamiaceae	2.1	6	gcrt, ms, ir, nmr	18
protoanemonin (**12**)	*Clematis* sp.	Ranunculaceae	0.2-2.3[f]	90	gcrt, ms, ir, nmr	19
cis-sabinene hydrate (**13**)	*Melaleuca alternifolia*[e,j,k]	Myrtaceae	1.0-3.0[g]	42	gcrt, ms, ir, nmr	20, 21
terpinolene (**14**)	*Melaleuca alternifolia*[e]	Myrtaceae	1.0-3.0[g]	46	gcrt, ms	22

[a] Leaf and terminal branchlets steam-distilled unless indicated otherwise. [b] Calculated as weight(g)/fresh weight (100g) unless indicated otherwise. [c] Maximum given where more than one sample has been examined. [d] gcrt = gas chromatographic retention time comparison with authentic sample, ms = mass spectrometry, ir = infrared spectroscopy, nmr = nuclear magnetic resonance. [e] Chemical variation within this species - this entry relates to one chemovar only. [f] Air dried. [g] Calculated as volume (mL)/fresh weight (100g) unless indicated otherwise. [h] Recent name change. [j] Volatiles extracted rather than distilled. [k] Leaf flush growth only.

1 **2** **3** **4** **5**

6 **7** **8** **9** **10**

11 **12** **13** **14**

15 **16** **17**
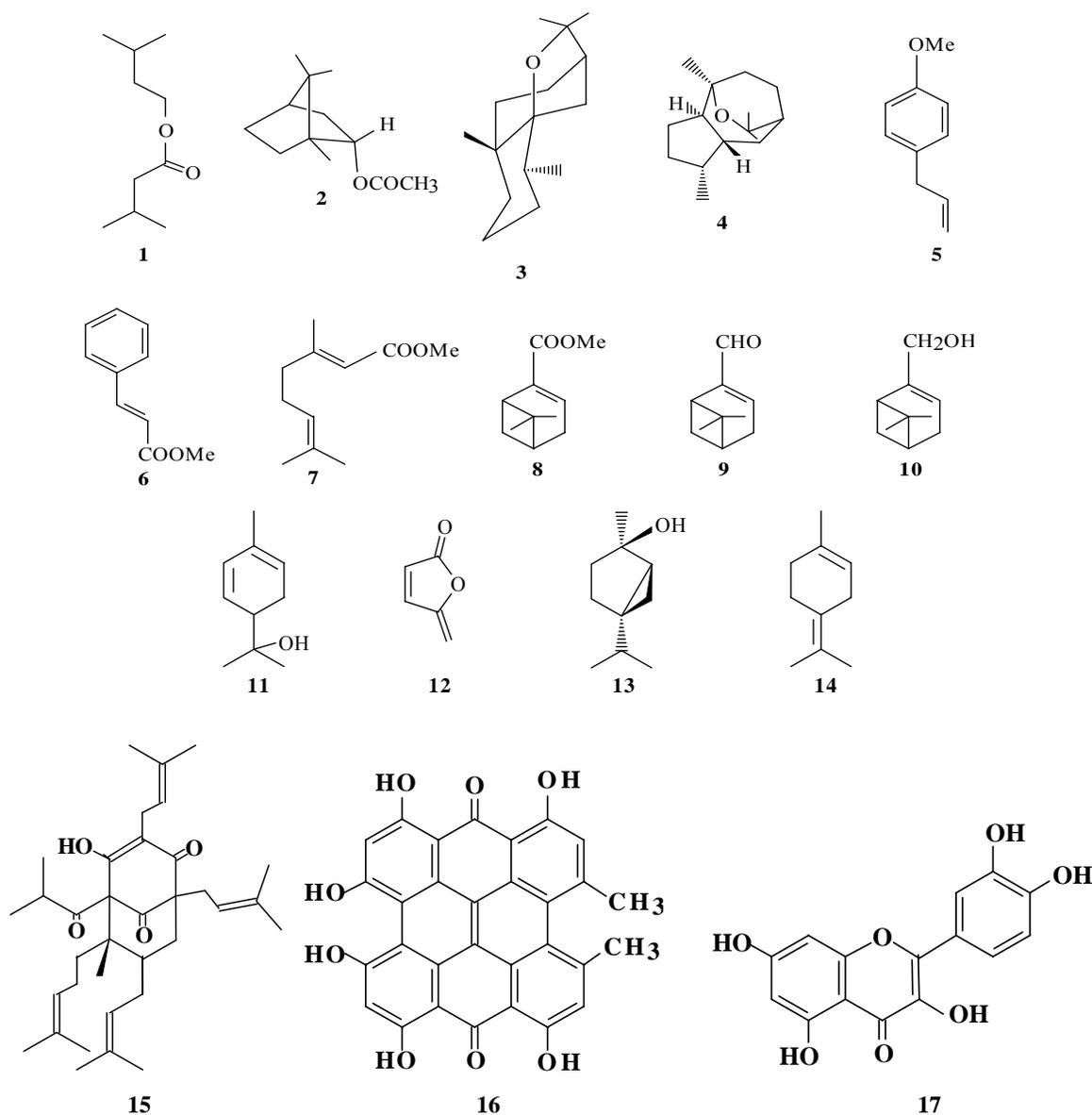
Medicinal Plant or Toxic Weed?

St John's wort (*Hypericum perforatum*) and cathead or puncture vine (*Tribulus terrestris*), two weed species introduced to Australia, are renowned for their toxicity to stock and are also used as herbal remedies. The photosensitization and even death of sheep subsequent to browsing on pastures infested with these species have long been a concern of pastoralists in the central west of New South Wales [25].

St John's wort has reached weed status in many countries, including Australia, especially because of photosensitizing symptoms imparted to browsing stock. As a medicinal plant, *Hypericum perforatum* preparations are used in the treatment of mild to moderately severe depression. Although the active ingredient is thought to be the phloroglucinol hyperforin (**15**), the photosensitizing agent has been identified as the naphthodianthrone hypericin (**16**). Querecitin (**17**) and related flavonoids are also known to be bioactive. *H. perforatum* is also known to possess antiviral and antiretroviral activity [26]. Hypericism is a disease of livestock affecting the unpigmented skin, depressing the central

nervous system (CNS) and making affected stock (sheep, cattle, horses, goats and swine) hypersensitive to temperature change and handling [27]. In our investigation, measurement of the hypericin showed that the narrow-leaf variety contained greater concentrations than the broad-leaf variety and that the toxin was concentrated in the flowering parts of the plant (Table 2) [28]. When measured over two seasons, it became clear that concentrations were such that sheep could be used for weed control if moved onto St John's Wort infested pastures during the winter months of June to September [26] (Fig 1).

Tribulus terrestris, commonly known in Australia as cathead, puncture vine or caltrop, is also well known for chronic poisoning in sheep [29] and medicinal plant use [30]. In sheep, the disease is characterized by a distressing irreversible asymmetric locomotor disorder. It was thought that the neurotoxic agent may be the β-carboline alkaloid, tribulusterine (**18**), which had been reported from the fruit of *T. terrestris* [31]. After exploring several approaches, a Pictet-Spengler cyclisation route was used to prepare tribulusterine (**18**) [32]. The cyclisation of tryptamine (**19**) and furaldehyde (**20**), obtained in turn from 3-furanmethanol, in dry benzene and the presence of p-toluenesulphonic acid afforded indole (**21**), which was then dehydrogenated using palladium on charcoal to yield (**22**), and then tribulusterine (**18**) after hydrolysis (Scheme 1).

**Table 2.** Hypericin concentration in different plant parts of Australian varieties.

Variety	Plant Part	Hypericin (ppm)
Broad-leaf	leaves	370
	stems	40
	flowers	2360
	capsules	730
Narrow-leaf	leaves	1110
	flowers	5030

Figure 1. Seasonal variation in hypericin concentrations in Australian St John's Wort
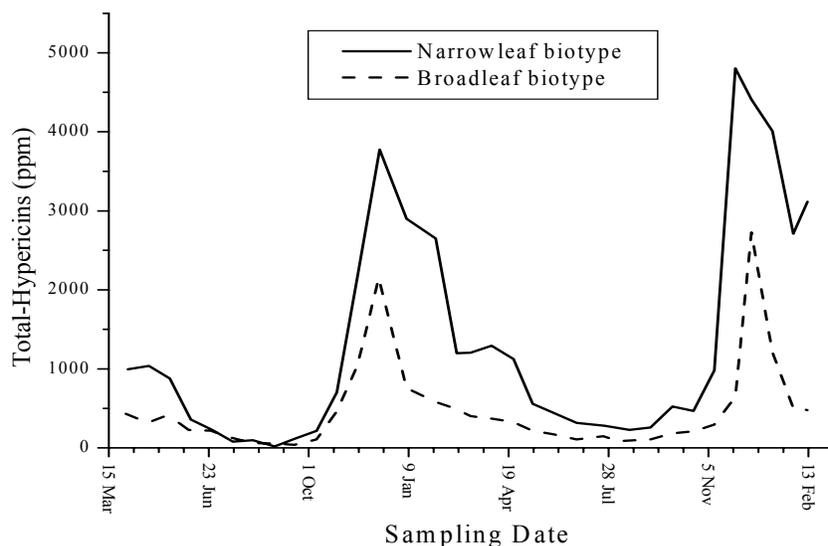
The structure of (**18**) was confirmed unequivocally by crystallography. NMR differences between the natural and synthetic materials however indicated that the natural product was probably perlolyrine (**23**) rather than tribulusterine. An independent synthesis of perlolyrine using the same procedure confirmed this [32]. As it is unlikely that the irreversible CNS effects are caused by perlolyrine, further analysis of the minor alkaloid and amide components of *Tribulus terrestris* is under investigation.

**Scheme 1.** The Synthesis of Tribulusterine
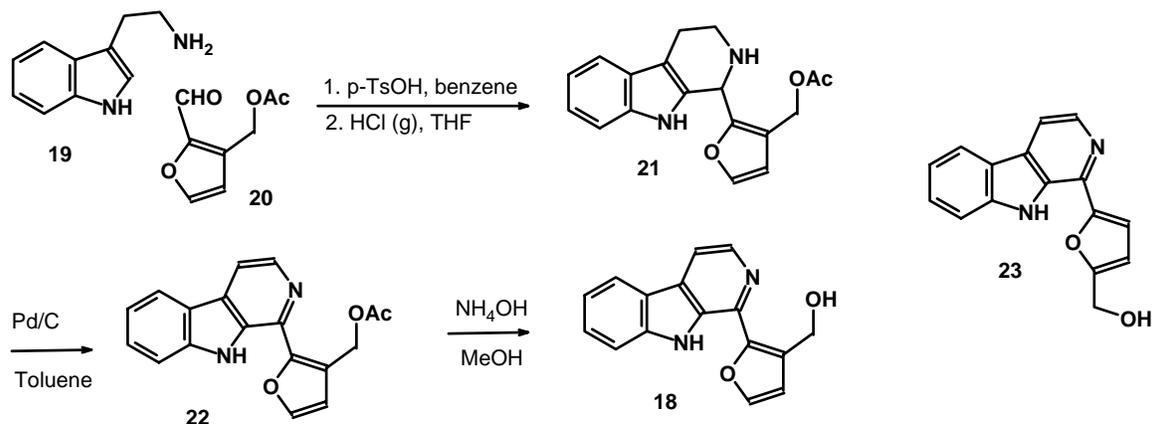
Tea Tree Oil

Teion has been a substantial industry on the north coast of NSW for the last two decades [33]. Plantation quality is dependant on the chemical variety of *Melaleuca alternifolia* planted. Measurement of this quality has been confounded by two factors discovered in our laboratories. Immature leaf of the commercial terpinen-4-ol variety was found, using microwave-assisted micro extraction techniques, to be rich in *cis* and *trans*-sabinene hydrate which was replaced by terpinen-4-ol and γ-terpinene as the leaf matured (Figure 2) [34].

Figure 2. Along the branch variation in the concentrations of cis-sabinene hydrate, terpinen-4-ol and γ-terpinene in individual leaves of M. alternifolia.
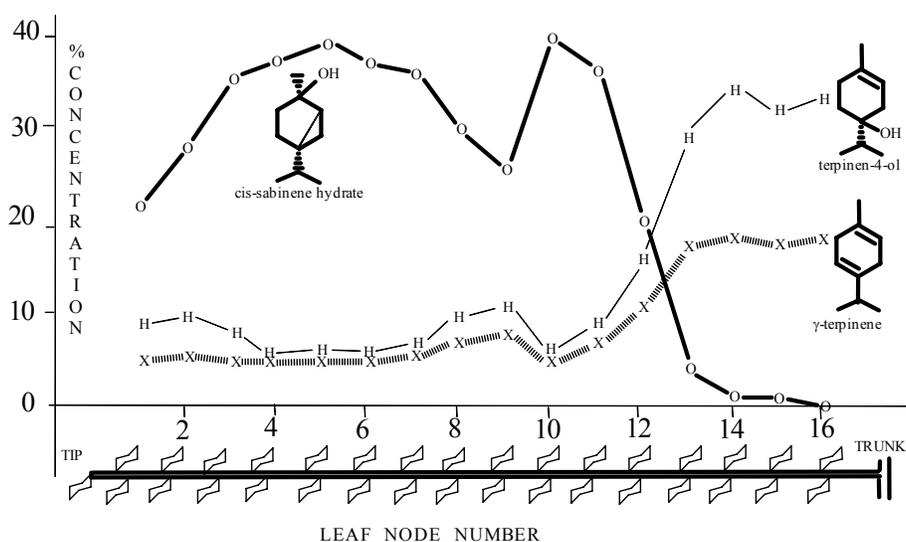
Commercial distillation of the immature leaf did not cause contamination because of the thermal mild acid-catalyzed conversion of the sabinene hydrates to terpinen-4-ol and γ-terpinene. In addition, the analysis of early stage tea tree seedling leaves was found to be misleading because of the sequential onset of different biogenetic pathways in *M. alternifolia* [35]. For example α-pinene and terpinolene were found to be produced in up to six week old seedlings [36] prior to the formation of the commercially important terpinen-4-ol (Figure 3). Consequently, recommendations to the industry included analyzing only mature, older seedling leaf for confirmation of quality prior to plantation establishment by transplanting seedlings [33].

Figure 3. Concentration of terpinolene (∇), α-pinene (□), terpinen-4-ol (×) and 1,8-cineole (■) in consecutive leaf sets 6 weeks after emergence**.**
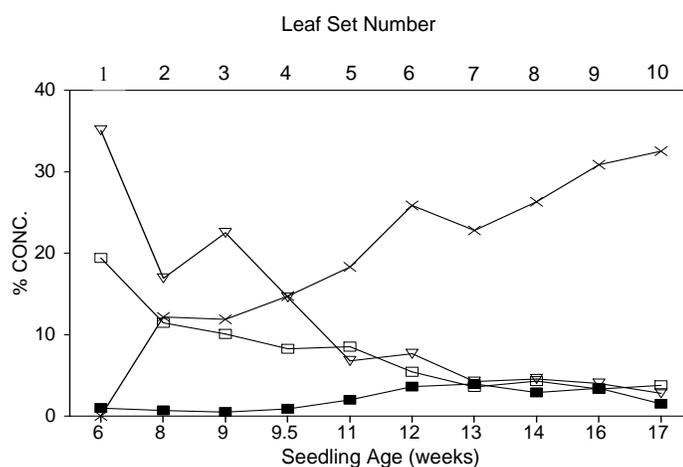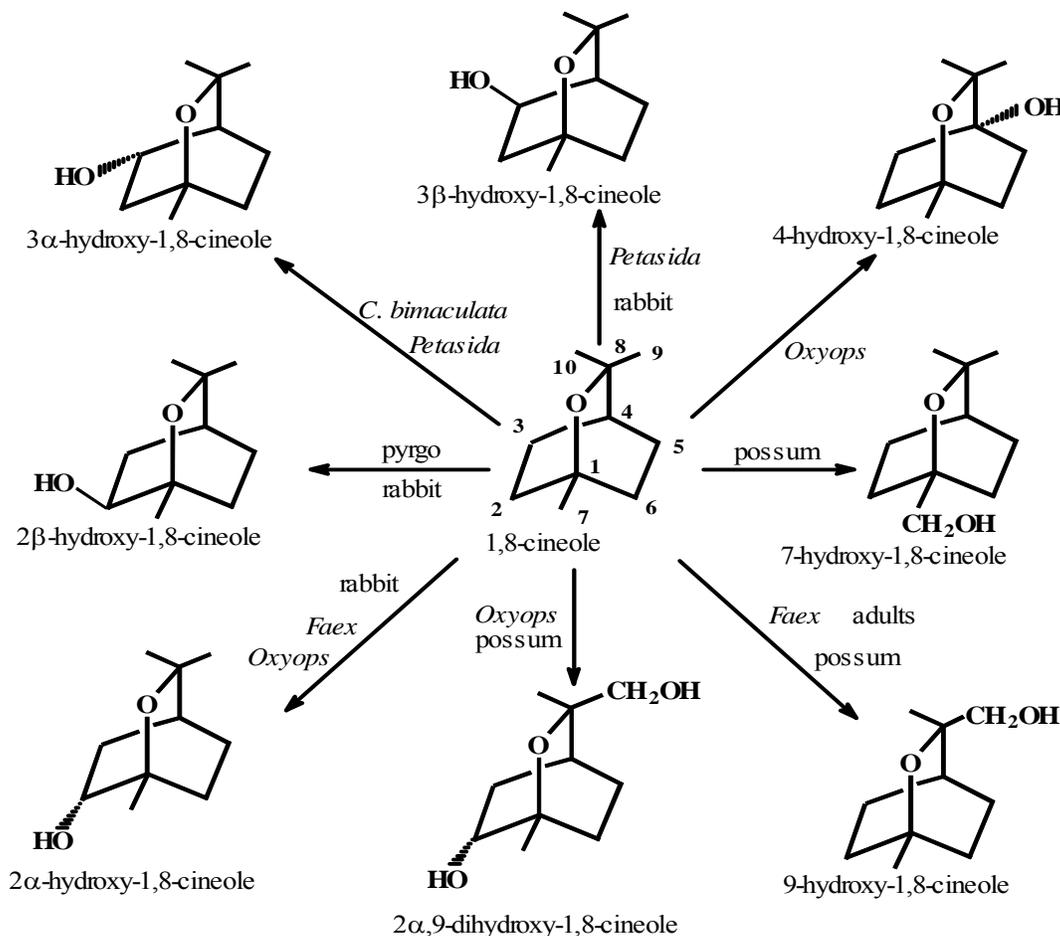

Insect Chemical Ecology

The major insect pest of tea tree plantations in north eastern New South Wales is the chrysomelid prygo beetle, *Paropsisterna tigrina*. Investigation of the frass volatiles of both adult and larval forms indicated that ingested oil was generally excreted unchanged except for 1,8-cineole which was metabolized predominantly to 2β-hydroxycineole with traces of the 2α, 3α and 9-hydroxycineole isomers [37, 38]. Examination of frass from other cineole ingesting herbivores has shown that this metabolism is species specific (Figure 4). Mammals were seen to preferentially oxidize the most sterically unhindered methyl substituents in the 1,8-cineole molecule. Amongst the marsupials, the koala oxidizes cineole more extensively (to dicarboxylic acids) than the possum and other generalist herbivores [39]. Insects however, favour hydroxylating the ring carbons. Even within the insect kingdom, hydroxylation varies with the species suggesting a pheromonal use for the metabolites [40]. This was confirmed with pyrgo beetle by the attraction of 25 out of 26 beetles toward crystals of 2β-hydroxycineole in a choice test with a blank [8].

North-eastern NSW grows a range of tropical horticultural crops such as macadamias, avocados, custard apples and lychees susceptible to attack by insect pests such as monolepta beetle, macadamia nut borer and fruit spotting bug. A homologous series of novel long chain olefinic ethers with the formula $CH_3(CH_2)_9CH=CH(CH_2)_6O(CH_2)_{12-16}CH_3$ was found in the cuticular wax of monolepta

(*Monolepta australis*) [41]. Current investigations are using SPME (Solid Phase Micro-Extraction) techniques coupled with GCMS (Gas Chromatography – Mass Spectrometry) to examine volatile constituents emitted from flowers and fruit that attract insect pests. These attractants, in addition to insect pheromones, are being identified and tested for use in lure-and-kill type traps as part of an IPM (Integrated Pest Management) strategy for the control of harmful horticultural crop insects.

Figure 4. Metabolism of 1,8-cineole by a variety of insects (pyrgo, *Chrysophtharta bimaculata, Petasida, Oxyops, Faex*) and mammals (rabbit, possum).

Acknowledgements

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