



BACTERICIDAL OILS OF *MELALEUCA*. A CHEMICAL REINVESTIGATION OF THE ESSENTIAL OIL *M. alternifolia* CHEEL (MYRTACEAE)

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Résumé : *les premiers chercheurs ont postulé que Melaleuca alternifolia existe sous trois variétés chimiques différentes par leur teneur de leur huile essentielle en 1, 8-cinéole. Considérant les groupes de monoterpènes biogénétiquement proches, plutôt qu'un seul constituant de l'huile, il a été montré que deux de ces groupes, basés sur les deux ions carbonium p-menthenyl portant leur charge respectivement en C4 ou C8, montrent une distribution clairement non linéaire dans la Nature. Ainsi M. alternifolia apparaît n'exister que sous deux variétés chimiques seulement, correspondant aux deux cas extrêmes où chacune des voies se poursuit à l'exclusion de l'autre.*

Abstract : *early workers postulated the existence of three chemical varieties of Melaleuca alternifolia characterized by different 1,8-cineole contents of their volatile leaf oils. By considering groups of biogenetically closely related monoterpenoids, rather than single oil constituents, it has been shown that two of these groups, based on the two isomeric p-menthenyl carbonium ions with their positive charge on C4 and C8 respectively, exhibit a significant non-linear distribution in Nature. Thus M. alternifolia appears to exist in two chemical varieties only, corresponding to the two extreme cases where each of these two pathways operates at a virtually exclusion of the other.*

Melaleuca alternifolia Cheel (Myrtaceae) is a shrub or small tree growing near watercourses and in swampy situations in coastal areas of Northern New South Wales and Southern Queensland.

Earlier workers have identified α -pinene, α - and δ -terpinene, p-cymene, terpinen-4-ol and 1,8-cineole as the principal constituents of the volatile leaf oil and proposed the existence of three chemical varieties based on the 1,8-cineole content of oils from a large number of single trees selected at random (1). Of these three varieties only the so-called 'type' oil containing less than 10% 1,8-cineole, but rich in terpinen-4-ol, has achieved commercial importance as a very powerful germicide (2-4).

Owing to renewed interest in the bactericidal properties of the terpinen-4-ol rich oils of this species it was decided to re-investigate the findings of previous workers, particularly since their cineole contents were obtained using Cocking's o-cresol method (following enrichment with 1,8-cineole in the case of cineole poor oils), a method known to give erroneous results if alcohols and other oxygenated compounds are present.

In the present investigation foliage from 23 individual trees, selected at random from a natural population of *M. alternifolia* growing near Grafton in Northern New South Wales, was extracted by means of cohobative water distillation and the oils analyzed using capillary GLC. Individual constituents were confirmed by co-injection with authentic compounds.

Graphing of all major monoterpene constituents showed considerable variations in most of them thus complicating the recognition of any distinct chemical varieties (Fig. 1). It was felt that this difficulty could be overcome by grouping together all those constituents which could be considered as having been derived from the same precursor. In this way the importance of quantitative variations due to transformations of a given precursor (such as loss



of a hydrogen ion yielding an alkene, hydroxylation leading to alcohols, etc) could be evened out and minimized.

Using accepted models of monoterpene biosynthesis as a starting point the monoterpenoids of *M. alternifolia* were grouped together as shown in Fig. 2. Terpinolene and β -cymene were not included as they may have originated from more than one precursor. GLC derived percentages of all oil constituents were converted into mole fractions. Relevant values were then combined into the proposed biogenetic groups, expressed as mole % of the total oil (Fig. 3).

Fig. 3 shows that variations in groups A, D and E are continuous and relatively small (group F has been omitted as its overall contribution is negligible). In contrast variations in groups B and C are large and discontinuous indicating that the two pathways operate in opposition to each other. Thus, Penfold's 'Type' (1,8-cineole < 14%) and 'Variety B' (1,8-cineole > 54%) correspond to the two extreme cases where one of the two pathways operates at the almost total exclusion of the other. Penfold's 'Variety A' (1,8 cineole: 31-45%) merely combines those few trees showing significant contributions from both pathways.

There are at least three further species of *Melaleuca* which are known to exist in terpinen-4-ol rich form. *M. dissitiflora* F. Muell. (5) and *M. linariifolia* Sm. exist in a terpinen-4-ol rich and a 1,8-cineole rich form. Both species are fairly closely related to *M. alternifolia* and the oils of these three species are qualitatively identical and indistinguishable from each other. *M. uncinata* R. Br. appears to exist in four forms: one rich in 1,8-cineole and containing all three isomers of eudesmol (α -, β - δ -), another rich in 1,8-cineole but devoid of any significant amounts of sesquiterpenoid alcohols and a third rich in α -pinene but deficient in both 1,8-cineole and sesquiterpenoid alcohols. A fourth form, only recently discovered, contains about 40% of terpinen-4-ol. Table 1 summarizes data on terpinen-4-ol contents of these three species.

Table 1:

Species	Terpinen-4-ol form		Reference
	Terpinen-4-ol	1,8-cineole	
<i>M. dissitiflora</i>	35 - 52 %	1.5 - 2.0 %	(5)
<i>M. linariifolia</i>	30 - 37 %	3.5 - 8.0 %	unpublished
<i>M. uncinata</i>	ca 40 %	ca 0.5 %	unpublished

Since terpinen-4-ol is the chief germicidal agent of *M. alternifolia* oil the terpinen-4-ol rich forms of these three additional *Melaleucas* should exhibit similar germicidal activity.

The complex nature of most volatile oils of plant origin (essential oils) often diminishes their value as taxonomic tools. This difficulty may, perhaps, be overcome by applying simplifications such as those proposed here, e.g. the recognition of a relatively small number of groups of biogenetically closely related compounds. The potential usefulness of this concept was briefly alluded to in earlier work on the leaf oils of the *Eucalyptus* subseries *Strictinae* [6].

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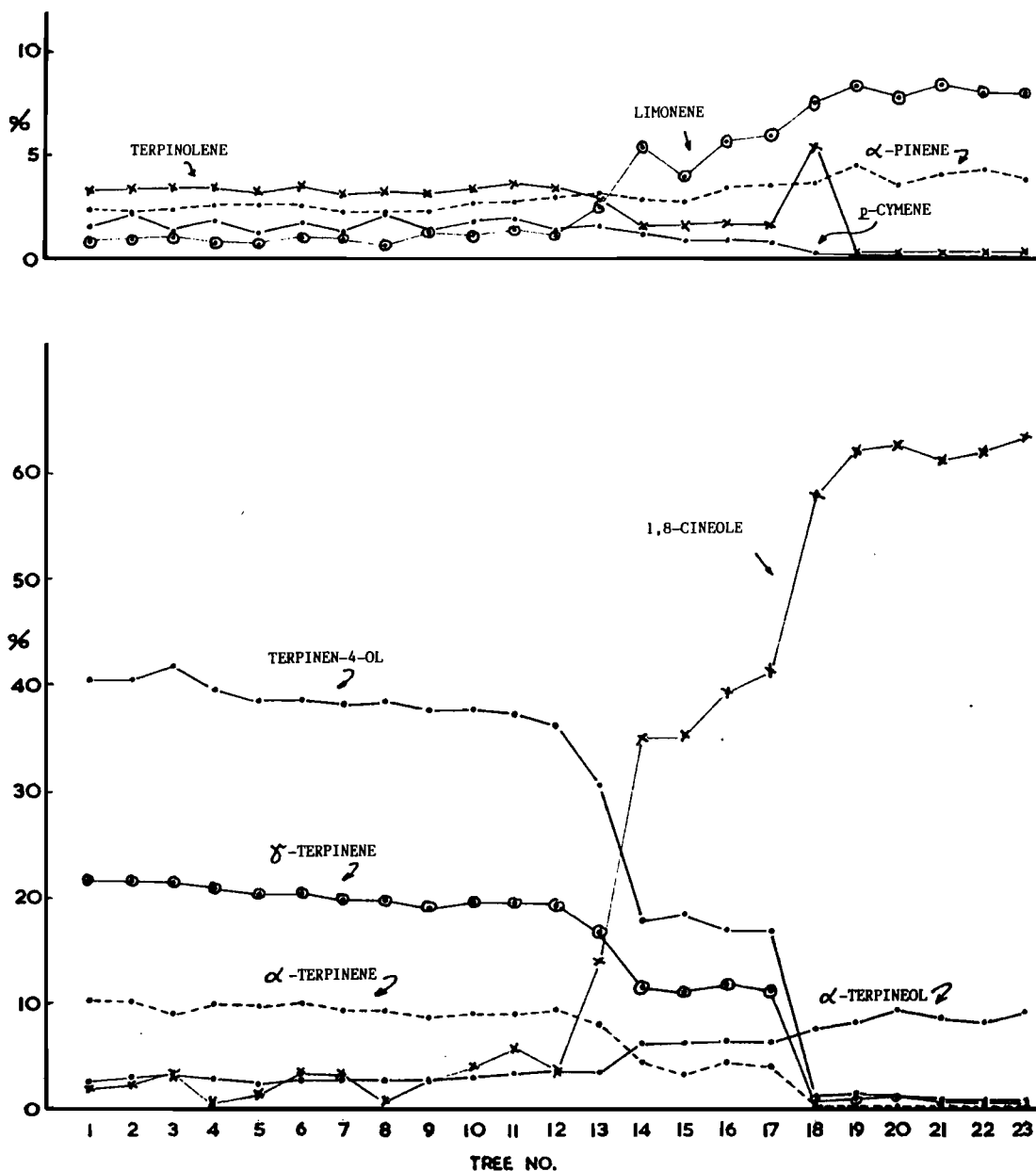


Figure 1 : Variation in major oil constituents

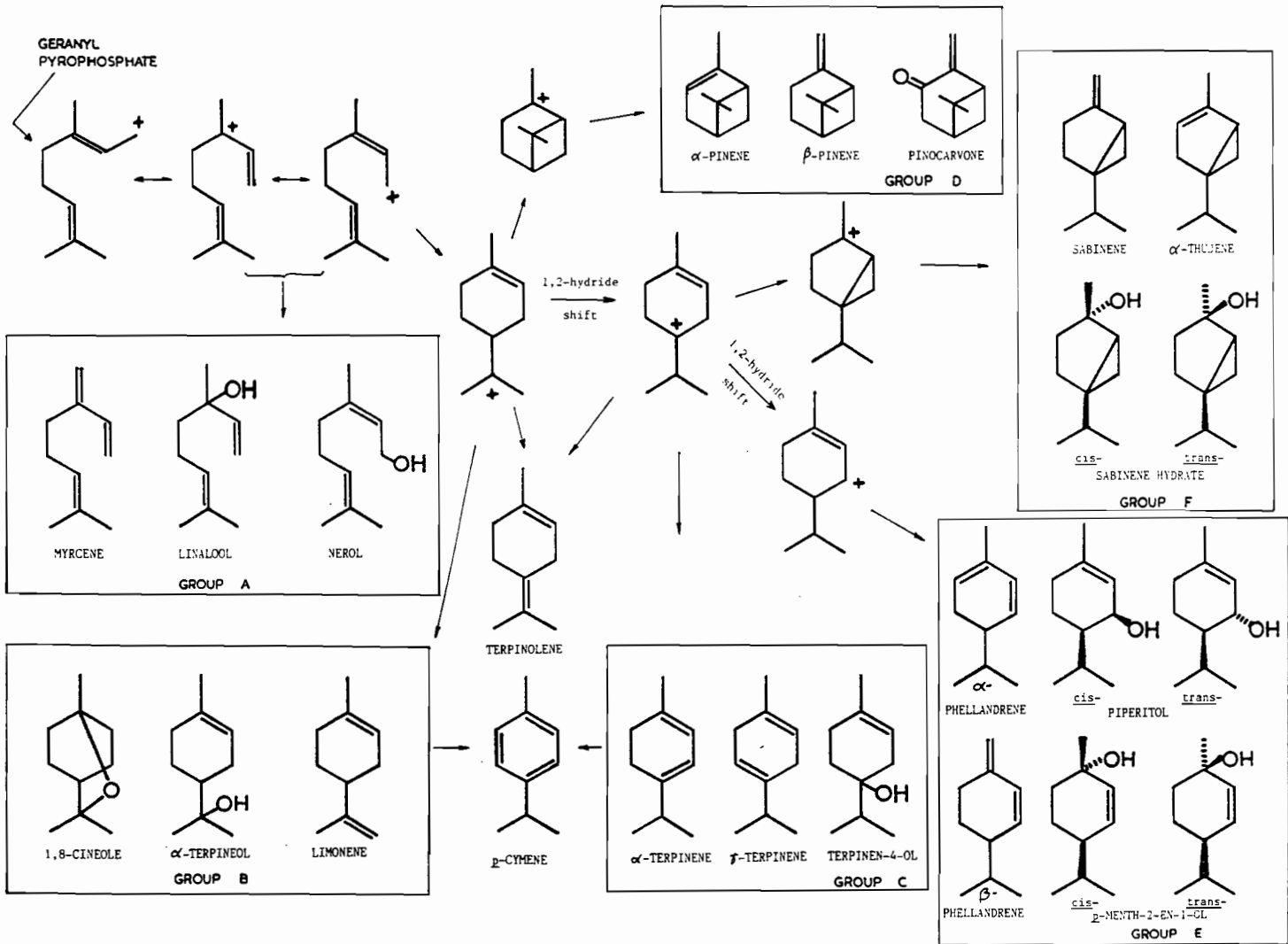


Figure 2: Monoterpeneoid biosynthesis

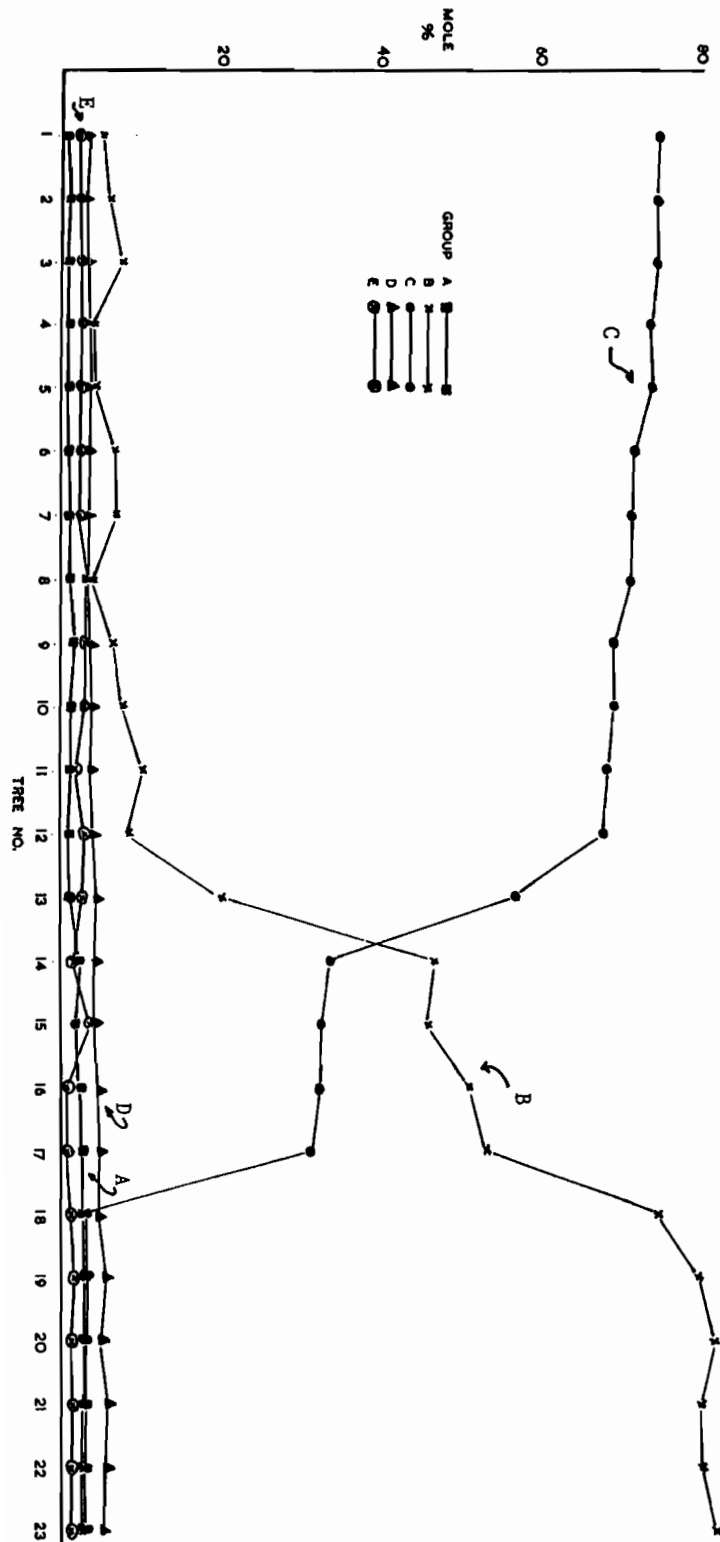
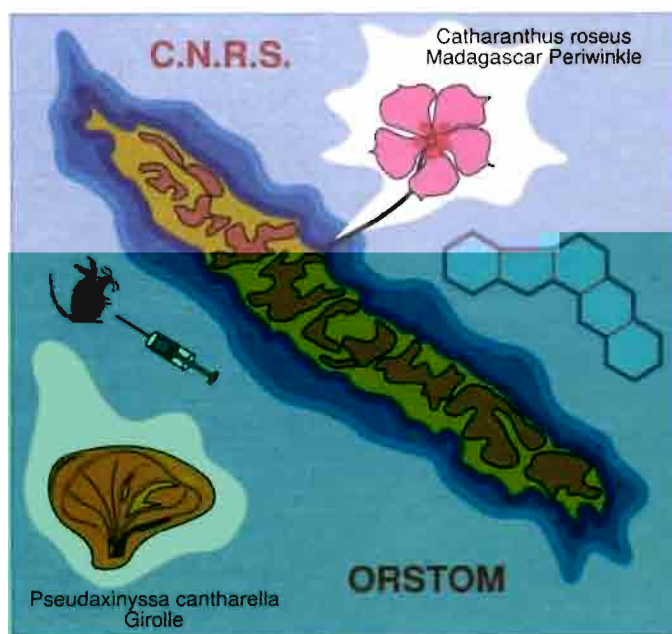


Figure 3 : Variation in "biogenetic groups"

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