Biosynthesis of Monoterpenes in Rose Petals

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ROSE petals assimilate and very efficiently utilize mevalonate for the synthesis of a range of acyclic monoterpene alcohols.[1] The *trans* allylic alcohol geraniol appears to be the first acylic non-phosphorylated monoterpene formed from mevalonate in rose petals. Incorporation of mevalonate stereospecifically labelled with tritium into the flower head of the hybrid tea rose Lady Seton[2] demonstrated that geraniol is formed by stereospecific elimination of the 4*S* proton of mevalonate (the 2*R* proton of isopentenyl pyrophosphate) during double bond formation as might be expected by analogy with other trans terpenoid systems [3] The *cis* isomer nerol is formed in the same manner again by 4*S* elimination rather than by elimination of the epimeric 4*R* proton as occurs in biosynthesis of the polycis-isoprene rubber in the latex of the rubber tree *Hevea brasiliensis*.[4] On the basis of the above findings it has been concluded that geranyl pyrophosphate (or a derivative) is converted to neryl pyrophosphate by *trans* to *cis* isomerization of the 2,3 double bond. This isomerization has not been demonstrated in terpenoid systems Cell free extracts from rose petals of Lady Seton and Fragrant Cloud contain a dehydrogenase that reversibly oxidizes/reduces geraniol (nerol) and the aldehydes geranial (neral) in the presence of NADP/NADPH. Addition of the sulphydryl compounds 2-mercaptoethanol or reduced glutathione to the system results in the rapid isomerization of the *trans* aldehyde geranial to an equilibrium mixture of the two isomers geranial/neral, and vice versa, the *trans* isomer predominating. The reversible dehydrogenase is capable, in the presence of NADPH, of reducing both aldehydes back to the corresponding allylic alcohols. This cycle of events results in the isomerization of the *trans*, 2,3 double bond of geraniol to the *cis* double bond of nerol, the aldehyde being the active intermediate. Thiols as well as adding to the double bond of conjugated ene-one systems are known to catalyse *trans*/*cis* isomerizations of the α/β double bond.[5] Similar mechanisms have been reported for the enzymatic formation of *cis*, *trans*-farnesol in a cell free system from orange flavedo[6] and the conversion of *trans*,*trans*-epoxyfarnesol to the *cis trans* isomer by the fungus *Helminthosporium sativum* [7]

A further series of reactions occurring in rose petals and petal extracts including reductions, glycosylations, and acylations lead to the formation of dihydro alcohols,[8] terpene glycosides and monoterpene fatty acyl esters. The overall interrelationship of these various reactions, which appear to occur mainly in the epidermal layer of cells account for the variety of monoterpene components found in the rose petal

[1] FRANCIS, M. J. O. and O'CONNELL, M. (1969) *Phytochemistry* **8**, 1705.
[2] FRANCIS, M. J. O., BANTHORPE, D V. and LE PATOUREL, G. N J. (1970) *Nature* **228**, 1005.
[3] POPJAK, G. (1964) *Metabolism and Physiological Significances of Lipids* (DAWSON, R. M C and RHODES, D. N., eds.), p. 45, Wiley, New York.
[4] ARCHER, B. L., BARNARD, D., COCKBAIN, E. G., CORNFORTH, J. W., CORNFORTH, R. H and POPJAK, G. (1966) *Proc. R. Soc.* **163B**, 519.
[5] JOCELYN, P. C. (1972) *Biochemistry of the SH Group*, Academic Press, New York.
[6] CHAYET, L., PONT-LEZICA, R., GEORGE-NASCIMENTO, C. and CORI, O. (1973) *Phytochemistry* **12**, 95.
[7] SUZUKI, Y. and MARUMO, S. (1972) *Tetrahedron Letters* 5101.
[8] DUNPHY, P. J. and ALLCOCK, C (1972) *Phytochemistry* **11**, 1887.

The Constituents of Ninde Oil

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NINDE oil (*Aeolanthus gamwelliae*; Labiatae) was first discovered in 1931 by the Misses Gamwell, who lived in what is now Zambia. The history of the development of this novel essential oil illustrates many of the problems involved in the development of such an oil, which is used as a substitute for palmarosa oil First, problems were encountered in propagating and growing the crop; when these were overcome, there was a collapse in the market for palmarosa oil which led to the abandonment of production of ninde.

Some 14 years later when the price of palmarosa oil had recovered, further efforts were made to develop the oil both in Zambia and in Malawi, in collaboration with the Tropical Products Institute, the staff of which analysed and assessed the oil and found a purchaser for it, thus resolving one of the major problems in establishing a new oil. Further developments in the techniques of growing ninde and distilling the oil have been made subsequently.

The major constituents of ninde oil have been isolated by column chromatography and gc and identified from mass spectra and IR data. Similar studies have been made on Indian and Brazilian palmarosa oil, as shown in Table 1.

TABLE 1. ESSENTIAL OILS CONSTITUENTS OF NINDE AND PALMAROSA

Constituent	Ninde	Indian palmarosa	Brazilian palmarosa	Constituent	Ninde	Indian palmarosa	Brazilian palmarosa
Myrcene	0·02	0·07	0·13	Neral	0·48	0·24	0·11
Limonene	T	0·26	0·06	Geranyl formate	0·34	—	—
Ocimene	0·07	0·11	0·28	Geranial	2·21	} 6·85	0·24
γ-Terpinene	—	0·35	1·08	Geranyl acetate	4·54		6·96
Methyl Heptenone	—	T	0·03	Nerol	0·55	0·33	0·30
Linalool	1·71	0·59	2·58	Geraniol	87·61	88·37	86·16
Caryophyllene	—	0·35	0·93	Caryophyllene oxide	T	T	0·14
α-Guaiene	1·23	—	—	Farnesol	—	0·07	0·17

T = Trace.

The resemblances between ninde oil and the two types of palmarosa are very striking, with constituents making up more than 95% of the oils being common to all.

Factors Influencing Production of Patchouli Sesquiterpenes in Cultured Cells and Regenerated Plantlets

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PATCHOULI oil is a complex mixture of sesquiterpenes obtained from the leaves of *Pogostemon cablin* Benth. The major components are patchouli alcohol, α-, β- and γ-patchoulene, α-guiaene and α-bulnescene. The oil is secreted in a variety of specialized glandular cells, both on the leaf surface, and within the leaf. The external glands (glandular trichomes) are similar to those of mint, but the internal glands appear to be a unique feature of the plant.[1] In both cases the glandular cells are closely associated with the photosynthetic cells of the leaf, although some internal glands also occur in stem and even root tissues. In these cases glands are located in the phloem.

Our problem was to grow patchouli cells in nutrient culture and attempt to induce patchouli oil synthesis. Although vigorous cultures were obtained, no success was obtained in detecting even minute traces of patchouli sesquiterpenes. However, patchouli cells in culture readily regenerated plantlets, and glandular trichomes were present from the earliest signs of organization. Analysis of gland contents showed only a series *n*-alkanes and no terpenoid hydrocarbons to be present although the glands appeared to possess normal morphology.[2]

Cultivated patchouli does not normally flower, and the crop is always vegetatively propagated. Volkhovrskaya[3] reported the induction of flowers in 8 hr days but no seed was set. We therefore do not know whethe-

[1] HENDERSON, W., HART, J. W., HOW, P. and JUDGE, F. J. (1970) *Phytochemistry* **9**, 1219.
[2] HART, J. W., WOODCOCK, G. J. and WILSON, L. (1970) *Ann. Bot.* **34**, 137, 789.
[3] VOLKHOVSKAYA, U. V. (1968) *Tr. Sukhum Opyt. Sta. Efirnomaslich. Kult.* **7**, 33.