juvenile seedling stages exist when patchouli oil biosynthesis is absent. Once switched on, synthesis apparently continues and oil bearing glands are present in large numbers on the second leaf primordium in the normal vegetative apex. Hence the youngest available cuttings always contain oil.

Plants derived from cultured material were grown on in growth rooms but possessed abnormal leaf morphology and did not accumulate patchouli sesquiterpenes. Two changes were made in the growth conditions earlier used.[1] Glucose was changed to sucrose in the medium and immediately there was good rooting, which had hitherto been poor, and normal leaf morphology Perfectly normal looking patchouli plants were obtained, but no sesquiterpenes appeared in the glands. These were allowed to grow in a glasshouse, where at that particular time of year they experienced long days. A full spectrum of patchouli oil sesquiterpenes was produced This immediately raised the question of whether daylength and/or light quality was an important regulation of sesquiterpene synthesis. In *Mentha*, long days and low night temperatures are required.[4,5] Our experiments indicated that both daylength and light quality may be important.

In one experiment regenerated plants were grown in 12 and 18 hr days at two light intensities with day temp 20° and night temp. 15°. Sesquiterpenes developed faster in long days than in short days, patchouli alcohol being the last major peak to appear. The time of first appearance of sesquiterpenes was not affected by light intensity. In order to test whether the daylength effect was phytochrome controlled, plantlets were grown in short days with red (R), and R followed by far-red (FR) night breaks No sesquiterpenes developed in either regime until the night temp. was lowered, when they appeared in both treatments. The system is therefore not phytochrome controlled.

The importance of low night temperature and long days suggest that reduction of respiratory drain is an important factor. In the light, acetyl CoA could be available for terpene synthesis, whereas in the dark it would be utilized in the Krebs' cycle. The close association of glandular trichomes with photosynthetic palisade cells of the leaf suggest rapid conversion of photosynthate into oil, which may subsequently be used as a respiratory substrate. Such a mechanism may be inferred from the results of Francis.[6]

From the complexity of the specialized structures involved and the interdependence with environment it seems unlikely that cultured patchouli cells will be induced to produce the sesquiterpenes typical of patchouli oil. However, the free regeneration of plantlets from culture could be utilized in conjunction with mutagenic treatments followed by selection for improved oil production in a crop, which being non flowering has little inherent variability.

[4] STEWARD, F. C., HOWE, K. J., CRANE, F. A. and RALESON, R R. (1962) *Cornell Univ Agr. Expt. Sta. Mem.* 379.

[5] LANGSTON, R. and LEOPOLD, A. C. (1954) *Proc. Am. Soc. Hort. Sci.* **63**, 347

[6] FRANCIS, M. J O. (1972) *Planta Med* **22**(2), 201.

Instrumental Techniques for the Analysis of Essential Oils

P. TEISSEIRE

Centre de Recherche, Société Roure-Bertrand-Dupont, Grasse, France

THE existence in an essential oil of components in very small amounts characterizes the organoleptic properties of that essential oil. Therefore, an intimate knowledge of these components is indispensable when it is desired to obtain a fully satisfactory artificial reproduction of the essential oil.

Such a study requires highly advanced instrumental techniques. In the present paper, I shall describe the chief techniques used. The primary object is to collect micro-samples of compounds and then to determine their various spectral properties for the purpose either of identifying them with known products, or of characterizing them. A detailed technological study of the instrumental means used in micropreparative GLC is described. The technology of the quantitative transfer of the micro-samples separated above to the various instruments used in spectrometry (IR, NMR) is given. Examples of the application of the above techniques to the study of the minor components of some essential oils follow.

Oil of Cedarwood Atlas (*Cedrus Atlantica*). Characterization of γ-himalachene, of dehydro-aryl-himalachene and of α-epoxy-6,7-himalachene.

Oil of Ylang-Ylang. Identification of copaene, caryophyllene, D-germacrene and α-farnesene We show, also, that the presence of all these four sesquiterpenic hydrocarbons in Oil of Basil from the Comoro Islands, Reunion or Madagascar provides proof of the adulteration of Oil of Basil with Oil of Ylang-Ylang.

Oil of Patchouli. (a) *Sesquiterpenic hydrocarbons.* 13 hydrocarbons were isolated, 8 were definitely identified: these are β-patchoulene, α-patchoulene, seychellene, α-bulnesene, α-guaiene, caryophyllene, β-elemene and α-humulene. (b) *Sesquiterpenic epoxides.* Epoxy-1α,5α-guaiene 4, epoxy-1,10-α-bulnesene 5 and epoxy-caryophyllene. (c) *Ethylenic alcohols and keto-alcohols.* Besides patchoulol and pogostol, there are at least 2 other ethylenic alcohols and 3 ethylenic keto-alcohols.