

## SHORT COMMUNICATION

### CHEMICAL VARIATION WITHIN *EUCALYPTUS DIVES*

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**Abstract**—An examination of the oils from four “physiological forms” (chemical varieties) of *Eucalyptus dives* by gas chromatography has shown the presence, in each oil, of sixty-two peaks which are identical with respect to their retention times. This supports the hypothesis that chemical varieties usually show quantitative rather than qualitative variations in oil constitution.

THE UTILIZATION of secondary plant constituents as adjuncts to classical morphological taxonomy has led to numerous attempts to establish relationships between the chemical composition and the systematic position of plants. One of the most comprehensive early attempts was made with the genus *Eucalyptus* (Myrtaceae) in which examination of the steam volatile oils of some 200 species indicated that the composition of the oil could be regarded as comparatively constant within each separate species.<sup>1</sup> In 1927,<sup>2</sup> however, it was found that in a number of different populations of *Eucalyptus dives* Schau. widely differing oil types apparently occur. For example, some trees showed on analysis oils containing about 45 per cent piperitone and 40 per cent of a hydrocarbon fraction, largely  $\alpha$ -phellandrene, *p*-cymene,  $\gamma$ -terpinene, myrcene and  $\alpha$ -thujene, whilst others, morphologically indistinguishable, contained 70 per cent cineole and only a small hydrocarbon fraction of different composition. The variants were given the name “physiological forms”, and, in order to distinguish the four separate and distinct oil forms, they were called “Type”, “Variety A”, “Variety B”, and “Variety C”, in order of discovery.<sup>2</sup> The use of the term “physiological form” has now been largely discontinued, but, although the problems involved in the terminology of infraspecific chemical variation in plants has been widely discussed,<sup>3-6</sup> no general agreement has proved possible, although the terms “chemovar” and “chemoforma” of Tétényi<sup>7</sup> for infraspecific chemical categories seem to provide a suitable basis. The existence of infraspecific chemical variation has been established in such varied genera as *Eucalyptus*, *Melaleuca*, *Leptospermum*, *Backhousia*, *Geijera*, *Euodia*, *Boronia* and *Zieria*<sup>8</sup> as well as in *Ocimum*,<sup>9</sup> *Mentha*, *Lippia* and *Satureja*,<sup>10</sup> and in *Cinnamomum*<sup>11</sup> and *Ocotea*.<sup>12</sup>

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<sup>1</sup> R. T. BAKER and H. G. SMITH, *A Research on the Eucalyptus, Especially in Regard to their Essential Oils*, Government Printer, Sydney (1920).

<sup>2</sup> A. R. PENFOLD and F. R. MORRISON, *J. Proc. R. Soc. N.S.W.* **81**, 54 (1927).

<sup>3</sup> R. HEGNAUER, *Pharm. Weekblad.* **92**, 860 (1957).

<sup>4</sup> G. DILLEMANN, *Pharm. Weekblad.* **92**, 853 (1957).

<sup>5</sup> P. TETENYI, *Taxon* **9**, 241 (1960).

<sup>6</sup> T. SWAIN (editor), *Comparative Phytochemistry*, Academic Press, London (1966).

<sup>7</sup> P. TETENYI, *Botan. Zh.* **47**, 1731 (1962).

<sup>8</sup> H. H. G. MCKERN, *J. Proc. R. Soc. N.S.W.* **98**, 1 (1965).

<sup>9</sup> P. ROVESTI, *Riv. Ital. Ess.* **34**, 247 (1952).

<sup>10</sup> G. FESTER, *Rev. Fac. Ing. Qim. Santa Fe* **31**, 39 (1962).

<sup>11</sup> N. HIROTA, *Mem. Ehime Univ. (Japan)* **2**, 99 (1955).

<sup>12</sup> O. R. GOTTLIEB, M. T. MAGALHAES and W. B. MORS, *Perf. Essent. Oil Rec.* **50**, 26 (1959).

The exact nature of these variants is still in some doubt although some writers, e.g. Flück,<sup>13</sup> have suggested that the variation may be purely quantitative and not qualitative.

We have now established that the oils of *Eucalyptus dives* "Type", "Var. A", "Var. B", and "Var. C", all contain the same constituents, but in markedly varying proportions. This tends to support Flück's hypothesis and indicates that monoterpene variation known in other eucalypts may be of the same type.

Leaf samples of the four variants, "Type", "Var. A", "Var. B", "Var. C", were collected from the original locations described by Penfold and Morrison.<sup>2</sup> The samples were examined for morphological consistency, even though they were in areas where the possibility of hybridism could be almost certainly excluded. Progeny grown from the seed collected from the experimental trees has shown uniformity of characters in both the juvenile and adult leaf stages.

The leaf samples were distilled and the oils dried with anhydrous Na<sub>2</sub>SO<sub>4</sub> and kept under N<sub>2</sub> at 5°. The physical constants of each oil were taken and the oils then separated into hydrocarbon and oxygenated fractions by elution from a silica gel column. A sample of each oil, hydrocarbon fraction and oxygenated fraction were passed through a high resolution Perkin Elmer Model 226 gas chromatograph with a 150 ft × 0.01 in. Golay column containing either silicone oil DC200 or Apiezon L as stationary phases and helium as carrier gas. The oil from each of the four variants was shown to have sixty-two peaks, which were identical with respect to their retention times on both stationary phases. Known major components (piperitone, cineole and  $\alpha$ -phellandrene) were identified in each oil; the remainder have only been recorded on the basis of their retention times. The quantitative variation as obtained by GLC agreed generally with the earlier data of Penfold and Morrison and of other workers.

It seems clear that the original separation of these chemical variants based on the major constituent in the oils is simply a reflection of a large quantitative variation within the species. It is also apparent that in the possible continuum of oil constitutions, certain combinations have been selected and have become isolated from the rest of the population. The occurrence of the variants of *E. dives* is quite widespread and each tends to occur in separate distinct populations, although some oil types do mingle, e.g. "Var. A" and "Type". Whether the extant variants have a selective advantage over the other possible combinations of oil constituents is unknown.

If the nature of the chemical variants in other genera is shown to be the same as for *E. dives*, the utilization of essential oil characters for classificatory purposes in those plant groups showing within species chemical variation would appear to have a certain degree of justification. However, it should be borne in mind that the determination of the oil composition of some plant species may be subject to an error introduced by the presence of artefacts arising during or subsequent to the isolation of the oil from the plant. For example, Clary Sage,<sup>14</sup> *Geijera parviflora*<sup>15</sup> and *Hedycarya angustifolia*<sup>16</sup> all show marked differences in oil composition according to the manner in which the oils are isolated. In addition, as substances formed in plants are only summaries of underlying biogenetic processes they should always be used as taxonomic markers with some care.

<sup>13</sup> H. FLÜCK, in *Chemical Plant Taxonomy* (edited by T. SWAIN), p. 169, Academic Press, London (1963).

<sup>14</sup> L. L. CRABALONA, *France Parf.* **2**, 26 (1959).

<sup>15</sup> R. V. H. JONES and M. D. SUTHERLAND, *Australian J. Chem.* **21**, 2255 (1968).

<sup>16</sup> R. V. H. JONES and M. D. SUTHERLAND, *Chem. Commun.* 1229 (1968).