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# CONTRIBUTION TO THE STUDY OF THE ESSENTIAL LEAF OILS IN *SEVERINIA BUXIFOLIA* (POIR.) TENORE

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**Abstract**—The individual components of the distilled essential leaf oils of *Severinia buxifolia* (Poir.) Tenore (Rutaceae) have been separated by gas–liquid chromatography and their retention volume and probable identification determined.

## INTRODUCTION

THERE is a large body of information concerning the botanical and horticultural aspects of *Severinia buxifolia* (Swingle[1]), but there is little reported in the literature concerning the chemical composition of the plant itself. The last decade has seen a rising interest in the chemical composition of plants. New methods like electrophoresis, immunodiffusion and various kinds of chromatography are enlisted by biosystematists to gain a better understanding of populations and their species concepts. Essential oils and terpenes have been utilized by von Rudloff[2] in the study of *Juniperus* and by Iconomou *et al.*[3] in the study of *Pinus*. Many other workers have tried to find genetic relationships based upon their chemical composition (Haagen-Smit[4]).

Encroachment of urban growth, disease infestation and mineral accumulation speed the search for new areas of citrus growing and for other rootstocks. *Severinia buxifolia* has been considered as a possible source for rootstocks because it shows tolerance to salt and boron accumulations, resistance to nematodes and ease of grafting with *Citrus*.

The author has therefore embarked upon the gas chromatographic analysis of the essential leaf oils of *Severinia buxifolia* in order to gain a wider understanding of its strains and populations, its chemical composition and the possible effects of its rootstock upon the essential oils of *Citrus* species.

## RESULTS

Uniform mature leaves from each of three trees were washed, ground and distilled in a Clevenger distilling apparatus in an atmosphere of $CO_2$ to inhibit oxidation. Steam distillation was found to be superior to solvent extraction, and the fresh oil examined by gas chromatography.

To identify the peaks obtained in the chromatograms, the retention data were first compared with those from literature and then with those of pure standards taken under the same parameters in the authors' laboratory. By subsequent implementation of known

[1] W. T. SWINGLE, *The Botany of Citrus and Its Wild Relatives of the Orange Subfamily*, p. 275. University of California Press, Berkley (1943).
[2] E. VON RUDLOFF, *Can. J. Chem.* **41**, 2876 (1963).
[3] N. ICONOMOU, G. VALKANAS and J. BUECHI, *J. Chromatog.* **16**, 29 (1964).
[4] A. J. HAAGEN-SMIT, *Ann. Rev. Plant Physiol.* **4**, 305 (1953).

quantities of pure standards to known quantities of the distilled oils, a large number of components present could be tentatively identified. Of several columns tested, the above gave the best separation (Fig. 1, Table 1). The area under the peaks was determined by the trapezoidal approximation method.

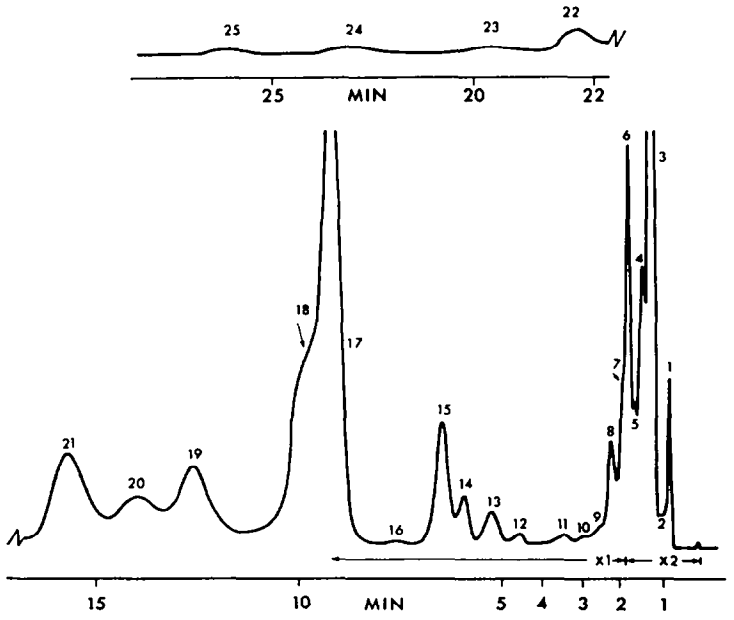


FIG. 1. GAS–LIQUID CHROMATOGRAM OF 1 $\mu$l OF DISTILLED LEAF OIL OF *Severinia buxifolia*. TENTATIVE PEAK IDENTITY AND PERCENT COMPOSITION OF THE INDIVIDUAL COMPONENTS IN TABLE 1.

One $\mu$l of the fresh oils was injected into an Aerograph A-90-P gas chromatograph with a thermo-detector (Wilkins Instrument and Research, Walnut Creek, Calif.). The conditions used were as follows: Column pyrex, 150 cm in length and 6 mm outside diameter; packing 60–80 mesh Chromosorb P with 20% LAC 446; carrier gas helium, 40 lb/in$^2$ pressure and 60 cc/min; injection temp. 210°, column temp. 160°, detector temp. 210°; filaments 200 mA; chart speed 0·5 in./min.

Of several genera of the Rutaceae tested, e.g. *Citrus*, *Poncirus*, *Hesperethusa*, *Atalantia*, *Murraya* and *Aeglopsis*, *Severinia buxifolia* lies well within the range of expected diversity as far as the leaf oils are concerned.

Repeated sampling at various stages of leaf-maturity in *Severinia* has shown that there is a change in the percent composition of the essential oil components. This shift in the quantitative relationships of the individual peaks to each other might well be due to the rebuilding of some components from precursors, primary substances or other essential oil components.

Slight changes in the terpenic fraction have been found in tissues which were distilled after wilting. This is explicable if one considers that plants, or plant parts, once removed from the ground, still undergo an active even though altered metabolism and that it is the metabolism that considerably influences the percent composition of the essential oil components. One can only speculate upon the role of the oils in the metabolism of the plant. Distillation of leaves after their natural abscission showed no remarkable loss of essential oil content. It is therefore doubtful that these oils serve as storage products. Moreover, it is known that the oils in related genera, as *Citrus* for example, are toxic to their own tissues and

make translocation improbable. However, oils might have evolutionary significance by repelling browsing animals and attracting others as pollinators.

TABLE 1. PERCENTAGE COMPOSITION OF THE ESSENTIAL LEAF OILS OF *Severinia buxifolia*

| Peak number | Relative retention time range | Relative retention time | Percentage retention volume | Component |
|---|---|---|---|---|
| 1 | 0·09–0·10 | 0·10 | 2·50 | α-Pinene |
| 2 | 0·13–0·14 | 0·13 | 0·95 | Camphene |
| 3 | 0·16–0·17 | 0·16 | 14·55 | β-Myrcene |
| 4 | 0·19–0·20 | 0·19 | 6·80 | Phellandrene |
| 5 | 0·21–0·22 | 0·22 | 2·14 | Heptanal |
| 6 | 0·24–0·26 | 0·25 | 8·59 | α-Limonene |
| 7 | 0·27–0·29 | 0·27 | 3·22 | γ-Terpinene |
| 8 | 0·30–0·34 | 0·33 | 1·31 | p-Cymene |
| 9 | 0·35–0·38 | 0·38 | 0·35 | Octanal |
| 10 | | 0·43 | 0·23 | Unidentified |
| 11 | 0·48–0·50 | 0·50 | 0·47 | Methyl heptanone |
| 12 | 0·68–0·69 | 0·69 | 0·58 | Nonanal |
| 13 | 0·78–0·81 | 0·80 | 0·95 | Furfural, octyl acetate |
| 14 | 0·89–0·91 | 0·91 | 1·19 | Decanal |
| 15 | 1·00 | 1·00 | 3·10 | Linalool |
| 16 | 1·17–1·20 | 1·17 | 0·35 | Nonyl acetate |
| 17 | 1·40–1·45 | 1·43 | 14·55 | Bornyl acetate |
| 18 | 1·51–1·55 | 1·51 | 18·01 | Neryl formate |
| 19 | 1·92–1·96 | 1·96 | 5·84 | Decyl acetate? |
| 20 | 2·20–2·22 | 2·20 | 3·69 | Geranyl formate |
| 21 | 2·44–2·48 | 2·46 | 6·08 | Neral |
| 22 | 2·60–2·70 | 2·68 | 1·67 | Geranyl acetate |
| 23 | 2·90–2·99 | 2·99 | 0·71 | 1-Carvone |
| 24 | 3·50–3·60 | 3·58 | 1·07 | Nerol |
| 25 | | 4·11 | 0·95 | Unidentified |

In summary, it may be said that *Severinia buxifolia* shows a consistent pattern of essential oil components when analyzed by means of gas chromatography. This pattern is determined by the relationship of the individual components to each other, rather than by individual peaks. Variability between trees exists and may be due to both genetic or non-genetic factors, and has to be taken into account in evaluating these chromatograms.

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Voucher specimens of *Severinia buxifolia* (Scora 5078a–c) are deposited at the UCR Herbarium.