

## ESSENTIAL LEAF OIL VARIABILITY IN GREEN, VARIEGATED AND ALBINO FOLIAGE OF *MYRTUS COMMUNIS*

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**Key Word Index**—*Myrtus communis*; Myrtaceae; leaf essential oils; variation in variegated and albino forms.

**Abstract**—Green, variegated and albino leaves of *Myrtus communis* yield mono- and sesqui-terpenes which show considerable variation in their ratios when compared to each other.

### INTRODUCTION

MONO- and sequi-terpenes of essential plant oils have often been considered to be secondary substances. Their value as reliable markers in systematic investigations has amply been demonstrated. In the past few years, however, much knowledge has been accumulated about the sensitivity of these essential oils to various outside influences. Influences such as: geography,<sup>1,2</sup> maturation,<sup>3</sup> nutrients,<sup>4-6</sup> temperature,<sup>7</sup> storage,<sup>8</sup> polyploidy,<sup>9</sup> rootstocks,<sup>10</sup> daylength,<sup>11,12</sup> photoperiodic effects,<sup>13</sup> and others.

An investigation on *Myrtus communis* L. showed unusually large variations in its essential leaf oils from repetitive samples. These randomly harvested samples contained green, albino and variegated foliage, each in varying amounts. Since all other conditions were equal and albinism the only variable, it was thought that this variable might have caused the change in terpene ratios. Therefore, an analysis of the various types of foliage was undertaken. The variegated foliage consisted primarily of the periclinal type, interspersed with few leaves of the sectorial type.

The oil of this *Myrtus* species has been previously investigated by a number of workers because of its importance in the food and cosmetic industries. Spanish myrtle oil.<sup>14,15</sup>

<sup>1</sup> E. KRUPSKI and L. FISCHER, *J. Am. Pharm. Asoc., Sci. Educ.* **39**, 433 (1950).

<sup>2</sup> D. M. SMITH and L. LEVI, *J. Agric. Food Chem.* **9**, 230 (1961).

<sup>3</sup> V. K. WATSON and J. L. ST. JOHN, *J. Agric. Food Chem.* **3**, 1033 (1955).

<sup>4</sup> J. V. BAIRD, *Agron. J.* **49**, 225 (1957).

<sup>5</sup> F. C. STEWARD, F. CRANE, K. MILLAR, R. M. ZACHARIUS, R. RABSON and D. MARGOLIS, *Symp. Soc. Exptl. Biol.* **13**, 148 (1959).

<sup>6</sup> F. C. STEWARD, K. J. HOWE, F. A. CRANE and R. RABSON, *Cornell Univ. Agric. Expt. Sta. Mem.* **379**, Parts I-VII (1962).

<sup>7</sup> R. H. BIGGS and A. C. LEOPOLD, *Proc. Am. Soc. Hort. Sci.* **66**, 315 (1955).

<sup>8</sup> F. W. HEFENDEHL, *Pharmazie Beih. Ergaenzungsband* **18**, 777 (1963).

<sup>9</sup> R. W. SCORA, J. W. CAMERON and J. BERG, *Taxon* **19**, 752 (1970).

<sup>10</sup> W. P. BITTERS and R. W. SCORA, *Bot. Gazette* **131**, 105 (1970).

<sup>11</sup> A. GRAHLE and C. HOELTZEL, *Naturwissenschaften* **50**, 552 (1963).

<sup>12</sup> C. HOELTZEL, Ueber Zusammenhaenge zwischen der Biosynthese der aetherischen Oele und dem photoperiodischen Verhalten der Pfefferminze (*Mentha piperita* L.), Dissertation, Eberhard-Karls Universitaet, Tuebingen (1964).

<sup>13</sup> R. LANGSTON and A. C. LEOPOLD, *Proc. Am. Soc. Hort. Sci.* **63**, 347 (1954).

<sup>14</sup> E. JAHNS, *Arch. Pharm. Ber. dt. Pharmaz. Gesellschaft* **227**, 174 (1889).

<sup>15</sup> J. T. OCHOA, *Inst. Forestal de Investigaciones y Experiencias Madrid* **23**, 97 (1952).

Calabrian, Sicilian and Sardinian myrtle oil,<sup>16</sup> Indian myrtle oil,<sup>17</sup> and that of Moroccan origin<sup>18</sup> were investigated. The physical, chemical and antibacterial properties of *Myrtus communis* leaf oils were also reported.<sup>19</sup>

#### RESULTS AND DISCUSSION

The amount of chlorophyll present in a leaf affects the rate of photosynthesis; since photosynthesis influences the essential oil metabolism, the yield of oil and the terpene ratios can be expected to show such influence. Albino foliage yielded a light opaque oil with hardly any smell as compared to more than four times as much light green oil with full-bodied bouquet from the green foliage. Each type of essential oil was found to have the same number of measurable individual components, namely 25.

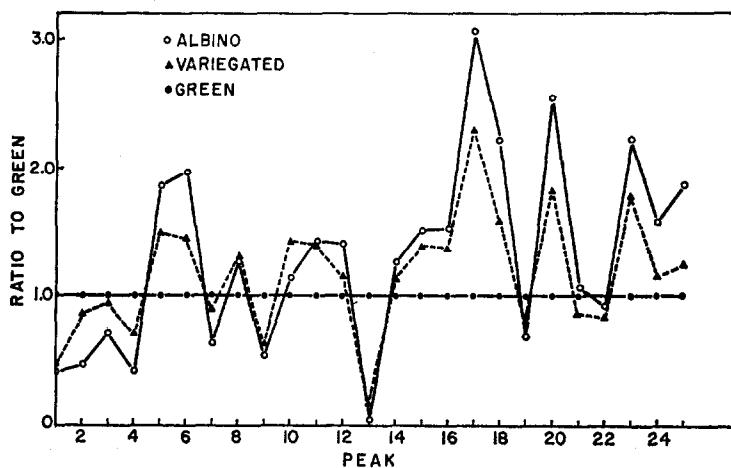


FIG. 1. RATIOS OF VARIEGATED AND ALBINO LEAF OILS TO THAT OF NORMAL GREEN LEAF OIL EXPRESSED IN PER CENT.

A one-way analysis of variance for each peak was calculated to compare the three groups: albino, variegated and green. The between-groups comparison was significant for all peaks except for No. 22. Next, the three groups were compared with Duncan's multiple range test to pinpoint the differences. The ratio of albino to green and of variegated to green was calculated for each peak and graphed to show which peaks were higher or lower in albino and in the variegated sample when compared to green. The ratio of albino to green is the average for the peak for the albino samples divided by the average for the peak for the green samples (Fig. 1).

The differences among the oil types were not qualitative, but quantitative; and these quantitative differences were so consistent that of the 25 components present in each type of oil, 24 were significantly different from one another (Table 1). In 8 out of 25 components it was the oil component of the green foliage which had the highest percentage; mostly, however, (in 14 cases) it was that of the albino oil. If the changes in individual oil com-

<sup>16</sup> G. L. BRUTO and M. CALVARANO, *Essence Derivati Agrumari* V. 29, 185 (1959).

<sup>17</sup> K. P. SINGH and G. N. GUPTA, *Indian Perfumers* V. 5, 23 (1961).

<sup>18</sup> B. M. LAWRENCE, S. T. TERHUNE and J. W. HOGG, *Am. Perfumer* 85, 53 (1970).

<sup>19</sup> A. P. DEGTYAROVA and V. YA POCHINOK, *Pharm. J., Kiev* 15, 47 (1960).

ponents between the oils from the green and albino foliage are due to the photosynthetic ability of the leaves, then the oil from the variegated foliage should take the intermediate position between the green and albino oil no matter if the individual components range from a higher to a lower percentage, or vice versa. This is clearly the case in 22 components, the exceptions being components 10, 21 and 22 (Table 1).

TABLE 1. COMPOSITION OF GREEN, VARIEGATED AND ALBINO LEAF OILS IN *Myrtus communis*

Peak No.	Components	Leaf oil mean %			1% Duncan's	1% L.S.D.
		Albino	Variegated	Green		
1	—	0.02	0.03	0.06	y,y,x	0.03
2	Tricyclene	0.02	0.05	0.06	y,xy,x	0.03
3	$\alpha$ -Pinene	25.50	32.58	35.13	z,y,x	2.01
4	$\beta$ -Pinene	0.60	0.96	1.39	z,y,x	0.13
5	$\beta$ -Myrcene	0.66	0.52	0.35	x,y,z	0.07
6	2-Methylbutyl-2-methyl butyrate	0.66	0.46	0.32	x,y,z	0.06
7	2-Methylbutanol	0.66	0.94	1.02	x,x,x	0.40
8	d-Limonene	28.16	26.42	21.08	x,y,z	0.42
9	1,8-Cineol	8.15	9.11	15.03	z,y,x	0.36
10	$\gamma$ -Terpinene	1.28	1.54	1.09	xy,x,y	0.30
11	<i>p</i> -Cymene	0.53	0.50	0.36	x,x,x	0.18
12	<i>trans-allo</i> Ocimene	3.17	2.50	2.20	x,y,y	0.50
13	Unidentified	0.02	0.12	0.69	z,y,x	0.07
14	Linalool	13.69	11.72	10.57	x,y,z	0.21
15	Linalyl acetate	1.20	1.08	0.78	x,x,y	0.20
16	Camphor	0.40	0.34	0.26	x,x,x	0.15
17	Bornyl acetate	1.68	1.20	0.54	x,y,z	0.19
18	Myrtenol	0.58	0.40	0.26	x,xy,y	0.21
19	Caryophyllene	0.05	0.06	0.07	x,x,x	0.02
20	$\alpha$ -Humulene	2.33	1.62	0.88	x,y,z	0.19
21	$\alpha$ -Terpineol	4.74	3.66	4.31	x,z,y	0.14
22	Myrtenyl acetate	0.14	0.13	0.14	x,x,x	0.07
23	Unidentified	0.39	0.31	0.17	x,x,y	0.13
24	Geranyl acetate	4.66	3.28	2.88	x,y,z	0.34
25	Myrtanyl acetate	0.72	0.50	0.38	x,y,y	0.17

## EXPERIMENTAL

Healthy mature leaves were collected from one bush of *Myrtus communis* containing regular green branches, albino branches of light yellowish tint and variegated branches. This bush was selected in order to have exactly the same genetic and environmental conditions for the green, variegated and albino samples. Of each type five samples were collected from different branches of the same bush. Each sample contained about 400 leaves. These were macerated, steam distilled and the resulting oils were analyzed by a Varian 1520 GLC. The columns used were  $305 \times 0.64$  cm ss filled with 20% LAC 446 on 60-80 mesh Chromosorb W. The temperature was non-linearly programmed from 50-182° in a 2.5 hr run. The areas under the peaks were calculated by a Varian 475 digital integrator. The large peaks were identified by MS and the smaller ones tentatively determined by comparison of their retention times with those of standards. *Myrtus communis* voucher specimen: Scora 3182 at UCR.

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