CHEMICAL COMPOSITION OF THE ESSENTIAL OILS
FROM CERTAIN NATIVE PLANTS OF THE SOUTHWESTERN DESERT

by
William Frank McCaughey

submitted to the faculty of the
Department of Agricultural Chemistry and Soils
in partial fulfillment of the requirements for the degree of
DOCTOR OF PHILOSOPHY
in the Graduate College, University of Arizona

1951

Approved: [Signature]
Director of Thesis
Date: May 21, 1951
THE PROBLEM OF THE SPONTANEOUS 
FORMATION OF OXYGEN FROM CERTAIN 
ADIABATIC RAYS OF THE STRATOSPHERE 

By

[Student's Name]

[Signature]

[Date]

[Institution]

[Department]

[Institution]

[Program]

[Institution]

[Date]

[Institution]

[Signature]
WILLIAM FRANK McCaughey

1921 Born Chicago, Illinois
1942 B.S. Purdue University
1943 Commissioned 2nd Lt., U.S. Army
1948 M.S. Northwestern University
1947-49 Richard Hudnut Fellow in Agricultural Chemistry and Soils
1951 Ph.D. University of Arizona
ACKNOWLEDGMENTS

To Prof. T. F. Buehrer, Head of the Department of Agricultural Chemistry and Soils, who, as director of this thesis, gave so generously his time and effort, I am greatly indebted.

For the Richard Hudnut Company and the Warner Institute for Therapeutic Research for providing the fellowship which made this investigation possible—

For the other members of the Department of Agricultural Chemistry and Soils for their advice and assistance—

For the members of the Department of Nutrition and the Department of Chemistry for aid and equipment in time of need—

For Dr. Frank Gould and Messrs. H. H. Haskell and E. B. Kurtz for their assistance and cooperation in the field—

For Magnus, Mabee and Reynard, Inc. and for Fritzsche Brothers, Inc., both of New York City, for their generosity in providing authentic essential oil samples—

And for my wife, Margie, who was a constant source of inspiration and whose assistance was invaluable during the course of this investigation—

I have the deepest sense of appreciation and gratitude.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>LITERATURE REVIEW</td>
<td>5</td>
</tr>
<tr>
<td>PLAN AND SCOPE OF THE INVESTIGATION</td>
<td>15</td>
</tr>
<tr>
<td>EXPERIMENTAL METHODS AND RESULTS</td>
<td>17</td>
</tr>
<tr>
<td><strong>PART I. PRELIMINARY INVESTIGATION OF SEVERAL OILS</strong></td>
<td></td>
</tr>
<tr>
<td>Exploratory Phase</td>
<td>17</td>
</tr>
<tr>
<td>Trial Steam Distillation</td>
<td>19</td>
</tr>
<tr>
<td>Methods of Analysis for Gross Constituents</td>
<td>48</td>
</tr>
<tr>
<td>Design of the Fractionation Apparatus</td>
<td>56</td>
</tr>
<tr>
<td><strong>PART II. INVESTIGATION OF THE OIL OF Haplopappus laricifolius</strong></td>
<td>64</td>
</tr>
<tr>
<td>Fractionation of the Bulk Extract</td>
<td>64</td>
</tr>
<tr>
<td>Determination of the Ultraviolet Absorption Spectra</td>
<td>69</td>
</tr>
<tr>
<td>Determination of Melting Points</td>
<td>73</td>
</tr>
<tr>
<td>Technique of Nitrogen Determinations</td>
<td>74</td>
</tr>
<tr>
<td>Identification of alpha-Pinene</td>
<td>76</td>
</tr>
<tr>
<td>Identification of beta-Phellandrene</td>
<td>79</td>
</tr>
<tr>
<td>Attempted Identification of Fraction 11</td>
<td>84</td>
</tr>
<tr>
<td>Attempted Identification of Fraction 14</td>
<td>87</td>
</tr>
<tr>
<td>Attempted Identification of Fraction 15</td>
<td>91</td>
</tr>
<tr>
<td>Identification of Phellandral</td>
<td>96</td>
</tr>
<tr>
<td>Attempted Identification of the Sesquiterpene Fractions</td>
<td>102</td>
</tr>
<tr>
<td><strong>PART III. INVESTIGATION OF THE OIL OF Tagetes lemmoni</strong></td>
<td>105</td>
</tr>
<tr>
<td>Fractionation of the Bulk Extract</td>
<td>105</td>
</tr>
<tr>
<td>Determination of the Ultraviolet Absorption Spectra</td>
<td>110</td>
</tr>
<tr>
<td>Determination of Degree of Unsaturation by Means of Iodine Absorption</td>
<td>112</td>
</tr>
<tr>
<td>Indication of the Ester Content of Fraction 1</td>
<td>114</td>
</tr>
<tr>
<td>Indication of the Probable Presence of Ocimene in Fraction 4</td>
<td>116</td>
</tr>
<tr>
<td>Attempted Identification of Fraction 6</td>
<td>120</td>
</tr>
<tr>
<td>Identification of 2,6-Dimethyl-7-octen-4-one</td>
<td>123</td>
</tr>
<tr>
<td>Identification of Tagetone</td>
<td>128</td>
</tr>
<tr>
<td>PART IV. INVESTIGATION OF THE OIL OF <em>Choisya dumosa</em></td>
<td>134</td>
</tr>
<tr>
<td>-----------------------------------------------------</td>
<td>-----</td>
</tr>
<tr>
<td>Fractionation of the Oil Extract</td>
<td>134</td>
</tr>
<tr>
<td>Determination of the Ultraviolet Absorption Spectra</td>
<td>137</td>
</tr>
<tr>
<td>Attempted Identifications of the Various Fractions</td>
<td>139</td>
</tr>
</tbody>
</table>

<p>| PART V. MISCELLANEOUS TESTS ON OTHER OIL EXTRACTS    | 140 |
| DISCUSSION                                          | 144 |
| SUMMARY                                             | 149 |
| BIBLIOGRAPHY                                         | 150 |</p>
<table>
<thead>
<tr>
<th>Number</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>Apparatus for exploratory small-scale steam distillation test</td>
<td>19</td>
</tr>
<tr>
<td>II.</td>
<td>Pressed plant specimens</td>
<td>22</td>
</tr>
<tr>
<td>III.</td>
<td>Pressed plant specimens</td>
<td>23</td>
</tr>
<tr>
<td>IV.</td>
<td>Pressed plant specimens</td>
<td>24</td>
</tr>
<tr>
<td>V.</td>
<td>Pressed plant specimens</td>
<td>25</td>
</tr>
<tr>
<td>VI.</td>
<td>Pressed plant specimens</td>
<td>26</td>
</tr>
<tr>
<td>VII.</td>
<td>Pressed plant specimens</td>
<td>27</td>
</tr>
<tr>
<td>VIII.</td>
<td>Pressed plant specimens</td>
<td>28</td>
</tr>
<tr>
<td>IX.</td>
<td>Pressed plant specimens</td>
<td>29</td>
</tr>
<tr>
<td>X.</td>
<td>Pressed plant specimens</td>
<td>30</td>
</tr>
<tr>
<td>XI.</td>
<td>Apparatus for steam distillation in bulk quantity</td>
<td>46</td>
</tr>
<tr>
<td>XII.</td>
<td>Original apparatus for vacuum fractional distillation, (a), and modifications</td>
<td>57</td>
</tr>
<tr>
<td>XIII.</td>
<td>Apparatus for high vacuum fractional distillation</td>
<td>59</td>
</tr>
<tr>
<td>XIV.</td>
<td>High vacuum fractionation apparatus including the modified column</td>
<td>61</td>
</tr>
<tr>
<td>XV.</td>
<td>Modified high vacuum fractionation apparatus</td>
<td>63</td>
</tr>
<tr>
<td>XVI.</td>
<td>Semimicro chromatographic adsorption column</td>
<td>94</td>
</tr>
<tr>
<td>XVII.</td>
<td>Semimicro hydrogenation apparatus</td>
<td>125</td>
</tr>
<tr>
<td>XVIII.</td>
<td>Macro fractional distillation apparatus</td>
<td>134</td>
</tr>
<tr>
<td>XIX.</td>
<td>Photomicrographs of leaf sections</td>
<td>143</td>
</tr>
<tr>
<td>XX.</td>
<td>Photomicrographs of leaf sections</td>
<td>143</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Number</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Quantitative distribution and physical properties of the essential oil fractions of <em>Haplopappus laricifolius</em></td>
</tr>
<tr>
<td>2.</td>
<td>Ultraviolet absorption spectra of the essential oil fractions of <em>Haplopappus laricifolius</em></td>
</tr>
<tr>
<td>3.</td>
<td>Quantitative distribution and physical properties of the essential oil fractions of <em>Tagetes lemmoni</em></td>
</tr>
<tr>
<td>4.</td>
<td>Ultraviolet absorption spectra of the essential oil fractions of <em>Tagetes lemmoni</em></td>
</tr>
<tr>
<td>5.</td>
<td>Quantitative distribution and physical properties of the essential oil fractions of <em>Choisya dumosa var. mollis</em></td>
</tr>
<tr>
<td>6.</td>
<td>Ultraviolet absorption spectra of the essential oil fractions of <em>Choisya dumosa var. mollis</em></td>
</tr>
<tr>
<td>7.</td>
<td>Visible absorption spectrum of volatile oil of <em>Artemisia carruthii</em> compared with spectrum of <em>S-guajazulene</em></td>
</tr>
</tbody>
</table>
CHEMICAL COMPOSITION OF THE ESSENTIAL OILS FROM CERTAIN NATIVE PLANTS OF THE SOUTHWESTERN DESERT

INTRODUCTION

Essential oils have long played an important rôle in our civilization. Even before the time of Christ, oil of turpentine was known and used as a therapeutic remedy. As time passed, the essential oils assumed a place of importance in the preparation of incense for religious ceremonies; and still later they formed the basis for the extensive field of perfumery. Down through the centuries the interest in essential oils has continued at an ever-increasing rate. Today the list of industries employing essential oils is an extensive one, including the manufacturers of perfumes, toilet goods, condiments, pharmaceuticals, foods, confections, soaps, insecticides, and soft drinks. These oils are of evident importance since they are a necessary constituent in the production of many millions of dollars of consumers' goods each year.

Essential oils, or volatile oils, are defined as oils which are liberated or volatilized by the action of steam on the flowers, leaves, stems, roots, or seeds of a plant. The oils of rose, wintergreen, sassafras, cinnamon, thyme, sage, fennel, rosemary, and lemongrass are among the common volatile oils which find wide use. Physically the essential oils are homogeneous liquids, miscible with alcohol, generally of
pleasant aroma, and usually yellow in color, although they sometimes are colorless, blue, green, or brown, and occasionally are solid in form.

Chemically the essential oils are a complex mixture of more or less volatile constituents. These constituents can be classified generally into three groups: (1) derivatives of benzene, C₆H₆, such as anisaldehyde, CH₃OC₆H₄CHO; cinnamic alcohol, C₆H₅CH=CH·CH₂OH; and acetophenone, C₆H₅COCH₃; (2) straight-chain compounds, such as ethyl acetate, CH₃COOC₂H₅, and methyl n-heptyl ketone, CH₃CO(CH₂)₆CH₃; (3) the terpenes, which are derivatives of isoprene, or isopentene, C₅H₈,

\[ \text{CH}_2 = \text{C-CH} = \text{CH}_2 \]

\[ \text{CH}_3 \]

The terpenes comprise the largest group of essential oil constituents. They include the monoterpenes, C₁₀H₁₆, made up of two isoprene units; the sesquiterpenes, C₁₅H₂₄, containing three isoprene units; the diterpenes, C₂₀H₃₂, with four units; and the polyterpenes containing "n" units. The name "terpene" in a more limited sense designates the C₁₀H₁₆ hydrocarbons and their oxygenated derivatives, C₁₀H₁₆O and C₁₀H₁₈O. A typical acyclic terpene hydrocarbon is myrcene:
Examples of monocyclic and bicyclic terpenes are alpha-terpinene and alpha-pinene respectively:

\[
\begin{align*}
\text{alpha-terpinene} & \quad \text{alpha-pinene} \\
\end{align*}
\]

Citral and menthone are examples of the oxygenated terpenes.

\[
\begin{align*}
\text{citral} & \quad \text{menthone} \\
\end{align*}
\]

The oxygenated terpenes—esters, ketones, aldehydes, and alcohols—have generally a more pleasing aroma and are less likely to resinify, hence are more desirable than the terpene hydrocarbons in the essential oil trade.

The pleasing and often even pungent aroma which pervades the desert after the summer rains is unmistakable evidence of
the presence of essential oils in the native plants of this region. Very little work has been reported in the literature on the essential oils of Arizona plants. Thus there appeared to be an extensive and virtually untouched source of plants in the desert and the mountain ranges of this region available for such a study. In 1943 the Richard Hudnut Company, a perfumery and cosmetic house in New York City, established a fellowship at the University of Arizona, through the Warner Institute for Therapeutic Research, for the investigation of some of the native plants of southern Arizona with a view to their possible cosmetic or therapeutic use. The results of the investigation to be presented in this dissertation constitute a contribution to our knowledge of the essential oil constituents of several of these plants. It may not be too much to surmise that such knowledge may in future prove to be of economic value to the farmers of Arizona if a market should be created for such oil extracts and if the plants yielding these oils can be cultivated economically as a specialty crop.
LITERATURE REVIEW

In 1899 Gildemeister and Hoffmann (23) published a three-volume set entitled The Volatile Oils, which contained information on the essential oil plants known up to that time. In 1922 the second edition was completed and translated from German into English. This set has been a standard reference work in the essential oil field since the early 1900's. In addition to the general chemical and physical properties of the various essential oils described in Volumes II and III, the specific chemical composition of many of the oils is reported. Since the plants described in these volumes are not restricted to a single locality, many occurring in various parts of the globe, it was to be expected that several would be found native to Arizona. Volume II contains descriptions of the following plants which, according to Kearney and Peebles (30), occur in Arizona:

<table>
<thead>
<tr>
<th>Plant Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abies concolor</td>
</tr>
<tr>
<td>Acacia farnesiana</td>
</tr>
<tr>
<td>Amorpha fruticosa</td>
</tr>
<tr>
<td>Asparagus officinalis</td>
</tr>
<tr>
<td>Brassica alba (B. hirta)</td>
</tr>
<tr>
<td>Brassica juncea</td>
</tr>
<tr>
<td>Brassica napus (B. campestris)</td>
</tr>
<tr>
<td>Brassica nigra</td>
</tr>
<tr>
<td>Capsella bursa pastoris</td>
</tr>
<tr>
<td>Chenopodium ambrosioides</td>
</tr>
<tr>
<td>Glycyrrhiza glabra</td>
</tr>
<tr>
<td>Juniperus communis</td>
</tr>
<tr>
<td>Lepidium sativum</td>
</tr>
</tbody>
</table>
Volume III treats of the following plants growing native in Arizona:

Achillea millefolium
Ambrosia artemisiafolia
Anagallis arvensis
Anethum graveolens
Anthemis cotula
Atriplex graveolens
Apocynum androsaemifolium
Artemisia annua
Artemisia biennis
Artemisia frigida
Coriandrum sativum
Datura stramonium
Dodecatheon meadia
Erigeron canadensis
Euphorbia pilulifera
Foeniculum vulgare
Lantana camara
Matricaria chamomilla
Matricaria discoidea
Mentha arvensis
Mentha spicata
Monotropa hypopitys
Nepeta cataria
Parthenium argentatum
Pastinaca sativa
Sium cicutaefolium
Solidago canadensis

In 1922 Parry (34) published a fourth edition of his volume containing monographs on essential oils among which are found the following plants native to Arizona:

Abies concolor
Acacia farnesiana
Amorpha fruticosa
Anethum graveolens
Atriplex graveolens
Apocynum androsaemifolium
Artemisia absinthium
Artemisia annua
Artemisia frigida
Artemisia ludoviciana
Brassica alba
Brassica juncea
Brassica nigra
Chenopodium ambrosioides
Coriandrum sativum
Erechtogen canadensis
Foeniculum vulgare
Humulus lupulus
Juniperus communis
Lantana camara
Matricaria chamomilla
Mentha arvensis
Mentha spicata
Monotropa hypopitys
Nepeta cataria
Pastinaca sativa
Pinus edulis
Pinus monophylla
Pinus palustris
Pinus ponderosa
Prunus virginiana
Pseudotsuga taxifolia
Pseudotsuga glauca
Sium cicutaefolium
Solidago canadensis
Trifolium pratense
Guenther (25) has published the most recent works on the essential oils with a view toward bringing the work of Gilde- meister, Parry, and Finnemore up to date. His third volume contains monographs concerning several genera in the families Rutaceae and Labiatae, of which the following plants also occur in Arizona:

- Audibertia incana
- Juniperus virginiana
- Mentha arvensis
- Mentha spicata
- Monarda menthaefolia
- Monarda pectinata
- Nepeta cataria
- Salvia carnosa

Naves and Mazuyer (33) brought forth a volume (English edition by Sagarin) in 1946 which treats of the extraction of flower oils by various methods—hot fat extraction, enfleurage, and extraction by means of cold, volatile solvents. The book contains monographs on many plant sources used as raw materials in the perfumery trade. A few of these plants occur in Arizona and are listed here:

- Acacia farnesiana
- Apium graveolens
- Aspidium filix-max
- Coriandrum sativum
- Humulus lupulus
- Juniperus communis
- Melilotus officinalis
- Philadelphus microphyllus
- Ricinus communis
- Trifolium melilotus officinalis

Members of other genera which occur in Arizona are: Buddleia, Ribes, Salvia, and Sambucus.

In 1947 a conference (39) was held in California at which papers were presented on the cultivation and nature of oil content of essential oil plants of the southwestern states.
Among the plants mentioned were the following which occur in Arizona:

- **Anaphalis margaritacea**
- **Anethum graveolens** (rare)
- **Artemisia spp.**
- **Chrysanthemum spp.**
- **Coriandrum sativum** (rare)
- **Gnaphalium spp.**
- **Isocoma spp.** (Haplopappus spp.)
- **Matricaria spp.**
- **Mentha arvensis**
- **Mentha spicata**
- **Pectis papposa**
- **Salvia spp.**
- **Trichostema sp.**

Hardy (27) lists species of plants in the genera *Teucrium*, *Salvia*, and *Foeniculum* as having an aromatic odor, all of which genera occur in Arizona. Shapter (41) describes the essential oil of *Chenopodium ambrosioides*. Fester (19), in discussing essential oil plants in the Argentine, mentions *Lippia*, *Hedeoma*, and *Tagetes* species as having a pleasing aroma. Two species of *Bursera* yield essential oils which are described by Narayan and Dutt (32). *Bursera odorata* and *B. microphylla* occur in Arizona. The latter species was distilled by the writer in the present investigation.

Kearney and Peebles' (30) volume on the flora of Arizona was used in this investigation as the authority on native plants as well as on their nomenclature. The plant descriptions in this volume frequently make mention of the aromatic character of a particular species and thus provide another source of plants of possible value to the investigator in this field. Plants in this category are as follows:

*The genera so indicated occur native in Arizona but are not the identical species as listed in the Proceedings.*
Langenau (4, I, pp. 344-8) has outlined a procedure for the examination and analysis of an essential oil:

"A representative sample of the oil to be investigated should be analyzed carefully. All physical and chemical properties should be determined, including specific gravity, optical rotation, refractive index, solubility and the percentages of esters, aldehydes, ketones, phenols, acids and alcohols."

"For an oil which has not been investigated previously, the first step is a general examination, followed by an investigation which endeavors to discover as many of the constituents as possible. This usually reveals those constituents which occur in substantial amounts. Frequently, indications of the occurrence of other constituents are thereby obtained, whose presence, however, cannot be established conclusively. A subsequent investigation directed solely to the isolation and identification of such individual constituents often will prove successful."

"After such preliminary treatment as indicated above, the oil should be fractionated, thus resulting in a separation of the oil into a low-boiling terpene fraction, an intermediate fraction, a fraction rich in oxygenated constituents, a second intermediate fraction, a fraction containing the sesquiterpene constituents, and a distillation residue. The residue usually contains polymerization products and high-boiling constituents, such as azulenec..."
compounds, and the naturally-occurring waxes in the case of citrus oils obtained by expression.

"Should the original analysis show a high ester content, it is usually best to fractionate the oil before saponification so that the ester may be obtained in a state of relative purity for a determination of physical properties. Its components may then be identified after saponification. Since the corresponding free alcohol usually is present with the ester, saponification of the whole oil (followed by fractionation) may be preferable, especially if only small amounts of ester are present.

"For the identification of individual constituents which have been separated and purified from the oil, two general procedures are employed: (1) The determination of physical properties including melting point (or congealing point), boiling point, specific gravity, optical rotation, refractive index and solubility in alcohol of varying strengths. (2) The preparation of suitable derivatives, preferably solid compounds of definite melting point capable of purification by recrystallization. In general, the identification may be considered established if no depression is observed in the melting point when a derivative of the constituent is mixed with the corresponding derivative of a sample of known purity and constitution. * * * * * In many cases compounds obtained by oxidation, reduction, and condensation may be used for identification.

"Other methods are often employed in establishing the identity of a constituent or derivative: combustion to determine the percentage of carbon and hydrogen and to establish the empirical formula; molecular weight determinations, especially by cryoscopic methods; molecular refraction; ignition of metallic salts, especially the silver salts of organic acids; determinations of the percentage of halogen in chlorides and bromides; and other procedures."

A procedure for the analysis of essential oils was described by Finlayson (20) in his investigation of the oil of Phebalium argenteum. The oil was obtained by steam distillation and was submitted to a systematic fractionation following treatment with a number of reagents which were used to remove
such compounds as might react therewith, thus simplifying the subsequent fractionation. The reagents were as follows: 5% caustic soda—followed by acidification of the aqueous layer to show the presence or absence of phenols; normal sodium sulfite—in order to separate the aldehydes citral and citronellal if present; sodium hydrogen sulfite (30% solution containing a small amount of free sulfur dioxide)—followed by distillation with aqueous sodium carbonate to separate any highly reactive ketones which might be present, such as, in this case, methyl heptyl ketone and methyl nonyl ketone; alcoholic caustic potash (10% solution)—in order to remove by saponification the esters present in the oil, which were shown by preliminary tests to boil over a wide range. This permitted examination of their acid and alcohol radicals separately. The unsaponifiable portion recovered from the alcoholic potash treatment (constituting the main bulk of the oil) was then submitted to a systematic fractionation.

Sievers and Marshall (44) reported on a preliminary investigation of the essential oil of Poliomintha incana (syn.: Hedeoma incana; common name, "mock pennyroyal"). The plant, which was grown in nursery experiments, is native to Arizona and has very aromatic foliage and flowers. The paper contains a description of the oil, its physical properties, chemical constants, and the results of a fractionation of the oil under atmospheric pressure. The physical constants of the main fraction indicate the principal constituent of
the oil to be pulegone.

In their paper on the essential oil of *Pectis papposa* Bradley and Haagen-Smit (26) discussed the growth factors of this plant, which occurs in Arizona. It was noted that the oil content was less in the more mature plant which had already dropped most of its seed and leaves, and that the oil was characterized by a somewhat sharper odor than that of the lush plant. The authors also discussed the fractionation of the oil, presenting a table of physical constants of the various fractions and stating that the oil of *P. papposa* contains by analysis 47% cumenaldehyde, 12% carvone, 27% beta-pinene, 2% alpha-pinene, 1% benzaldehyde, as well as small amounts of esters and acids. They also mentioned the interesting fact of the wide variation in composition between the oil content of *P. papposa* and the other reported *Pectis* species, *tellana* (1) and *elongata* (2). Cumenaldehyde is the predominating constituent of *papposa* while thymol and citral are the chief components of *tellana* and *elongata*, respectively. Neither of these last-named occurs in Arizona.

Buehrer, Mason, and Crowder (14) discuss the chemical composition of the rayless goldenrod, *Haplopappus hartwegi* (*H. tenuisectus*) as to its content of essential oil, alkaloids, rubber, etc. The oil content was determined on samples of fresh material to prevent partial loss on drying and grinding of the plant material. Prior treatment of the sample with 5% sodium hydroxide was necessary before a

*Genus Aplopappus is used in U. S. Government publications, but genus Haplopappus is the form preferred by the International Congress on Botanical Nomenclature.*
noticeable amount of oil could be obtained. Following this treatment a light oil with a sweet odor was obtained. The oil was identified by means of combustion analysis and physical constants, and seemed to be one of the menthane group of the terpenes, and to have the empirical formula, $C_{10}H_{18}$. The whole plant had an essential oil content of 1.24%.

- In their investigation of the essential oil of guayule, *Parthenium argentatum*, Haagen-Smit and Siu (26) studied the chemical and physical properties, the composition, and the distribution of the essential oil components. The leaves contained the highest essential oil content on the fresh weight basis (1.04%), followed next by the flowers (0.76%), then the bark (0.24%), and finally the wood (0.11%). The average yield for the whole plant was 0.45%. The oil was separated by distillation into ten fractions and refractionated into 45 fractions, and in this way could be divided into five large groups, viz.: 72.6% terpenes, 5.8% oxygenated terpenes, 9.3% sesquiterpenes, 6.3% oxygenated sesquiterpenes, and 5.9% residue. The following compounds were identified and their approximate amounts in the oil are listed: 60% alpha-pinene, 9% dipentene, 8% cadinene, 6% di-, tri-, and higher terpenaceous compounds, 4% elemol-like sesquiterpene alcohol, 4% phellandral (probably), 3% sesquiterpene alcohol with an azulene nucleus, 2% guajene-like sesquiterpene, 2% terpene ketone or aldehyde, 2% beta-pinene, and small amounts
of an easily oxidizable terpene. The authors also discussed the significance of these compounds in relation to the formation of rubber in the plant.

The title of this investigation included a study and analysis of the plants native to tropical regions, with the extraction and analysis of the plants collected, on the distribution of terpenes. The results of the investigation, and specific mention of the terpenes in the study, are shown in the following sections:

1. The title of this investigation includes an analysis of the plants native to tropical regions, with the extraction and analysis of the plants collected, on the distribution of terpenes. The results of the investigation, and specific mention of the terpenes in the study, are shown in the following sections:
2. The title of this investigation includes an analysis of the plants native to tropical regions, with the extraction and analysis of the plants collected, on the distribution of terpenes. The results of the investigation, and specific mention of the terpenes in the study, are shown in the following sections:
3. The title of this investigation includes an analysis of the plants native to tropical regions, with the extraction and analysis of the plants collected, on the distribution of terpenes. The results of the investigation, and specific mention of the terpenes in the study, are shown in the following sections:
4. The title of this investigation includes an analysis of the plants native to tropical regions, with the extraction and analysis of the plants collected, on the distribution of terpenes. The results of the investigation, and specific mention of the terpenes in the study, are shown in the following sections:
5. The title of this investigation includes an analysis of the plants native to tropical regions, with the extraction and analysis of the plants collected, on the distribution of terpenes. The results of the investigation, and specific mention of the terpenes in the study, are shown in the following sections:
PLAN AND SCOPE OF THE INVESTIGATION

The plan of the investigation included a survey and collection of plants native to southern Arizona, trial distillations of the plants collected, steam distillation in bulk of some of the more desirable volatile oils, fractionation of some of the bulk extracts, and identification of the constituents making up those fractions. Extensive use was made of the University Herbarium in this connection.

On the basis of their aromatic characteristics as noted in the field, thirty-five plants were steam distilled in trial quantities. These were chosen from a group of possibly a hundred plants which were examined in the field. On the basis of percentage yield and characteristics of each oil obtained by trial distillation, certain of the plants were later collected in larger quantities to obtain the volatile extract in sufficient amount for fractionation and subsequent identification of its constituents.

The following scheme of analysis was carried through on the bulk extracts of *Tagetes lemmoni*, *Haplopappus laricifolius*, and *Choisy a dumosa var. mollis*.

I. Determination of the physical properties of the oil.

a. Density
b. Refractive index
c. Optical rotation
d. Boiling range (determined in the fractionation procedure)
e. Ultraviolet absorption spectrum of the whole oil
II. Determination of the chemical properties of the oil.
   a. Acid number
   b. Ester number
   c. Alcohol content by acetylation
   d. Aldehyde and ketone content by the hydroxylamine method

III. Fractionation of the oil in vacuo.

IV. Determination of physical properties of the individual fractions.
   a. Boiling point or range
   b. Density
   c. Refractive index
   d. Optical rotation

V. Determination of the chemical composition of the individual fractions by use of physical data and characteristic chemical reactions.
   a. Determination by means of classification tests of the functional groups of the compounds making up the fractions
   b. Measurement of the ultraviolet absorption spectra of the various fractions as an aid in the determination of structure and consequently aiding in the identification of constituents occurring in the fractions
   c. Use of special techniques, such as molecular weight determination, and the determination of carbon, hydrogen, and nitrogen content of a given fraction
   d. Preparation of one or two solid derivatives of each constituent as a means of final identification
EXPERIMENTAL METHODS AND RESULTS

PART I. PRELIMINARY INVESTIGATION OF SEVERAL OILS

Exploratory Phase

The exploratory work consisted of a survey of many plants in the field in order to determine those desirable for further study. In general, plants were selected which yielded an aromatic odor by the single expedient of crushing the green leaves or flowers between the fingers. Field trips to many areas in the southern part of Arizona were made during this exploratory phase. In addition to examination of the common desert plants a search was made in the Santa Catalina, Chiricahua, Tucson, Pinaleno, Huachuca, Pajarito, Tinajas Altas, and Santa Rita Mountain ranges. Plants of many families were tested including the Compositae, Labiatae, and Leguminosae. Of all the plants tested thirty-five were selected for trial steam distillation. These represent a fair cross-section of the typical families occurring in southern Arizona.

Kearney and Peebles' (30) volume and the manual by Benson and Darrow (5) contributed considerable data on the aromatic character of some of the native plants. A search of the literature was made for perfume plants of Europe and other essential oil-producing sections of the world of which
the same species, or other species in the same genera, occur in Arizona. Of particular value in this instance were Gildemeister and Hoffmann's (23) classic work and other journal articles previously mentioned.
Trial Steam Distillation

There are three general methods by which essential oils are extracted from plant material: steam distillation, solvent extraction, and enfleurage. The first procedure is used on leaves, stems, bark, roots, seeds, and some flowers. The second and third methods, since they are less drastic and less likely to alter the composition of the oil, are used in extracting flower oils.

In the course of this investigation only one plant, Acacia constricta, was analyzed for the oil content of its flowers. The rest of the plants either were not investigated during their flowering period or did not bear flowers of noteworthy aroma. Therefore, since the plant material employed was usually leaves and stems, steam distillation was used as a means of extracting the essential oils. Two different methods of steam distillation are possible. The first is the one shown in Plate I. Water is placed in the still pot with the plant material and is heated directly by means of a burner. The other method employs a separate flask containing boiling water as a source of steam which is piped into the still pot holding the plant material. Both methods were used on the first few plants with little difference in yield with but one exception. In the distillation of Tagetes lemmoni a separate source of steam was used and a negligible amount of oil was obtained. An excellent yield resulted, however, when water was added to the still and a direct fire
Plate I. Apparatus for exploratory small-scale steam distillation test.
was used. In the trial runs, the apparatus consisted of a six-liter glass flask, a spiral Graham condenser, and a collecting flask as shown in Plate I.

The method of treatment of the plant material previous to the steam distillation was varied from fine grinding to no grinding. Grinding or chopping up of the plant material allows a more intimate mixture of the steam with the oil glands, which is necessary for the oil to be volatilized and liberated from the plant cells. In the case of *Dalea lumholtzii*, chopping of the plant material prior to distillation was found to double the yield of volatile oil. In the trial distillations using small quantities of material, grinding or chopping was employed in most cases in order to increase the yield of oil. In the bulk quantity distillations, however, this treatment was not used due to the time factor involved and the possibility of the loss of the lighter oil fraction during grinding.

Another method of pretreatment of the plant material was tried on a few of the first trial distillations. Buehrer, Mason, and Crowder (14) noted that prior treatment with a solution of sodium hydroxide was necessary to steam distill the volatile oil of *Haplopappus hartwegi*. This treatment was tried on several plants; however, only in the distillation of *Pectis papposa* did the use of a solution of sodium hydroxide result in a yield of oil where none was obtained without this treatment. This procedure would seem undesirable
since the action of the sodium hydroxide during the steam distillation could be sufficiently harsh as to cause saponification of any esters present, thereby altering the composition of the oil extract.

The length of time during which the distillation was carried out was dependent upon the particular plant being distilled. The normal procedure was to halt the distillation when the oil globules in the water distillate were observed to taper off to a minimum. This required two to three hours for most of the plants investigated.

In Table 1 are listed the native plants on which trial distillations were made, their botanical names, families, and growth characteristics. The botanical data are taken from Kearney and Peebles (30) or from Benson and Darrow (5). Accompanying the descriptions are photographs of pressed specimens collected by the writer as shown in Plates II to X.
Table 1. Growth Characteristics of the Various Plants

Acacia constricta Benth. (Family: Leguminosae)
Plants shrubs or small trees, bearing numerous, small orange-yellow balls of very fragrant flowers, and well-armed with long, slender, straight, white spines. The plant is found in Greenlee, Gila, Yavapai, Cochise, Pima, and Yuma Counties, 2500 to 5000 feet, in shallow "caliche" soil on dry slopes and mesas, May to August (flowering period).

Achillea lanulosa Nutt. (Family: Compositae)
Perennial herbs, usually less than 0.5 m. high, thinly or densely pilose, sometimes silky-canescent, leafy, with creeping rootstocks. The plant is found in Apache, Navajo, and Coconino Counties to Cochise and Pima Counties, 5300 to 11,300 feet, mostly in the mountains, common in yellow pine forests, June to September. A. millefolium L., an European species extensively naturalized in the eastern United States, contains achilleine, a drug sometimes used in acute suppression of the menses. It was formerly prescribed as a tonic and in urinary disorders. It has been reported that a decoction of the leaves and flowers of A. lanulosa is used in family medicine in Arizona.

Artemisia carruthii Wood var. wrightii (A. Gray) Blake (Family: Compositae)
Herbs; leaves alternate, entire to once, twice, or thrice pinnatifid; heads small, discoid or disciform, usually very numerous, spicate, racemose or panicled. The plant is found in the White, Pinaleno, Pinal, and Chiricahua Mountains, 6000 to 8000 feet, in open pine forest, August to October.

Artemisia ludoviciana Nutt. (Family: Compositae)
Same general description as A. carruthii but smaller leaves and of a more silvery sheen. The plant occurs in the mountains of Cochise and Pima Counties, 2400 to 8000 feet, dry slopes and canyons, often in open pine forests, August to October.
Plate II.

Acacia constricta

Achillea lanulosa

Artemisia carruthii

Artemisia ludoviciana
Table 1. (continued)

**Baccharis glutinosa** Pers.  
(Family: Compositae)  
The plant is a dioecious shrub, leaves alternate, toothed,  
heads numerous and panicled, and grows to 4 or 5 feet  
in height. It is found in Coconino, Cochise, Santa  
Cruz, Pima, and Yuma Counties, below 5500 feet, mostly  
along water courses; March to December.

**Baccharis sarothroides** A. Gray  
(Family: Compositae)  
This plant is also a dioecious shrub with numerous  
broom-like flower heads and no leaves at flowering  
time, growing to about 4 to 6 feet in height. It is  
found in Yavapai, Gila, Maricopa, Pinal, Santa Cruz, and  
Pima Counties, 1000 to 4000 feet, hillsides and bottom  
lands; September to February.

**Brickellia californica** (Torr. & Gray) A. Gray  
(Family: Compositae)  
This shrub has alternate leaves, flower heads medium-  
sized, discoid, usually whitish, pappus of numerous  
capillary bristles. It is very common throughout the  
state, 3000 to 7000 feet, July to October. It is called  
"pachaba" by the Hopi Indians, who are reported to rub  
it on the head for headache.

**Bursera microphylla** A. Gray  
(Family: Burseraceae)  
Shrubs or small trees, unarmed, strongly aromatic;  
young bark smooth and brown, the older bark exfoliating;  
leaves alternate, pinnate, deciduous; flowers small,  
solitary or in very few-flowered clusters. This plant  
is found in the mountains of southwestern Arizona from  
the Salt River Mountains (Maricopa County) to the Gila  
and Tinajas Altas Mountains (Yuma County), 2500 feet or  
lower, locally abundant on arid rocky slopes, July.  
The trees reach a height in Arizona of 20 feet and a  
trunk diameter of 1 foot. The plant cannot withstand  
much cold. The bark contains tannin, while the gum was  
used for treating venereal diseases.
Baccharis glutinosa

Baccharis sarothroides

Brickellia californica

Bursera microphylla

Plate III.
Table 1. (continued)

**Choisya dumosa** (Torr.) A. Gray var. mollis (Standl.) L. Benson
(Family: Rutaceae)
Aromatic shrubs, often prominently glandular; found in
mountains of Santa Cruz County near Nogales and Ruby,
Sycamore Canyon, 3500 to 4000 feet, dry slopes and
sides of canyons.

**Cowania mexicana** Don var. stansburiana (Torr.) Jepson
(Family: Rosaceae)
Plants shrubby or arborescent, often resinous and strong
smelling, the stems usually erect and rather stiff;
leaves evergreen, thick; found in Apache County to
Mohave County; south to Cochise and Santa Cruz Counties,
3500 to 8000 feet, very common on dry slopes and mesas,
especially in the juniper-pinyon association, often on
limestone, April to September.

**Croton texensis** (Klotzsch) Muell. (Family: Euphorbiaceae)
This plant is an annual herb with alternate leaves,
petiolate, simple; dioecious flowers and racemose
inflorescence. It is found in Apache, Yavapai, Cochise,
Santa Cruz, and Pima Counties, 1200 to 7000 feet; common
to roadsides, fields, and dry stream beds. The plant
has a strong, disagreeable odor and has been reported
as being poisonous to cattle. The Hopi Indians use it
as an emetic and an eyewash.

**Dalea greggii** A. Gray
(Family: Leguminosae)
This perennial is glandular-punctate with odd-pinnate
leaves, clawed petals, pod small, indehiscent, and
grows about one foot high. It is found in Pima and
Santa Cruz Counties on rocky hills, 3000 to 5000 feet,
February to May.
Choisya dumosa

Cowania mexicana

Croton texensis

Dalea greggi

Plate IV.
Table 1. (continued)

Dalea lumholtzi Robins. and Fern. (Family: Leguminosae)
The plant is a glandular-punctate herb growing about 10
to 30 cm. in height. It grows in the Santa Catalina
and Baboquivari Mountains most abundantly in the months
September through November, in rocky canyon washes. It
has a very evident odor of lemon.

Dodonea viscosa Jacq. var. angustifolia (L. f.) Benth.
(Family: Sapindaceae)
This plant is a shrub with viscid foliage, simple,
short-petioled leaves, the blades narrow, entire and
linear and flowers yellowish, in small lateral corymbs,
fruits very conspicuous, dry with two to four broad
wings. The plant grows in southern Yavapai County to
Cochise and Pima Counties, 2000 to 4000 feet, fairly
common on dry rocky slopes and in canyons, often on
limestone, February to October.

Encelia farinosa A. Gray (Family: Compositae)
Low, branching shrubs; leaves alternate, ovate or oblong,
entire; plant found in Mohave County to western Gila,
Maricopa, Pinal, Pima, and Yuma Counties; up to 3000
feet, very abundant on dry, rocky slopes, November to
May.

Eriogonum fasciculatum Benth. var. polifolium (Benth.) Torr. &
Gray (Family: Polygonaceae)
A xerophilous shrub up to about three feet high with whitish
or pinkish slightly fragrant flowers; Coconino and Mohave
Counties to Pima and Yuma Counties, 2000 to 4500 feet,
on dry rocky slopes, March to June.
Dalea lumholtzii

Encelia farinosa

Plate V.
Table 1. (continued)

**Franseria ambrosioides Cav.** (Family: Compositae)
The plant is a shrub growing about 1 m. high; branches reddish-brown with long, conspicuous but not dense, white hairs; the leaves are green and are covered with rather harsh hairs, as well as with a dry, sticky material. The shrub grows abundantly in sandy washes and rocky or gravelly canyon bottoms and slopes in the desert.

**Franseria dumosa A. Gray** (Family: Compositae)
This shrub is weedy, monoecious and with dissected, alternate leaves bearing pistillate involucres armed with spines or prickles; it grows about one to two feet high and is found in Mohave, Pinal, and Pima Counties up to 3000 feet, very common on dry plains and mesas, April to November.

**Gutierrezia lucida Greene** (Family: Compositae)
Perennial herbs, more or less glutinous; leaves alternate, linear to narrowly oblanceolate, entire; heads small, yellow, radiate, usually numerous and crowded. Plants are of dry, stony plains, mesas, and slopes. These are worthless plants which are of little value in retarding soil erosion and which are more or less poisonous to sheep and goats when eaten in quantity. They are found almost throughout the state, 1200 to 6000 feet, June to October.

**Haplopappus cuneatus A. Gray var. spathulatus (A. Gray) Blake**
(Family: Compositae)
Perennial Shrub; leaves spatulate, entire; flower heads small, discoid; Sycamore Canyon (Santa Cruz County), Baboquivari Mountains (Pima County), Pinaleno Mountains (Graham County); 3500 to 5000 feet, rock ledges, September and October.
Franseria ambrosioides

Gutierrezia lucida

Haplopappus cuneatus

Plate VI.
Table 1. (continued)

**Haplopappus laricifolius** A. Gray  
(Family: Compositae)  
Perennial shrub; leaves filiform, entire; Mohave, Yavapai, Gila, Pinal, Cochise, and Pima Counties, 3000 to 6000 feet, mesas, slopes, and canyons, August to November.

**Haplopappus tenuisectus** (Greene) Blake  
(Family: Compositae)  
Perennial shrub; western Cochise and Pima Counties, 2000 to 4000 feet, plains, August to October.

**Heteropogon melanocarpus** (Ell.) Benth.  
(Family: Gramineae)  
An annual grass growing usually more than one meter high, flat leaf blades and solitary terminal racemes, this plant grows in Santa Cruz and Pima Counties, fields and waste places. It gets its common name, "sweet tanglehead", from the pineapple-like fragrance of the fresh foliage.

**Heterotheca subaxillaris** (Lem.) Britt. and Rusby  
(Family: Compositae)  
This species is an annual herb growing to about two feet with relatively small yellow flower heads. It grows abundantly along roads and ditches from March to November. The fresh plant has a slight odor of camphor or ginger when macerated between the fingers. It is sometimes known as "camphor-weed".
Plate VII.

Haplopappus laricifolius

Haplopappus tenuisectus

Heteropogon melanocarpus

Heterotheca subaxillaris
<table>
<thead>
<tr>
<th>Species</th>
<th>Family</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Hymenoclea monogyra</em> Torr. and Gray</td>
<td>Compositae</td>
<td>Low, much-branched shrub, monoecious; leaves alternate, linear-filiform and entire; flower heads small; characteristic and abundant shrub of sandy stream beds and washes; small forage value; Gila, Pinal, Santa Cruz, and Pima Counties, 2000 to 4000 feet, usually in sandy soil, September.</td>
</tr>
<tr>
<td><em>Hyptis emoryi</em> Torr.</td>
<td>Labiatae</td>
<td>A shrub, usually canescent, the hairs branched; leaves ovate; flowers several in axillary, woolly cymules. Common in Mohave, Yuma, and Pima Counties; also found in Yavapai, Graham, Maricopa, and Pinal Counties, up to 5000 feet (usually lower), dry rocky slopes and canyons, flowering almost throughout the year at lower elevations.</td>
</tr>
<tr>
<td><em>Larrea divaricata</em> Cav.</td>
<td>Zygophyllaceae</td>
<td>This shrub (called &quot;creosote bush&quot;) is much-branched, grows up to 11 feet tall, and has small, resinous leaves which are strong-scented. It grows in Pima County at 5000 feet or lower on the dry plains and mesas, flowering from time to time throughout the year but most profusely in the spring.</td>
</tr>
<tr>
<td><em>Lippia wrightii</em> A. Gray</td>
<td>Verbenaceae</td>
<td>Perennial plant, shrubby; flowers in slender elongate spikes; a graceful shrub with aromatic foliage, responding well to cultivation. The flowers are reported to yield excellent honey. Grand Canyon (Coconino County) and northern Mohave County to Greenlee, Cochise, and Pima Counties (King Canyon), 2000 to 6000 feet, common on dry rocky slopes, August to October.</td>
</tr>
</tbody>
</table>
Hymenoclea monogyra

Hyptis emoryi

Larrea divaricata

Lippia wrightii

Plate VIII.
Table 1. (continued)

Parthenium incanum H. B. K.  (Family: Compositae)
A small shrub, branching; leaves alternate, pinnatifid; heads small, white, cymose-panicled. Grand Canyon; Yavapai, Cochise, and Pima Counties (King Canyon), 3000 to 6000 feet, dry plains and mesas, usually in "caliche" soil, June to October.

Pectis papposa Harv. and Gray  (Family: Compositae)
The annual or perennial herb is a low, slender-stemmed plant with leaves opposite, entire, dotted with pellucid glands; flower heads small, radiate, yellow, the rays often purplish beneath. The plant grows in Pima County at 3000 feet or lower, on sandy-gravelly plains and mesas. The best season for growth is from July to October. The plant grows in scattered stands and was particularly sparse two years ago due to the extreme lack of summer rains. The odor of the plant is quite aromatic, similar to the odor of the oil distilled from it.

Porophyllum gracile Benth.  (Family: Compositae)
Perennial; leaves opposite, with conspicuous translucent oil glands in the tissue; heads medium-sized, discoid, whitish, or purplish; Grand Canyon and Mohave County to western Cochise, Pima, and Yuma Counties, 4000 feet or lower, dry rocky slopes and canyons, March to October.

Prosopis juliflora (Swartz) DC. var. velutina (Woot.) Sarg.  (Family: Leguminosae)
This plant is a shrub or a small tree armed with straight spines, leaves with 2 to 4 pinnae, and numerous narrow leaflets; flowers in cylindric spikes, small, greenish yellow, somewhat fragrant. It grows in Coconino County to Mohave County, southward to Cochise, Santa Cruz, Pima, and Yuma Counties, up to 5000 feet, common chiefly along streams and where the water table is relatively high, April to August.
Parthenium incanum

Porophyllum gracile

Pectis papposa

Prosopis juliflora

Plate IX.
Table 1. (continued)

<table>
<thead>
<tr>
<th>Plant Name</th>
<th>Family</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psilostrophe cooperi (A. Gray) Greene</td>
<td>Compositae</td>
<td>Plant a shrub, densely white-woolly; leaves linear, entire; heads mostly solitary at tips of branches, slender-peduncled; Mohave and Yavapai Counties to Graham, Pima, and Yuma Counties, 2000 to 5000 feet, mesas and plains, common, flowering during most of the year.</td>
</tr>
<tr>
<td>Tagetes lemmoni A. Gray</td>
<td>Compositae</td>
<td>This plant is herbaceous with pinnately-divided leaves which are dotted with oil glands. It grows in height from 30 to 60 cm. and has a strong, aromatic odor which is especially noticeable while it is being gathered. The plant grows in the Santa Catalina Mountains from 6000 to 7500 feet, in rocky washes. The best growing season is from August to October, although good specimens of the plant were collected in late December.</td>
</tr>
<tr>
<td>Vauquelinia californica (Torr.) Sarg.</td>
<td>Rosaceae</td>
<td>This plant is a large shrub or a small tree with thick, evergreen leaves; flowers around May or June. The leaves are not resinous. The shrub grows in the mountains of Pima County from 2500 to 5000 feet.</td>
</tr>
</tbody>
</table>
Psilostrophe cooperi

Tagetes lemmoni

Vauquelinia californica

Plate X.
Descriptions of the trial steam distillations of the various native plants investigated follow in succeeding paragraphs.

**Acacia constricta.**

One mile east of Gates Pass, Tucson Mountains, along roadside.

About 150 g. of flower heads were picked slightly past the full flowering stage in the late afternoon, wrapped in a dampened cloth, and stored in a refrigerator over night. The next day they were placed in a 5 l. flask equipped with a reflux condenser. About 600 ml. of petroleum ether (30-60°C.) was added to the charge, and the mixture was gently refluxed for 45 minutes. The solvent, containing waxes, pigments, and flower oils, was decanted into a flask, stoppered, and placed in the cold. A second batch of solvent was added to the flower charge and the procedure repeated. The two extracts were then combined, concentrated under reduced pressure, and evaporated to dryness, using two receivers in an ice bath to reclaim most of the ether. The residue was an orange, waxy mass of a rather fragrant odor, and weighed about 0.9 g., or a 0.6% yield on fresh weight basis. To this residue was added 25 ml. of 95% ethanol. The mixture was refluxed for one hour when apparently no more of the residue would dissolve. The alcoholic solution was then filtered, cooled overnight, and filtered cold, removing the alcohol-insoluble portion from the alcoholic solution of flower oil.
Achillea lanulosa.—
Treasure Park on the Swift Trail, Mt. Graham, Graham County.
About 300 g. of fresh, unchopped and unground plant was steam-distilled using a direct heat. The yield was about 0.2 g. of an oil which did not have the pleasant, mint-like odor of the herbage, but was very penetrating probably because of its concentration. This oil was very unusual in the respect that it was colored, not yellow or red or green as essential oils frequently are, but deep blue by transmitted light and black by reflected light.

Artemisia carruthii var. wrightii.—
Sugarloaf Trail, Chiricahua Mountain range.
About 200 g. of fresh, unchopped plant material was steam-distilled. A blue oil was obtained with a yield of 1.2 g. or 0.6% on a fresh weight basis. This oil was also a very dark blue, similar to that of Achillea, except that it had a noticeably pleasant odor. Distillation of a large quantity of the plant (1950 g.) yielded only 4.7 g. of oil of heavy, sweet odor.

Artemisia ludoviciana.—
Mount Lemmon road below Soldiers' Canyon, Catalina Mountains.
Steam-distillation of about 125 g. of fresh plant material, consisting of unchopped leaves, stems, and a few roots, yielded only a drop of oil. About 184 g. of the same material coarsely chopped was distilled, yielding only 0.055 g. of an oil of disagreeable odor. Evidently chopping does
not increase the yield of oil from this plant materially.

Baccharis glutinosa.---
Soldiers' Canyon, Catalina Mountains.

The unchopped, dry leaves and stems of the plant were steam-distilled, and a green syrupy oil was obtained. From a large quantity of unchopped, partially air-dried material (1900 g.), 6 g. of this green oil of a sweet, persistent odor was obtained.

Baccharis sarothroides.---
Mount Lemmon road, below the prison camp.

About 4900 g. of the female plant, consisting of the stems of the plant but no leaves, was steam-distilled in the fresh, unground state. The yield was 3.2 g. of a yellow oil with a rather unpleasant odor.

Brickellia californica.---
Sabino Canyon, Catalina Mountains.

About 3100 g. of fresh plant material, unground, was steam-distilled yielding about 0.5 g. of light green oil of pungent odor, suggestive of pepper.

Bursera microphylla.---
Tinajas Altas, Yuma County.

Grind oil with the odor of fresh, green leaves was obtained.

About 200-300 g. of air-dried leaves was steam-distilled using direct heat. The yield of light yellow oil was 0.4 g.

The odor of the oil was identical with that of the leaves, resembling the odor of oil of Eucalyptus.
**Choisya dumosa var. mollis.**—Sycamore Canyon.

Three distillations were made using leaf material only. The quantities used were 600 g., 445 g., and 460 g., chopped to medium size with a miniature ensilage cutter. They were steam-distilled using direct heat, and 4.2 g. of a practically colorless oil was obtained. The oil had a strong, fruity rather pleasant odor.

**Cowania mexicana var. stansburiana.**—Patagonia Road, 1 to 2 miles west of Sonoita.

Steam-distillation of about 720 g. of air-dried plant material, not ground, with direct heat yielded about 1.2 g. of a brownish-yellow oil with a heavy, not unpleasant odor. The leaves of the plant were very resinous-appearing and were covered with small white specks which may have been oil glands. Due to the relatively low yield of essential oil, these specks are believed to be indicative of resin ducts.

**Croton texensis.**—Cortaro Road, one-half mile west of Casa Grande Highway.

About 1900 g. of air-dried, unground plant material was steam-distilled over a period of three hours. A yellowish-green oil with the odor of fresh, green leaves was obtained in an amount of 3 g.

**Dalea greggii.**—Mount Lemmon Road just below Soldiers' Canyon.

About 73 g. of leaves, fresh, unground, was steam-distilled yielding about 0.1 g. of a light-yellowish oil.
with an odor faintly suggestive of lemon.

Dalea lumholtzii.—
Soldiers' Canyon, Catalina Mountains.

Approximately 1000-1200 g. of the whole plant was steam-distilled in four batches yielding a yellow oil with a pleasant odor of lemon verbena. The yield was about 2 g. of oil or 0.2%. The last batch after being exhausted of oil was steam-distilled with 100 ml. of 5% sodium hydroxide, but no additional oil was obtained. Another batch of 690 g. of plant material collected one month later was chopped fine and steam-distilled, yielding 2.5 g. of oil or about 0.4%. Another batch collected the next year but one month earlier than the first collection yielded about 0.9% of oil on being chopped fine and steam-distilled.

Dodonea viscosa var. angustifolia.—
Sabino Canyon, Catalina Mountains.

Steam-distillation of about 250 g. of air-dried, unground plant material yielded no oil.

Encelia farinosa.—
Rattlesnake Pass, Tucson Mountains.

A total of 2120 g. of leaves, air-dried, and not ground, was steam-distilled with direct heat. The yield was about 6.6 g. of a yellow oil with the odor of green leaves.

Eriogonum fasciculatum var. polifolium.—
Tinajas Altas Mountains, Yuma County.

Steam-distillation of 140 g. of air-dried leaves, ground
medium, yielded a yellow oil in an amount of 0.7 g. The odor of this oil resembled somewhat that of oil of Eucalyptus.

**Franseria ambrosioides.**—
Hillito Creek at Campbell Avenue, Tucson.

About 500 g. of leaves and stems was steam-distilled using a separate source of steam. A very small amount of oil with a disagreeable odor distilled during a two-hour period. The addition of 240 ml. of 2 N sodium hydroxide neither increased the ratio of oil to water in the distillate, nor did it noticeably change the odor of the oil.

**Franseria dumosa.**—
North edge of Organpipe Cactus National Monument, Pima County.

Approximately 3500 g. of unchopped, air-dried material was steam-distilled yielding only 2.0 g. of a green oil with a foul-smelling odor. The plant material was mostly stems, being very sparsely leafed.

**Gutierrezia lucida.**—
Papago reservation, Haiwana Nakya Village, Pima County.

About 525 g. of air-dried, finely-chopped plant material consisting of leaves and stems was distilled using direct heat. The yield was about 2.9 g. of a yellow oil of a rather pleasant odor resembling that of menthol.

**Haplopappus cuneatus var. spathulatus.**—
Sycamore Canyon.

Steam-distillation of one batch of medium-chopped leaf
Haploppappus laricifolius.--
Mount Lemmon Road just below the prison camp, Catalina Mountains.

About 300 g. of fresh, new leaves and stem tips, not ground, was steam-distilled with direct heat. The yield was 3.2 g. of a yellow oil. The oil had an odor resembling a combination of menthol and oil of Eucalyptus.

Haploppappus tenuisectus.--
Two miles east of Gates Pass, Tucson Mountains.

About 1800 g. of leaves, stems, and some roots, fresh, unground, was steam-distilled in the large still. The yield was 5.0 g. of a yellow oil with a pungent odor suggesting that of camphor.

Heteropogon melanocarpus.--
Ashburn ranch near Sonoita.

About 900 g. of the slightly fragrant, fresh, unground grass was steam-distilled using a direct source of heat, but no yield of oil was obtained even after two hours' distillation.

Heterotheca subaxillaris.--
Rillito Creek at Campbell Avenue, Tucson.

About 215 g. of the plant--stems, leaves, and flowers--was ground fine, steam-distilled for two hours, then for two more hours following the addition of 500 ml. of 1 N sodium.
hydroxide. The yield was about five drops of yellow oil of unpleasant odor. No increase in yield of oil was noted after addition of alkali.

**Hymenolea monogyra.**—Desert Grassland Station, Santa Rita Mountains.

About 385 g. of air-dried, finely-chopped plant material—leaves and stems—was distilled using direct heat. The yield was about 0.75 g. of a yellow oil having a faint odor suggestive of menthol but an unpleasant background odor.

**Hyptis emoryi.**—Tinajas Altas, Yuma County.

About 610 g. of air-dried leaves and small stems were chopped fine and steam-distilled using direct heat. The yield of essential oil was 2.42 g. of a yellow oil with a mint-like odor.

**Larrea divaricata.**—Desert north of Rillito Creek at Campbell Avenue, Tucson.

About 400 g. of plant material consisting of leaves and small twigs was air-dried, chopped, and steam-distilled for two hours using a separate source of steam. Water was then added to the still and a direct heat applied for two more hours. No oil was obtained during either period of distillation.

**Lippia wrightii.**—King Canyon, Tucson Mountains.

**Trial I.** About 25 g. of air-dried coarsely-chopped, pleasant-smelling leaves and fine stems was steam-distilled
for one and one-half hours using direct heat. The yield was 0.54% of a colorless, light oil of a sweet, mint-like odor.

Trial II. About 435 g. of finely-chopped leaves and fine stems was steam-distilled using an indirect source of steam, no heat being applied directly to the stillpot. The yield of oil was 0.695 g. (0.16%). After the distillate had become homogeneous, water was added to the residue in the still and the distillation continued over a direct heat. About 2.46 g. of oil was obtained (0.72%), which showed the advantage of direct heat as the source of steam.

*Parthenium incanum.*—
King Canyon, Tucson Mountains.

Steam-distillation of about 370 g. of air-dried leaves and small stems chopped fine, using direct heat yielded about 0.5 g. of a yellow oil of a rather pleasant odor.

*Pectis papposa.*—
Rillito Creek at Campbell Avenue, Tucson.

About 300 g. of the whole plant was steam-distilled using a separate source of steam. No oil was observed in the distillate until after the addition of 500 ml. of 2 N sodium hydroxide, when a small amount of oil with a pleasant odor similar to that of cuminaldehyde distilled over.

*Porophyllum gracile.*—
Sandy wash on the Tucson Mountain Park Road, Tucson Mountains.

Steam-distillation of 1300 g. of air-dried plant material
using direct heat resulted in 0.2 g. of a yellow oil of a fresh odor, similar to that of the crushed plant stems.

**Prosopis juliflora var. velutina.**—Anklam Road east of Gates Pass, Tucson Mountains.

Steam-distillation of 110 g. of fresh leaves, unground, yielded no oil after four hours of distillation.

**Psilostronhe cooperi.**—Mount Lemmon roadside in the foothills of the Catalina Mountains.

Steam-distillation of about 2500 g. of whole plant, air-dried, unground, yielded only 0.7 g. of oil of a rather unpleasant odor.

**Tagetes lemmoni.**—Soldiers' Canyon, Mount Lemmon Road, Catalina Mountains.

About 330 g. of plant material, mainly leaves and a few stems, air-dried two weeks, was steam-distilled using a separate source of steam, but no appreciable oil yield was obtained. When water was added to the still and a direct heat applied, a yield of yellow oil amounting to about 3 g. was readily obtained.

**Vauquelinia californica.**—Soldiers' Canyon, Catalina Mountains.

Steam-distillation of 300 g. of chopped leaves and small twigs yielded no oil even after four hours' distillation.
In Table 2 are listed the plants of which the foregoing trial distillations were made, their common names, the general character of the oil extracts, and approximate yields.

**Table 2. Character and Yield of the Volatile Extracts**

<table>
<thead>
<tr>
<th>Plant</th>
<th>General Character of the Oil</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acacia constricta &quot;whitethorn acacia&quot;</td>
<td>Alcoholic extract of natural flower oil; pleasant odor.</td>
<td>0.6</td>
</tr>
<tr>
<td>Achillea lanulosa &quot;yarrow&quot;</td>
<td>Oil dark blue, very volatile; odor, penetrating.</td>
<td>0.07</td>
</tr>
<tr>
<td>Artemisia carruthii &quot;sagebrush&quot;</td>
<td>Oil very blue; odor heavy, pleasant, somewhat minty.</td>
<td>0.24</td>
</tr>
<tr>
<td>Artemisia ludoviciana &quot;sagebrush&quot;</td>
<td>Oil yellow-colored; disagreeable odor.</td>
<td>0.03</td>
</tr>
<tr>
<td>Baccharis glutinosa &quot;seepwillow&quot;</td>
<td>Green, syrupy oil; sweet, persistent odor.</td>
<td>0.32</td>
</tr>
<tr>
<td>Baccharis sarothroides &quot;desert broom&quot;</td>
<td>Yellow oil of rather unpleasant odor.</td>
<td>0.07</td>
</tr>
<tr>
<td>Brickellia californica &quot;pachaba&quot;</td>
<td>Light green oil; unpleasant, pungent odor.</td>
<td>0.02</td>
</tr>
<tr>
<td>Bursera microphylla &quot;elephant tree&quot;</td>
<td>Light yellow oil; pleasant odor similar to oil of Eucalyptus.</td>
<td>0.16</td>
</tr>
<tr>
<td>Choisya dumosa &quot;star leaf&quot;</td>
<td>Colorless oil; strong, fruity, pleasant odor.</td>
<td>0.21</td>
</tr>
<tr>
<td>Cowania mexicana &quot;cliffrose&quot;</td>
<td>Brownish-yellow oil; persistent odor.</td>
<td>0.17</td>
</tr>
<tr>
<td>Croton texensis &quot;doveweed&quot;</td>
<td>Yellow oil; odor of green leaves.</td>
<td>0.16</td>
</tr>
</tbody>
</table>
Table 2. (continued)

<table>
<thead>
<tr>
<th>Species</th>
<th>Oil color</th>
<th>Odor description</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dalea greggii</td>
<td>Oil of a yellowish tint;</td>
<td>Odor faintly suggestive of lemon.</td>
<td>0.15</td>
</tr>
<tr>
<td>Dalea lumholtzii</td>
<td>Yellowish oil; pleasant</td>
<td>Odor of lemon verbena.</td>
<td>0.4</td>
</tr>
<tr>
<td>Dodonea viscosa</td>
<td>No yield.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Encelia farinosa</td>
<td>Yellow oil; fresh odor.</td>
<td></td>
<td>0.31</td>
</tr>
<tr>
<td>Eriogonum fasciculatum</td>
<td>Yellow oil; odor resembling</td>
<td>Oil of Eucalyptus.</td>
<td>0.5</td>
</tr>
<tr>
<td>Franseria ambrosioides</td>
<td>Unpleasant odor; very small</td>
<td>Yield of oil which partly polymerized after few weeks' standing.</td>
<td></td>
</tr>
<tr>
<td>Franseria dumosa</td>
<td>Greenish oil; very foul</td>
<td>Odor.</td>
<td>0.06</td>
</tr>
<tr>
<td>Gutierrezia lucida</td>
<td>Yellow oil; rather pleasant</td>
<td>Odor of menthol.</td>
<td>0.55</td>
</tr>
<tr>
<td>Haplopappus cuneatus</td>
<td>Yellow oil with a pleasant</td>
<td>Reddish-brown oil; strong, varnish-like odor.</td>
<td>0.5</td>
</tr>
<tr>
<td>Haplopappus laricifolius</td>
<td>Yellow oil; odor resembling</td>
<td>A mixture of menthol and Eucalyptus.</td>
<td>1.07</td>
</tr>
<tr>
<td>Haplopappus tenuisectus</td>
<td>Yellow oil; pungent odor</td>
<td>Odor suggestive of camphor.</td>
<td>0.28</td>
</tr>
<tr>
<td>Heteropogon melanocarpus</td>
<td>No yield.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterotheca subaxillaris</td>
<td>Unpleasant odor.</td>
<td></td>
<td>0.1</td>
</tr>
<tr>
<td>Hymenolea monogyra</td>
<td>Yellow oil; faint odor</td>
<td>Odor suggestive of menthol with an unpleasant background</td>
<td>0.19</td>
</tr>
</tbody>
</table>
Table 2. (continued)

<table>
<thead>
<tr>
<th>Plant Name</th>
<th>Description</th>
<th>Yield (fresh weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyptis emoryi &quot;desert lavender&quot;</td>
<td>Yellow oil; pleasant, mint-like odor very similar to oil of Lippia wrightii.</td>
<td>0.4</td>
</tr>
<tr>
<td>Larrea divaricata &quot;creosotebush&quot;</td>
<td>No oil obtained; foliage derives odor from resins.</td>
<td>---</td>
</tr>
<tr>
<td>Lippia wrightii</td>
<td>Colorless oil; sweet, mint-like odor.</td>
<td>0.5-0.7</td>
</tr>
<tr>
<td>Parthenium incanum &quot;mariola&quot;</td>
<td>Yellow oil; not unpleasant odor.</td>
<td>0.14</td>
</tr>
<tr>
<td>Pectis papposa &quot;chinchweed&quot;</td>
<td>Oil has a pleasant odor suggestive of cumenaldehyde.</td>
<td>0.1</td>
</tr>
<tr>
<td>Porophyllum gracile &quot;yerba-del-venado&quot;</td>
<td>Yellow oil; odor very similar to that of crushed stems of plant.</td>
<td>0.015</td>
</tr>
<tr>
<td>Prosopis juliflora &quot;mesquite&quot;</td>
<td>No yield.</td>
<td>---</td>
</tr>
<tr>
<td>Psilostrophe cooperi &quot;paper daisy&quot;</td>
<td>Greenish-yellow oil; rather unpleasant odor.</td>
<td>0.028</td>
</tr>
<tr>
<td>Tagetes lemmoni</td>
<td>Yellow oil with a pleasant odor.</td>
<td>0.9</td>
</tr>
<tr>
<td>Vauquelinia californica</td>
<td>No oil obtained, although leaves soaked in hot water have prune odor.</td>
<td>---</td>
</tr>
</tbody>
</table>

On the basis of the aromatic characteristics and percentage yield of the volatile extract obtained during the trial steam-distillation, the plant was either eliminated or retained for future investigation. A plant yielding an oil of pleasant aroma with a yield of about 0.1% or more on a fresh weight basis, was considered worthwhile for further
However, the choice of plants to be investigated was influenced by other factors: their abundance throughout the southern part of the state, leaf area, and position of the oil glands in the leaves. These factors are important since only a relatively small yield can at best be expected in the extraction of essential oils. In this investigation, yields of from 0.1% to 1% on a fresh weight basis have been obtained; and since 100 g. or more of the oil is required for the fractionation and identification procedures, it is apparent that from 10 to 100 kg. of plant material must be collected for the investigation of each plant. Regardless of the excellence of the oil obtained, it is of little importance if the plant cannot be found in sufficient abundance to supply a large enough quantity of oil for purposes of identification. Since a very small- and sparsely-leaved plant will yield a low total weight of oil per unit weight of whole plant, the time involved in collecting a sufficient quantity of plant material would make the plant of little value in this investigation. And finally, a plant in which the oil glands are situated deep within the leaf tissue generally requires fine chopping to make the glands more accessible to the steam and thus to obtain the full yield of oil from the material. Fine chopping is a time-consuming process; therefore, in this investigation, plants in which the oil glands were on or near the surface of the leaves were considered much more desirable.
On the basis of the above-mentioned criteria the following plants were selected for distillation in large quantities. Haplopappus tenuisectus is an abundant desert plant, easily collected, and from it a fair yield of oil is obtained. Haplopappus laricifolius is also an abundant plant growing at altitudes of 3000 feet and higher, and it produces a high yield of oil. Tagetes lemmonii grows along moist washes at elevations of from 4000 to 8000 feet and produces a good yield of oil of a pleasant odor. The excellent yield from this plant is attributed to the fact that the oil glands are located on the surfaces of the leaves and stems.

Several other plants were extracted in smaller quantities than those listed above and some 10 to 20 g. of each of the volatile oils collected. This amount of oil was not large enough to allow fractionation of its various constituents, but it was sufficient for the determination of some of its physical and chemical properties. Among these plants was Dalea lumholtzii, the essential oil of which has an interesting odor similar to lemon verbena, due probably to citral and citronellal. The plant, however, was difficult to collect in quantity since it was of small size and of relative scarcity. Lippia wrightii yielded an oil with a strong, minty aroma; but the total yield of oil was low since the plant had a small leaf area and consisted of much woody stem and branch material containing little or no oil. Another plant, Choisya dumosa var. mollis, yielded an oil of a rather
fruity odor, characteristic of esters. As will be noted in a subsequent table of chemical properties, this extract had the highest ester value of all of the oils tested. *Hyptis emoryi* yielded a volatile oil of a rather minty odor, but the yield in bulk distillation proved considerably less than that obtained in the trial distillation, probably because only the leaves and very small stems were included in the trial run.

The method of obtaining the volatile extracts in larger quantities than was possible in the trial distillations required a modification of the apparatus. A large still was constructed as shown in Plate XI. It consisted of a 10-gallon milk can which served as the still pot, a tin funnel held on by C-clamps as the top, a glass-tubing vapor take-off, a glass Allihn condenser, and a separatory funnel as the collecting vessel, surrounded by an inverted bell jar containing ice and water to keep the distillate cold and minimize loss of the light fraction of the volatile extract. The collecting vessel was connected to a U-tube which allowed the distillate to be maintained at constant level. This arrangement permitted the oil, which was lighter than water, to collect above the water distillate and the excess of the latter to be drained out through the U-tube.

In Table 3 are compiled the physical constants of the extracts obtained in sufficient amounts to allow determination of these constants.
Plate XI. Apparatus for Steam Distillation in Bulk Quantity.
Table 3. Physical Constants of the Volatile Oil Extracts

<table>
<thead>
<tr>
<th>Plant</th>
<th>$n_D^{20}$</th>
<th>$d_{30}^{**}$</th>
<th>$\lambda_D^{***}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bursera microphylla</td>
<td>1.4802</td>
<td>0.9145</td>
<td>----</td>
</tr>
<tr>
<td>Choisya dumosa</td>
<td>1.4686</td>
<td>0.8709</td>
<td>-20.57$^{31}$</td>
</tr>
<tr>
<td>Cowania mexicana</td>
<td>1.5077</td>
<td>0.9566</td>
<td>----</td>
</tr>
<tr>
<td>Dalea lumholtzii</td>
<td>1.4659</td>
<td>0.8762</td>
<td>11.65$^{33}$</td>
</tr>
<tr>
<td>Encelia farinosa</td>
<td>1.4800</td>
<td>0.8678</td>
<td>-5.88$^{31}$</td>
</tr>
<tr>
<td>Eriogonum fasciculatum</td>
<td>1.4810</td>
<td>0.8865</td>
<td>----</td>
</tr>
<tr>
<td>Gutierrezia lucida</td>
<td>1.4806</td>
<td>0.8865</td>
<td>34.99$^{31}$</td>
</tr>
<tr>
<td>Haplopappus cuneatus</td>
<td>1.5010</td>
<td>0.9379</td>
<td>----</td>
</tr>
<tr>
<td>Haplopappus laricifolius</td>
<td>1.4842</td>
<td>0.8631</td>
<td>-14.06$^{34}$</td>
</tr>
<tr>
<td>Haplopappus tehuisectus</td>
<td>1.4777</td>
<td>0.8195</td>
<td>-2.57$^{34}$</td>
</tr>
<tr>
<td>Hymenoclea monogyra</td>
<td>1.4872</td>
<td>0.8943</td>
<td>14.40$^{31}$</td>
</tr>
<tr>
<td>Hyptis emoryi</td>
<td>1.4864</td>
<td>0.9348</td>
<td>-10.60$^{30}$</td>
</tr>
<tr>
<td>Lippia wrightii</td>
<td>1.4764</td>
<td>0.8584</td>
<td>15.61$^{30}$</td>
</tr>
<tr>
<td>Parthenium incanum</td>
<td>1.4929</td>
<td>0.9176</td>
<td>2.37$^{30}$</td>
</tr>
<tr>
<td>Porophyllum gracile</td>
<td>1.4650</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>Psilostrophe cooperi</td>
<td>1.4810</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>Tagetes lemmoni</td>
<td>1.4580</td>
<td>0.8608</td>
<td>6.63$^{33}$</td>
</tr>
</tbody>
</table>

* with Abbe refractometer.
** density in g./ml. at 30° C. unless otherwise stated.
*** observed rotation using a 1 dcm. x 2 mm. tube at temperature indicated by superscript.
Methods Used in Analysis for Gross Constituents of the Oils

The procedures listed below were carried out as described by Guenther (25).

Determination of the acid content

The acid number of an oil is defined as the number of milligrams of potassium hydroxide required to neutralize the free acids in one gram of the oil. The procedure (25, I, pp. 263-5) used in this determination was as follows:

"Weigh accurately about 2.5 g. of the oil into a 100 cc. saponification flask. Add 15 cc. of neutral 95% alcohol and 3 drops of a 1% phenolphthalein solution. Titrate the free acids with a standardized 0.1 N aqueous sodium hydroxide solution, adding the alkali dropwise at a uniform rate of about 30 drops per minute. The contents of the flask must be continually agitated. The first appearance of a red coloration that does not fade within 10 seconds is considered the end point."

The acid number was calculated from the following formula:

\[
\text{Acid number} = \frac{5.61 \times (\text{no. of cc. of 0.1 N NaOH})}{\text{wt. of sample in grams}}
\]

Determination of the ester content

The ester number of an oil is defined as the number of milligrams of potassium hydroxide required to saponify the esters present in one gram of oil. The procedure (25, I, pp. 265-7) used in this determination was as follows:

"Into a 100 cc. alkali-resistant saponification flask weigh accurately about 1.5 G. of the oil. Add 5 cc. of neutral 95% alcohol and 3 drops of a 1% alcoholic solution of phenolphthalein, and neutralize the free acids with standardized 0.1 N aqueous sodium hydroxide solution. Then add 10 cc.}
of 0.1 N alcoholic sodium hydroxide solution, measured accurately from a pipette or a burette. Attach a glass, air-cooled condenser to the flask, 1 m. in length and about 1 cm. in diameter, and reflux the contents of the flask for 1 hour on a steam bath. Remove and permit to cool at room temperature for 15 minutes. Titrate the excess alkali with standardized 0.5 N aqueous hydrochloric acid. A further addition of a few drops of phenolphthalein solution may be necessary at this point. A blank determination must be run observing the same conditions but omitting the oil."

The ester number was calculated from the following formula:

\[
\text{Ester number} = \frac{28.05 \times a}{s}
\]

where \( a \) = number of cc. of 0.5 N sodium hydroxide used in the saponification;
\( s \) = sample weight in grams.

The ester percentage was calculated from the following formula:

\[
\text{Ester percentage} = \frac{a \times m}{20 \times s}
\]

where \( a \) = number of cc. of 0.5 N sodium hydroxide used in saponification;
\( m \) = molecular weight of the ester predominant in the oil;
\( s \) = sample weight in grams.

On the basis of an average ester molecular weight of 225, which represents a fair average of the molecular weights of esters commonly present in the essential oils, the ester content of *Choisya dumosa* is about 34 per cent, a value quite high in comparison with that of the six other extracts tested. The ester number is normally calculated on the basis of the major ester constituent present.
Determination of the free alcohol content

The difference between the ester number of an oil and its ester number after acetylation is an indication of the free alcohol content of the oil, since acetylation results in the esterification of all free alcohol groups. Subsequent saponification allows a quantitative determination of the amount of free alcohols in the sample. The procedure (25, I, pp. 271-4) for acetylation as used in this investigation was as follows:

"Introduce into a 100 cc. acetylation flask 10 cc. of the oil (measured from a graduated cylinder), 10 cc. of acetic anhydride (similarly) measured and 2.0 g. of anhydrous sodium acetate. Attach the air condenser, and boil the contents of the flask gently for exactly 1 hour on a sand bath suitably heated by an open Bunsen flame or an electric hot-plate. Permit the flask to cool for 15 minutes and introduce 50 cc. of distilled water through the top of the condenser. Heat the flask on a steam bath for 15 minutes with frequent shaking to destroy the excess of acetic anhydride. Transfer the contents of the flask to a separatory funnel and rinse the flask with two 10 cc. portions of distilled water; add these rinsings to the separatory funnel. Shake thoroughly to assure good contact of the aqueous layer with the oil. When the liquids have separated completely, reject the aqueous layer and wash the remaining oil repeatedly with 100 cc. portions of saturated salt solution, until the washings are neutral to litmus; this usually requires three washings. Dry the resulting oil with anhydrous sodium sulfate and filter. (If the oil has been washed properly, not more than 0.2 cc. of 0.1 N aqueous sodium hydroxide solution should be required per gram of acetylated oil in order to neutralize the remaining trace of acetic acid.)"

The dried, acetylated oil is then saponified by the procedure as mentioned above for determination of ester number. The
ester number after acetylation is calculated from the same formula used to calculate the ester number. The percentage of free alcohols in the oil may then be calculated from the following formula:

\[
\text{Percentage of free alcohols} = \frac{d}{m} m \text{ mol wt of alcohol; } d = (\text{ester number after acetylation} - \text{ester number})
\]

If an average molecular weight of the free alcohols is taken to be 150, the oil of Dalea lumholtzii contains approximately 46 per cent of free alcohols, about twice the amount in the oil of Tagetes lemmoni, which contains the next largest amount of free alcohols. The molecular weight used in this calculation is usually that of the free alcohol occurring in the oil in largest proportion.

**Determination of the aldehyde and ketone content**

An estimation of the aldehyde and ketone content of an extract can be made by use of the hydroxylamine procedure. This determination is based on the combination of the aldehyde or ketone with hydroxylamine hydrochloride, accompanied by release of an equivalent of hydrochloric acid and the subsequent titration of the hydrochloric acid with standard base. The initial reaction is as shown in the following equation:

\[
R-\text{CHO} + \text{NH}_2\text{OH}\cdot\text{HCl} \rightarrow R-\text{CH}=\text{N-OH} + \text{H}_2\text{O} + \text{HCl}
\]
The percentage of aldehyde and ketone present is then calculated on the basis of the amount of standard base required to neutralize the released hydrochloric acid and the molecular weight of the known carbonyl constituent or, if the identity is unknown, on the basis of an average molecular weight.

The procedure (25, I, pp. 285-7) used in this determination was as follows:

"Into a 100 cc. saponification flask weigh accurately the requisite amount of oil or synthetic and add 35 cc. of 0.5 N hydroxylamine hydrochloride solution, measured from a graduated cylinder. Permit the flask to stand at room temperature for the proper length of time (15 minutes used in all cases) and titrate the liberated hydrochloric acid with standardized 0.5 N alcoholic sodium hydroxide. The titration is continued until the original greenish shade of the hydroxylamine solution is obtained. A second flask containing 35 cc. of hydroxylamine hydrochloride solution may be used as a blank to assure a more accurate color match."

"Preparation of 0.5 N Hydroxylamine Hydrochloride Solution: Dissolve 275 g. of recrystallized hydroxylamine hydrochloride in 300 cc. of distilled water; warm to a temperature of 65° on a steam bath to yield a clear solution. Add this solution slowly to 2 gal. of 95% alcohol, and mix thoroughly. Then add 125 cc. of a 0.1% solution of bromphenol blue indicator in 50% alcohol, and sufficient 0.5 N alcoholic sodium hydroxide solution to change the yellow color of the solution to a greenish shade; this usually requires about 20 to 25 cc. of the alkali. The proper degree of neutralization is attained when 35 cc. of the solution shows a distinct greenish shade which changes to a distinct yellow upon the addition of 1 drop of 0.5 N hydrochloric acid. A stable solution of hydroxylamine hydrochloride is thus obtained which is approximately 0.5 N; an exact adjustment is unnecessary.

For lesser quantities of solution, dissolve 34.75 g. of recrystallized hydroxylamine hydrochloride in 40 cc. of distilled water and make up to 1 liter with 95% alcohol; add 15 cc. of the bromphenol blue solution and neutralize."
The percentage of aldehyde or ketone can then be calculated from the following formula:

\[
\text{Percentage of aldehyde or ketone} = \frac{a \cdot m}{20 \cdot s}
\]

where

- \( a \) = number of cc. of 0.5 N sodium hydroxide used for neutralization;
- \( m \) = molecular weight of the aldehyde or ketone;
- \( s \) = sample weight in grams.

Assuming an average of 150 grams per mole for the carbonyl compound, six of the extracts listed in Table 4 were tested for aldehyde and ketone content. \textit{Dalea lumholtzii} was found to contain about 22 per cent, more than twice that of the oil of the next highest aldehyde and ketone content, \textit{Tagetes lemmonii}.

In Table 4 are listed the approximate amounts of gross constituents, based on the preceding methods, of several oils which were available in sufficient quantity to permit determination of these properties.
<table>
<thead>
<tr>
<th>Plant</th>
<th>Acid Value</th>
<th>Ester Number</th>
<th>Ester No. after Acetylation</th>
<th>Free Alcohols</th>
<th>Aldehyde and Ketone Compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Choisya dumosa</em></td>
<td>1.0</td>
<td>76.2%</td>
<td>34</td>
<td>121</td>
<td>13</td>
</tr>
<tr>
<td><em>Dalea humholtzii</em></td>
<td>3.4</td>
<td>22.0%</td>
<td>10</td>
<td>174</td>
<td>46</td>
</tr>
<tr>
<td><em>Haplopappus laricifolius</em></td>
<td>3.7</td>
<td>7.8%</td>
<td>4</td>
<td>40</td>
<td>9</td>
</tr>
<tr>
<td><em>Haplopappus tenuisectus</em></td>
<td>2.2</td>
<td>21.7%</td>
<td>10</td>
<td>49</td>
<td>8</td>
</tr>
<tr>
<td><em>Hyptis emorvi</em></td>
<td>1.7</td>
<td>29.9%</td>
<td>13</td>
<td>70</td>
<td>11</td>
</tr>
<tr>
<td><em>Lippia wrightii</em></td>
<td>3.5</td>
<td>9.3%</td>
<td>4</td>
<td>47</td>
<td>10</td>
</tr>
<tr>
<td><em>Tagetes lemmoni</em></td>
<td>0.7</td>
<td>47.2%</td>
<td>21</td>
<td>127</td>
<td>22</td>
</tr>
</tbody>
</table>
Determinations of the ester number before and after acetylation and of aldehyde and ketone content are of use in the evaluation of a whole oil as to its suitability for use in perfumery. These data indicate the amount of oxygenated constituents, which are more important than the terpenes in contributing to the attractive qualities of the odor of an essential oil.
Design of the Fractionation Apparatus

Since the volatile oil as steam-distilled from the plant is a complex mixture, the first step in elucidating the chemical composition of the oil is the fractional distillation in vacuo. In general, the terpene hydrocarbons are quite stable to distillation at atmospheric pressure. However, the oxygenated derivatives of the terpenes—aldehydes, esters, alcohols, and ketones—usually must be distilled at low pressures in order to minimize polymerization, decomposition, or other chemical changes. By this means the components, which generally have different boiling points, are isolated in a fairly pure state in the various fractions. The purity of these fractions will depend upon the efficiency of the fractionation apparatus and the number of refractionations made, since frequently the boiling point difference of two succeeding fractions amounts to only a few degrees and this difference becomes even smaller when operating in vacuo.

Since the quantities of volatile oils available initially were limited, it was necessary to construct a semimicro distillation apparatus which required only 1 to 10 ml. of oil for a fractionation. The original apparatus was constructed along the lines of Shrader and Ritzer's microdistillation apparatus (42). The apparatus was modified by including a cold-finger reflux condenser and a thermometer by means of which the boiling range of the fractions could be observed.
The completed apparatus is shown in Plate XIIa. By use of this equipment a fractional distillation of the oil of *Haplopappus tenuisectus* was made in which three fractions were obtained. The physical constants of the fractions are listed in Table 5.

Table 5. Fractional Distillation of Oil of *Haplopappus tenuisectus*

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Pressure</th>
<th>Boiling Range</th>
<th>Volume</th>
<th>Color</th>
<th>Refractive Index</th>
<th>Density</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mm. Hg</td>
<td>°C.</td>
<td>ml.</td>
<td></td>
<td></td>
<td>g./ml.</td>
</tr>
<tr>
<td>Original oil</td>
<td></td>
<td>2.27</td>
<td>2.5</td>
<td>colrls</td>
<td>1.4692</td>
<td>.7836</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>78-81</td>
<td>1.0</td>
<td>yellow</td>
<td>1.4840</td>
<td>.9114</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>96-100</td>
<td>0.3</td>
<td>yellow</td>
<td>1.4952</td>
<td>.9425</td>
</tr>
</tbody>
</table>

Residue: a dark-brown, polymerized substance.

Stearic acid was used as an anti-foam agent, with two glass boiling tubes and 1 to 2 g. of clean sand to promote boiling. The first fraction was taken off using an 8-inch Vigreux column; the second and third fractions were obtained by the use of a 3-inch Vigreux column (Plate XIIb).

In an effort to obtain a more clean-cut fractionation, a fractionating column packed with glass helices was employed,
Plate XII. Original apparatus for vacuum fractional distillation, (a), and modifications.
as shown in Plate XIIc; and trial fractionations were run on the oils of *Haplopappus tenuisectus* and *Tagetes lemmonii*. The helix-packed column was used to gain more intimate contact between the ascending vapors and the descending condensate, thus giving a sharper fractionation. However, both trial runs resulted in the same rough fractionation as that obtained by use of the Vigreux column. In order better to control the cuts taken during the fractionation, a partial take-off reflux still head was constructed after the design of Whitmore and Lux (56). This apparatus permitted the withdrawal of oil fractions which distilled at definite temperature increments, according to the method of Haagen-Smit and Siu (26). The apparatus was constructed as shown in Plate XIIId; and a trial fractionation was made on the oil of *Haplopappus laricifolius* under a pressure of 4 mm. of mercury. The apparatus gave satisfactory results; but the high-boiling fractions decomposed or polymerized at this pressure, an occurrence which was also noted during the trial fractionation of the oil of *Tagetes lemmonii* mentioned above.

Since the fractional distillations could not in most cases be performed satisfactorily in the vacuum obtainable with the apparatus at hand, it was necessary to build a high vacuum line and to modify the fractionation apparatus. The high vacuum line, employing a mercury-vapor pump, a Cenco oil pump as a booster, and a McLeod gauge to measure the vacuum obtained, was designed and built with reference to
Sanderson's (40) book on high vacuum technique. The distillation apparatus was modified by discarding the original receiver with its rubber tubing connection to the vacuum pump, and by constructing a receiver consisting of four stopcocks and a collecting flask with a ground glass joint. The arrangement of the stopcocks made it possible to disconnect the collecting flask and remove the oil fraction to another container without breaking the vacuum in the system. After the pin-hole leaks in the system were located by means of a high voltage Tesla coil and sealed, it was possible to obtain as low a pressure as 5 microns of mercury (0.005 mm.). The high vacuum system is pictured in Plate XIII. The system contained a trap surrounded by a freezing mixture of solid carbon dioxide and chloroform which served to freeze out any vapors failing to condense in the collecting flask and thus prevented those vapors from passing into and damaging the pumps.

By means of the high vacuum distillation apparatus described above, a trial fractionation was made on the oil of *Tagetes lemmoni*. It was found that the low-boiling fraction could not satisfactorily be distilled under a pressure as low as 200 microns. At room temperature (32° C.) that fraction diffused as the gas phase through the apparatus into the trap where it was frozen out. The physical constants of these fractions are recorded in Table 6.
Plate XIII. Apparatus for high vacuum fractional distillation.
Table 6. Fractional Distillation of Oil of \textit{Tagetes lemmoni}

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Pressure (mm. Hg)</th>
<th>Boiling Range (°C.)</th>
<th>Volume</th>
<th>Color</th>
<th>Refractive Index</th>
<th>Density (g/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>In trap</td>
<td>0.200</td>
<td>32</td>
<td>0.25</td>
<td>colrs</td>
<td>1.4671^30</td>
<td>0.8520^32</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>34-36</td>
<td>1.50</td>
<td>colrs</td>
<td>1.4631^30</td>
<td>0.8240^32</td>
</tr>
<tr>
<td>2</td>
<td>0.250</td>
<td>56-61</td>
<td>1.50</td>
<td>colrs</td>
<td>1.4662^20</td>
<td>0.8123^32</td>
</tr>
<tr>
<td>3</td>
<td>0.250</td>
<td>63-70</td>
<td>5.0</td>
<td>yellow</td>
<td>1.4585^20</td>
<td>0.8372^32</td>
</tr>
</tbody>
</table>

Residue: a brown substance with a charred odor.

The fraction which diffused into the trap under high vacuum contained some of the higher-boiling as well as the low-boiling constituents, as is apparent from a comparison of the density of that fraction with the density of the whole oil. During this fractionation the problems of foaming and "bumping" were again encountered. The foaming was reduced by the use of Dow Antifoam DC, a silicone compound. After trying many boiling agents, steady ebullition was finally obtained by the use of a small chip of pumice.

In succeeding fractionations the low-boiling fractions were collected under a pressure of 15 to 25 mm. of mercury by use of the oil pump alone, while the higher-boiling fractions.
were taken off under as low a pressure as possible by use of the mercury pump, following the method of Haagen-Smit and Siu (26). This procedure tended to hold the polymerization and decomposition of the high-boiling fractions to a minimum and permitted the fractionation of the low-boiling constituents, which is possible only when there is a liquid phase in contact with a gas phase.

Following the suggestion of Bradley (11) an electrically-heated fractionating column was designed and built. This column through the maintenance of a uniform heat throughout its length had an improved efficiency of fractionation. The high vacuum line including this modified column is shown in Plate XIV.

With the apparatus as described above another quantity of oil of *Tagetes lemmoni* was fractionated. Only two fractions were obtained, as noted in Table 7 below.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Pressure (mm. Hg)</th>
<th>Boiling Range (°C.)</th>
<th>Weight (g)</th>
<th>Weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>23</td>
<td>80-85</td>
<td>25.6</td>
<td>62.5</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>43-46</td>
<td>8.8</td>
<td>21.5</td>
</tr>
<tr>
<td>Residue</td>
<td>--</td>
<td>--</td>
<td>6.6</td>
<td>16.1</td>
</tr>
</tbody>
</table>
Plate XIV. High vacuum fractionation apparatus including the modified column.
These two fractions were then refractionated at intervals of 2 to 3 degrees in an attempt to separate the constituents as pure fractions or at least as groups of constituents distilling in a narrow temperature range. The results obtained showed that insufficient oil was available to carry the distillation to the higher temperature range within which the oxygenated terpenes distill over. As a result, a small percentage of oxygenated terpene derivatives, sequiterpenes, and oxygenated sesquiterpene derivatives remained in the residue and perhaps appeared only as impurities in the fractions obtained. The fractions together with their physical properties are listed in Table 8.

Table 8. Refractionation of Essential Oil Fractions of *Tagetes lemmoni*

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Pressure</th>
<th>Boiling Range</th>
<th>Weight</th>
<th>$n_D^{25}$</th>
<th>$d^{27}$</th>
<th>$\lambda_D^{27}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mm. Hg</td>
<td>°C.</td>
<td>g.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>18</td>
<td>74-76</td>
<td>3.6</td>
<td>1.4594</td>
<td>0.8210</td>
<td>+ 7.14</td>
</tr>
<tr>
<td>1a</td>
<td>1</td>
<td>36-38</td>
<td>1.0</td>
<td>1.4442</td>
<td>0.8273</td>
<td>+ 8.51</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>38-40</td>
<td>4.9</td>
<td>1.4370</td>
<td>0.8226</td>
<td>+ 9.63</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>40-43</td>
<td>5.1</td>
<td>1.4298</td>
<td>0.8249</td>
<td>+ 10.53</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>43-46</td>
<td>1.4</td>
<td>1.4287</td>
<td>0.8280</td>
<td>+ 10.52</td>
</tr>
</tbody>
</table>
Following this fractionation a slight modification was made in the fractionation apparatus in order to facilitate the distillations. The alterations consisted of a steeper drop in the condenser in order that the fractions would pass through more quickly, and a copper coil surrounding the condensing section through which ice water was circulated to provide better condensation of the low-boiling fractions. The modified apparatus is shown in Plate XV.
Plate XV. Modified high vacuum fractionation apparatus.
PART II. INVESTIGATION OF THE OIL OF Haplopappus laricifolius

Fractionation of the Bulk Extract

About 160 g. of oil of Haplopappus laricifolius was fractionated using the modified apparatus in an attempt to obtain a quantitative distribution curve from the fractionation. Data from the preliminary fractionation are shown in Table 9. The oil was freshly steam-distilled prior to the fractionation and dried over anhydrous sodium sulfate.

Table 9. Preliminary Fractionation of Oil of Haplopappus laricifolius

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Pressure</th>
<th>Distillation Temperature</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mm. Hg</td>
<td>°C.</td>
<td>g.</td>
</tr>
<tr>
<td>1</td>
<td>18</td>
<td>55-65</td>
<td>52.4</td>
</tr>
<tr>
<td>2</td>
<td>18</td>
<td>65-70</td>
<td>45.3</td>
</tr>
<tr>
<td>3</td>
<td>0.200</td>
<td>33-38</td>
<td>5.3</td>
</tr>
<tr>
<td>4</td>
<td>0.200</td>
<td>38-48</td>
<td>7.2</td>
</tr>
<tr>
<td>5</td>
<td>0.250</td>
<td>48-58</td>
<td>12.4</td>
</tr>
<tr>
<td>6</td>
<td>0.250</td>
<td>58-68</td>
<td>9.6</td>
</tr>
<tr>
<td>7</td>
<td>0.250</td>
<td>68-85</td>
<td>2.9</td>
</tr>
<tr>
<td>8</td>
<td>0.250</td>
<td>85-90</td>
<td>1.9</td>
</tr>
<tr>
<td>9</td>
<td>0.250</td>
<td>90-100</td>
<td>1.6</td>
</tr>
<tr>
<td>Residue</td>
<td></td>
<td></td>
<td>20.0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>158.6</td>
</tr>
</tbody>
</table>
The nine fractions obtained above were then carefully fractionated into 2-, 3-, and 4-degree fractions as shown in Table 10, which also includes the physical constants of those fractions. The densities were determined with a 0.1 ml. pycnometer at room temperature. The refractive indices were measured with an Abbe refractometer at 20° C. The optical rotations were determined at room temperature (30° C.) by means of a Zeiss-Winkel polarimeter.

The physical data included in Table 10 are shown graphically in Figure 1 in the form of a quantitative distribution chart and three physical measurement curves.
Table 10. Physical Constants and Quantitative Distribution of Various Fractions of Oil of Haplopappus laricifolius

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Pressure</th>
<th>Distillation Temperature</th>
<th>Weight (g)</th>
<th>Weight (%)</th>
<th>$n_{D}^{20}$</th>
<th>$d_{15}^{30}$</th>
<th>$(A)_{D}^{30}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>19</td>
<td>28-58</td>
<td>1.3</td>
<td>0.92</td>
<td>1.4704</td>
<td>0.8620</td>
<td>+2.1</td>
</tr>
<tr>
<td>1b</td>
<td>19</td>
<td>58-60</td>
<td>3.8</td>
<td>2.70</td>
<td>1.4748</td>
<td>0.8542</td>
<td>-2.0</td>
</tr>
<tr>
<td>2</td>
<td>19</td>
<td>60-62</td>
<td>16.9</td>
<td>12.00</td>
<td>1.4768</td>
<td>0.8576</td>
<td>-7.9</td>
</tr>
<tr>
<td>3</td>
<td>19</td>
<td>62-64</td>
<td>11.5</td>
<td>8.16</td>
<td>1.4788</td>
<td>0.8558</td>
<td>-16.5</td>
</tr>
<tr>
<td>4</td>
<td>19</td>
<td>64-66</td>
<td>8.6</td>
<td>6.10</td>
<td>1.4803</td>
<td>0.8558</td>
<td>-18.3</td>
</tr>
<tr>
<td>5</td>
<td>19</td>
<td>66-68</td>
<td>18.4</td>
<td>13.06</td>
<td>1.4817</td>
<td>0.8558</td>
<td>-18.3</td>
</tr>
<tr>
<td>6</td>
<td>19</td>
<td>68-70</td>
<td>31.7</td>
<td>22.46</td>
<td>1.4835</td>
<td>0.8488</td>
<td>-21.0</td>
</tr>
<tr>
<td>7</td>
<td>0.200</td>
<td>28-34</td>
<td>1.1</td>
<td>0.78</td>
<td>1.4842</td>
<td>0.8488</td>
<td>-19.9</td>
</tr>
<tr>
<td>8</td>
<td>0.200</td>
<td>34-36</td>
<td>1.8</td>
<td>1.28</td>
<td>1.4840</td>
<td>0.8504</td>
<td>-18.6</td>
</tr>
<tr>
<td>9</td>
<td>0.200</td>
<td>36-38</td>
<td>1.7</td>
<td>1.21</td>
<td>1.4843</td>
<td>0.8543</td>
<td>-18.1</td>
</tr>
<tr>
<td>10</td>
<td>0.200</td>
<td>38-40</td>
<td>1.9</td>
<td>1.35</td>
<td>1.4852</td>
<td>0.8698</td>
<td>-14.1</td>
</tr>
<tr>
<td>11</td>
<td>0.250</td>
<td>40-42</td>
<td>5.6</td>
<td>3.97</td>
<td>1.4844</td>
<td>0.8620</td>
<td>-14.8</td>
</tr>
<tr>
<td>12</td>
<td>0.250</td>
<td>42-46</td>
<td>0.5</td>
<td>0.35</td>
<td>1.4857</td>
<td>0.8994</td>
<td>-8.5</td>
</tr>
<tr>
<td>13</td>
<td>0.250</td>
<td>46-48</td>
<td>0.7</td>
<td>0.50</td>
<td>1.4832</td>
<td>0.9221</td>
<td>-6.4</td>
</tr>
<tr>
<td>14</td>
<td>0.250</td>
<td>48-50</td>
<td>5.2</td>
<td>3.69</td>
<td>1.4832</td>
<td>0.9178</td>
<td>-3.8</td>
</tr>
<tr>
<td>15</td>
<td>0.250</td>
<td>56-58</td>
<td>13.6</td>
<td>9.65</td>
<td>1.4868</td>
<td>0.9532</td>
<td>-9.7</td>
</tr>
<tr>
<td>16</td>
<td>0.250</td>
<td>60-62</td>
<td>0.1</td>
<td>0.01</td>
<td>1.4908</td>
<td>0.9587</td>
<td>----</td>
</tr>
<tr>
<td>17</td>
<td>0.250</td>
<td>62-64</td>
<td>0.1</td>
<td>0.01</td>
<td>1.4911</td>
<td>0.9540</td>
<td>----</td>
</tr>
<tr>
<td>18</td>
<td>0.250</td>
<td>64-68</td>
<td>0.7</td>
<td>0.50</td>
<td>1.4920</td>
<td>0.9509</td>
<td>-11.4</td>
</tr>
<tr>
<td>19</td>
<td>0.250</td>
<td>68-70</td>
<td>0.3</td>
<td>0.21</td>
<td>1.4929</td>
<td>0.9509</td>
<td>----</td>
</tr>
<tr>
<td>20</td>
<td>0.250</td>
<td>70-74</td>
<td>0.3</td>
<td>0.21</td>
<td>1.4939</td>
<td>0.9543</td>
<td>----</td>
</tr>
<tr>
<td>21</td>
<td>0.250</td>
<td>74-78</td>
<td>0.2</td>
<td>0.14</td>
<td>1.4948</td>
<td>0.9473</td>
<td>----</td>
</tr>
<tr>
<td>22</td>
<td>0.250</td>
<td>78-80</td>
<td>0.5</td>
<td>0.35</td>
<td>1.4957</td>
<td>0.9517</td>
<td>-2.3</td>
</tr>
<tr>
<td>23</td>
<td>0.250</td>
<td>80-84</td>
<td>0.7</td>
<td>0.50</td>
<td>1.4974</td>
<td>0.9426</td>
<td>+ 3.5</td>
</tr>
<tr>
<td>24</td>
<td>0.250</td>
<td>84-86</td>
<td>0.6</td>
<td>0.43</td>
<td>1.4988</td>
<td>0.9434</td>
<td>+ 7.7</td>
</tr>
<tr>
<td>25</td>
<td>0.250</td>
<td>86-90</td>
<td>1.1</td>
<td>0.78</td>
<td>1.5000</td>
<td>0.9603</td>
<td>+ 13.3</td>
</tr>
<tr>
<td>26</td>
<td>0.250</td>
<td>90-94</td>
<td>0.8</td>
<td>0.57</td>
<td>1.5020</td>
<td>0.9371</td>
<td>+ 22.5</td>
</tr>
<tr>
<td>27</td>
<td>0.250</td>
<td>94-96</td>
<td>0.8</td>
<td>0.57</td>
<td>1.5030</td>
<td>0.9356</td>
<td>+ 26.9</td>
</tr>
<tr>
<td>28</td>
<td>0.250</td>
<td>96-100</td>
<td>0.8</td>
<td>0.57</td>
<td>1.5038</td>
<td>0.9371</td>
<td>+ 27.1</td>
</tr>
<tr>
<td>Residue</td>
<td>0.250</td>
<td>113-126</td>
<td>8.4</td>
<td>5.92</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>139.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 1. Quantitative distribution and physical properties of the essential oil fractions of *Haplopappus laricifolius*. 

- **Optical Rotation**: The graph shows a curve indicating optical rotation values for different fractions. The rotation values range from approximately +20 to +10 degrees.

- **Refractive Index**: The graph depicts the refractive index values for different fractions, with values ranging from 1.470 to 1.504.

- **Specific Gravity**: The graph illustrates specific gravity values for different fractions, ranging from 0.840 to 0.940.

- **Quantitative Distribution**: The bar chart displays the weight per cent of fraction for different distillation temperatures, showing the distribution of the essential oil fractions.
The fractionation data from the work on guayule essential oil by Haagen-Smit and Siu (26) indicate that the terpenes can be roughly classified as follows:

<table>
<thead>
<tr>
<th>Class</th>
<th>Boiling Range</th>
<th>Density (at 25°C)</th>
<th>Refractive Index (at 25°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{10} terpene</td>
<td>50-70°C</td>
<td>0.855</td>
<td>1.466</td>
</tr>
<tr>
<td>hydrocarbons</td>
<td>at 21 mm. Hg</td>
<td>0.837</td>
<td>1.479</td>
</tr>
<tr>
<td>C_{10} oxyterpenes</td>
<td>50-73°C</td>
<td>0.929</td>
<td>1.479</td>
</tr>
<tr>
<td>at 0.25 mm. Hg</td>
<td>0.953</td>
<td>1.492</td>
<td></td>
</tr>
<tr>
<td>C_{15} sesquiterpene</td>
<td>73-96°C</td>
<td>0.908</td>
<td>1.495</td>
</tr>
<tr>
<td>hydrocarbons</td>
<td>at 0.25 mm. Hg</td>
<td>0.929</td>
<td>1.505</td>
</tr>
<tr>
<td>C_{15} sesquiterpene</td>
<td>96-126°C</td>
<td>0.936</td>
<td>1.502</td>
</tr>
<tr>
<td>alcohols</td>
<td>at 0.25 mm. Hg</td>
<td>0.973</td>
<td>1.510</td>
</tr>
</tbody>
</table>

By comparing the above classification with the physical constants listed in Table 10 for the various fractions of the oil of Haplopappus laricifolius, Fractions 1a through 13 would seem to be terpene hydrocarbons, Fractions 14 through 19 oxygenated terpenes, and Fractions 20 through 28 sesquiterpene hydrocarbons, in the order of increasing boiling point. These three groups are present in approximately the following proportions: 75% terpenes, 14% oxyterpenes, 4% sesquiterpene hydrocarbons, and 7% residue and loss.

In the three groups just mentioned Fractions 2, 6, 11, 14, 15, 18, and 25, the "peak" fractions according to the
quantitative distribution chart, were assumed to be of greater purity than the intervening fractions. This is corroborated in some instances where the "peak" fractions in Figure 1 correspond to maxima or minima of the physical measurement curves. On the basis of their physical properties and of qualitative tests for esters, alcohols, aldehydes, and ketones, the "peak" fractions in the oxyterpene group, Fractions 14, 15, and 18, consisted mainly of terpene aldehydes and ketones. The qualitative tests are here briefly described.

According to Cheronis and Entrikin (15), an ester will react with hydroxylamine to form an alcohol and a hydroxamic acid which in acid solution will combine with ferric chloride to give a wine-colored ferric hydroxamate. The wine color indicates a positive test. The same authors describe the xanthate test for alcohols, in which the alcohol combines with potassium hydroxide to form the potassium alkoxide which is then converted to the potassium alkyl xanthate by reaction with carbon disulfide. The xanthate appears as a pale-yellow precipitate, indicating a positive test. The test for aldehydes and ketones according to Shriner and Fuson (43) involves the reaction of the aldehyde or ketone with an alcoholic solution of 2,4-dinitrophenylhydrazine to form a red, orange, or yellow precipitate of a 2,4-dinitrophenylhydrazone. The methone test for aldehydes described by Cheronis and Entrikin (15) involves the reaction of 5,5-dimethylcyclohexane-1,3-dione, "dimethone", and an aldehyde to give an addition product.
appearing as a milky suspension, which indicates a positive test. The reagent does not react with ketones.

Solid addition products were then prepared in an endeavor to ascertain the identity of the main constituent in each of the seven "peak" fractions. The derivatives used depended upon the type of compound as follows: for the terpene hydrocarbons—the nitrosocloride, nitrosite, tetrabromide, nitrosate, nitrolamine, and the maleic anhydride adduct; for the terpene aldehydes or ketones—the 2,4-dinitrophenylhydrazone, semicarbazone, and p-nitrophenylhydrazone; and for the sesqui-terpene hydrocarbons—the nitrosocloride, hydrochloride, and picrate.

Determination of the Ultraviolet Absorption Spectra

The ultraviolet absorption spectra of the various fractions of the oil of Haplopappus laricifolius were measured on a Model DU Beckman Spectrophotometer. The fractions were diluted in 95% ethanol to an approximate concentration of 0.05%. The concentration was not determined exactly since only the qualitative aspect was of interest here. The sample to be measured and a quantity of pure solvent were placed in quartz cells standard to the instrument. With the cell containing pure solvent in position in front of the beam emanating from the hydrogen tube, the sensitivity knob turned to maximum sensitivity, and the wave length dial at the desired setting, the
galvanometer needle was zeroed by narrowing the slit width. The cell containing the sample was then placed in the beam and the galvanometer needle zeroed by manipulating the optical density dial. This process was repeated at each desired wave length. Readings were taken every two millimicrons, when a maximum or minimum seemed imminent, and every five or ten millimicrons otherwise. Plotting the optical density against the wave length gave a curve containing maxima at certain wave lengths characteristic of the molecular structure of the compound being measured.

Braude (13) states that the maximum extinction at characteristic wave lengths in the ultraviolet is due to the presence of one or more chromophores in the molecule. For example, butadiene, containing two conjugated double bonds, exhibits a maximum at 217 millimicrons, as does crotonaldehyde which is an alpha-beta-unsaturated aldehyde. Other authors have presented data which show that in larger molecules containing substitutions around these chromophores a shift of the characteristic wave length occurs, usually in the direction of the higher wave lengths. For example, Van Os and Dykstra (50) state that carvone, an alpha-beta-unsaturated cyclic ketone, exhibits a maximum extinction at 225 millimicrons and that cinnamaldehyde, an alpha-beta-unsaturated aromatic aldehyde, exhibits a maximum at 224 millimicrons. Cooke and MacBeth (16) list a maximum for piperitone, an alpha-beta-unsaturated cyclic ketone, at 235 millimicrons in alcohol and 225
millimicrons in hexane, and a maximum for cryptone (4-isopropyl-2-cyclohexen-1-one) at 226 millimicrons in alcohol and 220 millimicrons in hexane. These last values also show the effect of the solvent on the position of the maximum, and it is verified by Woodward (57), who indicates an average increase of 7 millimicrons in changing from hexane to alcohol as a solvent. This author also states some generalizations, based on measurements of a large series of compounds, concerning the effect of substitution on alpha-beta-unsaturated carbonyl compounds, as follows:

\[
\begin{align*}
R - C - C &= C \stackrel{\beta}{<} \beta \\
\end{align*}
\]

<table>
<thead>
<tr>
<th>Substituted</th>
<th>Wave length (max.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{L or } \text{B} )</td>
<td>225 ± 5</td>
</tr>
<tr>
<td>( \text{L} \beta \text{ or } \beta \text{B} )</td>
<td>239 ± 5</td>
</tr>
<tr>
<td>( \text{L} \beta \beta )</td>
<td>254 ± 5</td>
</tr>
</tbody>
</table>

Concerning the substituted dienes, Booker, Evans, and Gillam (10) list a maximum at 224 millimicrons for myrcene (2-methyl-6-methylene-2,7-octadiene) and a maximum at 232 millimicrons for beta-phellandrene (1-methylene-4-isopropyl-2-cyclohexene).

The absorption spectra of the various fractions of the oil of Haplopappus laricifolius are shown in Figure 2. The units along the ordinate axis were chosen arbitrarily with regard to scale since it was desired to show the similarities or differences among the maxima of the various fractions. The spectra for Fractions 2 through 13 show maxima at 232
Figure 2. Ultraviolet absorption spectra of the essential oil fractions of *Haplopappus loricifolius*. 
millimicrons, which according to Woodward (57) and Booker, Evans, and Gillam (10) indicates a conjugated diene system with two substituted groups and one of the conjugated double bonds in an exocyclic position. This indication was substantiated by the subsequent identification of beta-phellandrene in Fraction 6. The maximum at 232 millimicrons for Fraction 2 would appear to result from the presence of some beta-phellandrene in that fraction since, according to Van Os and Dykstra (50), alpha-pinene, later identified in Fraction 2, exhibits a continuous absorption spectrum containing no maxima. For Fraction 11, which could not be identified, there exist two possibilities: either the main constituent has a structure similar to that of beta-phellandrene, or the main constituent exhibits a continuous spectrum and there is sufficient beta-phellandrene present to show a maximum at 232.

A maximum absorption occurs at 236 millimicrons for Fraction 14, which, according to Woodward (57), could indicate the presence of an alpha-beta-unsaturated ketone. Fraction 14 contains a ketone, but its identity could not be established. The spectra for Fractions 16 through 18 show maximum absorption at 231 millimicrons, which result verifies the presence of phellandral in Fraction 18. Fractions 20 through 28 showed only continuous spectra giving no indication of the structure of the sesquiterpenes present in those fractions. An arrest at 235 millimicrons in the spectrum of Fraction 23 may be
Due to the presence of a conjugated double bond system.

Determination of Melting Points

The melting points of the derivatives were determined by means of a Fisher-Johns melting point apparatus. This device consists of a metal heating block with a circular depression milled into the top and carrying a 300-degree thermometer mounted in a metal thermometer sheath, and a magnifying lens for viewing the sample. A transformer with an output of 4, 8, 12, 16, 20, or 24 volts is provided to control the amount of heat desired, as well as a rheostat for fine control of temperature. The thermometer tube is mounted at right-angles to the heating block and a hole is bored into the block so that the thermometer projects into the block. The thermometer is held in intimate contact with the block by means of a cap and spring which screw into the outer end of the thermometer sheath.

The melting point of a compound was determined by placing a very small quantity of the compound between two 18 mm. cover glasses, which were placed in the depression on top of the block, and raising the temperature of the block rapidly to within 10 or 20 degrees of the melting point as determined by a previous trial. The rate of increase in temperature was then reduced to about 1 or 2 degrees per minute and the melting-point range noted. The apparatus was calibrated by comparing the melting points of some standard compounds as
determined with the apparatus with the melting points of the same compounds as determined with a set of Anschütz thermometers in a melting-point bath.

**Technique of Nitrogen Determinations**

In this investigation the micro-Dumas method according to Pregl (38) was used to determine the nitrogen content of some of the solid addition products by which the constituents of the various fractions were characterized.* The method involves the conversion of nitrogenous compounds to gaseous nitrogen with copper oxide and copper metal by heating the compound in a stream of carbon dioxide and measuring the gaseous nitrogen in a nitrometer. Solid carbon dioxide in a Dewar flask was used as the source of carbon dioxide gas. The combustion tube contained the usual permanent filling of coarse copper oxide and reduced copper in the form of a rolled piece of copper screen 4 cm. long and a temporary filling of about 5 cm. of coarse copper oxide, 3-4 cm. of finely powdered copper oxide containing the sample, and 2-3 cm. of coarse copper oxide.

The sample weight was of the order of 2-4 mg. The sample was weighed in a weighing tube and transferred to a mixing tube. It was intimately mixed with fine copper oxide and transferred to the combustion tube. The apparatus was flushed out with carbon dioxide for one minute. The permanent

---

*Nitrogen analyses determined by Margie McCaughey, Department of Chemistry, University of Arizona.*
filling was heated for two minutes in a stream of carbon dioxide, and the system was connected to the nitrometer and tested for micro-bubbles, the presence of which indicated the exclusion of air from the system. The system was closed and the sample ignited for six minutes, after which time the liberated nitrogen was swept into the nitrometer filled with 50% potassium hydroxide, which served to absorb the carbon dioxide. The volume of nitrogen was read after allowing eight minutes for the potassium hydroxide to drain, and the nitrogen percentage for the sample was calculated.
Identification of alpha-Pinene

The physical constants and the odor of Fraction 2 suggested the presence of pinene. The fraction was shown to consist mainly of alpha-pinene, identified by preparation of the nitrosochloride, which showed no depression in its melting point when mixed with the same derivative of an authentic sample of alpha-pinene, and by determination of the nitrogen percentage of the nitrosochloride. Alpha-pinene was also identified in Fractions 1b and 3 by preparation of the nitrosochlorides which gave no depression of the melting point on admixture with pinene nitrosochloride.

From the fractionation data it was estimated that the volatile oil of Haplopappus laricifolius contains from 15 to 20% alpha-pinene, probably as the dl-form.

Preparation of Fraction 2 nitrosochloride
The preparation of the nitrosochloride was carried out according to Wallach (53) in the following manner: 1.25 ml. of Fraction 2, 0.9 ml. of glacial acetic acid, and 1.2 ml. of amyl nitrite were mixed and cooled in an ice-salt bath. To this mixture was added 0.6 ml. of 32% hydrochloric acid dropwise and with shaking. The acid was added slowly enough to maintain the green color of the solution. After the addition of the acid was complete, the solution was kept at -20° C. for 10 hours at which time a layer of fine, white crystals was observed. The derivative was filtered by suction, washed with cold methanol, and recrystallized six times by dissolving in a small amount of chloroform and precipitating with the addition of methanol. The final product was in the form of colorless plates, melting at 106-7°. The melting point of a mixture of the derivative and authentic alpha-pinene nitrosochloride gave no depression. Examination under the microscope showed the crystals from both sources to be identical in appearance.

The evidences for the identification of alpha-pinene are shown in Table 11.
<table>
<thead>
<tr>
<th>Property</th>
<th>Pure alpha-Pinene</th>
<th>Fraction 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boiling point (°C.)</td>
<td>155-156 (49)</td>
<td>157/700 mm. Hg</td>
</tr>
<tr>
<td></td>
<td>52/20 mm. Hg (52)</td>
<td>58-60/19 mm. Hg</td>
</tr>
<tr>
<td>Density</td>
<td>d\textsuperscript{20} 0.8590 (49)</td>
<td>d\textsuperscript{30} 0.8576</td>
</tr>
<tr>
<td>Index of refraction</td>
<td>n\textsubscript{D} 1.4670 (49)</td>
<td>n\textsuperscript{D} 1.4768</td>
</tr>
<tr>
<td>Nitrosochloride</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melting point (°C.)</td>
<td>106-107</td>
<td>106-107</td>
</tr>
<tr>
<td>Mixed melting point (°C.)</td>
<td></td>
<td>106-107</td>
</tr>
<tr>
<td>% Nitrogen</td>
<td>6.94 (calc.)</td>
<td>6.98</td>
</tr>
</tbody>
</table>
Identification of beta-Phellandrene

![Chemical Structure](image)

The physical constants of Fraction 6 indicated the possible presence of phellandrene, sylvestrene, terpinolene, delta-4-carene, or beta-pinene. The last three terpenes were eliminated by the preparation of a solid nitrosochloride of Fraction 6, since their nitrosochlorides are not reported in the literature. The possibility of sylvestrene being present was eliminated by the immediate formation of a solid nitrosite which is a positive indication of the presence of phellandrene according to Wallach and Gildemeister (54). The presence of the beta-isomer of phellandrene rather than the alpha-isomer was established in two ways: (a) by the failure of Fraction 6 to form a maleic anhydride adduct according to Goodway and West (24), and (b) by the formation of a solid nitrosochloride, since according to West (55) and to the findings of the present writer alpha-phellandrene yields no solid nitrosochloride. In order further to characterize Fraction 6 the conversion of the nitrosochloride to the nitrolphenylethylamine was attempted. The failure of the
nitrolamine to form was possibly caused by the instability of the nitrosochloride and of the terpene itself. A sample of the nitrosochloride decomposed on standing in a vacuum desiccator after only one day, and refrigeration failed to prevent decomposition in another sample. In addition, no reference could be found in the literature to a nitrolamine of beta-phellandrene.

The presence of beta-phellandrene in Fraction 5 was established by the immediate formation of a solid nitrosite and by preparation of the nitrosochloride, which gave no depression of the melting point on admixture with Fraction 6 nitrosochloride. According to the fractionation data, the volatile oil of Haplopappus laricifolius contains about 30 to 35% 1-beta-phellandrene.

The ultraviolet absorption spectrum of Fraction 6 was determined as previously described and is shown in Figure 2. It exhibited a maximum extinction in ethanol solution at a wave length of 232 millimicrons. According to Booker, Evans, and Gillam (10) a sample of beta-phellandrene in alcohol showed a maximum at 232 millimicrons.
Beta-phellandrene nitrosite was prepared in the following manner: 3 ml. of Fraction 6 was dissolved in 6 ml. of petroleum ether and added carefully to 6 ml. of a solution of 5 g. of sodium nitrite in 8 ml. of water so as to form two layers. This mixture was cooled in an ice-water bath and shaken gently while to it was added 3 ml. of glacial acetic acid. The characteristic voluminous, white, flocculated nitrosite was formed in the aqueous layer as the last ml. of acid was being added. This precipitate was filtered, washed with water, and then with methanol and air-dried. It was found to melt at 89-91°.
Preparation of Fraction 6 nitrosochloride

Beta-phellandrene nitrosochloride was prepared in exactly the same manner and with the same concentration of reagents as was used in the preparation of pinene nitrosochloride. After standing overnight at -20°C, the white, crystalline product was filtered, washed with cold methanol, and air-dried. It was recrystallized twice from acetone as white prisms and melted at 101.5-102°C. An authentic sample of alpha-phellandrene, treated in an identical manner, failed to yield a solid nitrosochloride, thus verifying the occurrence of the beta-isomer in Fraction 6 as mentioned in the discussion above.

Attempted preparation of Fraction 6 maleic anhydride adduct

An attempt was made to prepare the maleic anhydride addition product of beta-phellandrene by the method of Goodway and West (24). Two g. of Fraction 6 and 1 g. of maleic anhydride were refluxed in 5 ml. of ether for 30
minutes, at the end of which time no product had formed. The negative result indicated the presence of the beta-isomer rather than the alpha-isomer.

**Attempted preparation of Fraction 6 nitrolphenylethylamine**

An attempt to prepare beta-phellandrene nitrolphenylethylamine from the nitrosochloride was unsuccessful. About 500 mg. of the nitrosochloride of Fraction 6, 2 ml. of alcohol, and 1.5 ml. of phenylethylamine were mixed and warmed slightly, resulting in no apparent reaction. About 10 ml. of water was added and the mixture was shaken and placed in the cold. On standing overnight a gummy, light-brown oil settled out, but no crystallization occurred after two weeks in the refrigerator. As mentioned previously, this result was probably caused by the instability of both the beta-phellandrene and its nitrosochloride.

The evidences for the occurrence of beta-phellandrene are listed in Table 12.
### Table 12. Identification of beta-Phellandrene in Oil of *Haplopappus laricifolius*

<table>
<thead>
<tr>
<th>Property</th>
<th>Pure beta-Phellandrene</th>
<th>Fraction 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boiling point (°C.)</td>
<td>178-9/758 mm. Hg (48)</td>
<td>169-170/703 mm. Hg</td>
</tr>
<tr>
<td></td>
<td>78/24 mm. Hg (48)</td>
<td>70/19 mm. Hg</td>
</tr>
<tr>
<td>Density</td>
<td>d$_{15.5}$ 0.843 (6)</td>
<td>d$_{15}$ 0.8488</td>
</tr>
<tr>
<td>Index of refraction</td>
<td>n$_{D}^{20}$ 1.4826 (6)</td>
<td>n$_{D}^{20}$ 1.4835</td>
</tr>
<tr>
<td>Melting point of nitrosochloride (°C.)</td>
<td>101-102 (21)</td>
<td>101.5-102</td>
</tr>
<tr>
<td>Immediate formation of solid nitrosite</td>
<td>Positive (54)</td>
<td>Positive</td>
</tr>
<tr>
<td>Formation of maleic adduct under given conditions</td>
<td>Negative (24)</td>
<td>Negative</td>
</tr>
</tbody>
</table>

**Attempted Identification of Fraction 11**

Attempts to identify Fraction 11 were unsuccessful since the quantity of the fraction available was small and sufficient characteristic solid derivatives could not be prepared. The nitrosochloride was prepared, but the amount obtained was not sufficient to determine a constant melting point by successive recrystallizations. The boiling point
of the fraction (173-4°/703 mm. Hg) and the melting point of the nitrosochloride (102.5-103°) suggested the presence of limonene (b.p. 177-8°; m.p. of nitrosochloride, 103-4°, 105-6°); however, the odor of Fraction 11 did not resemble that of limonene nor did the density and refractive index correspond closely to the values for limonene. The amount of bromine absorbed in the attempt to prepare the tetrabromide suggested the presence of one double bond. Limonene, however, contains two double bonds. This fact suggested the possible presence of delta-3-carene, which contains one double bond and forms a nitrosochloride melting at 101-2°. The physical constants compared favorably except for the refractive index (nD for Fraction 11, 1.4844; nD for delta-3-carene, 1.468). Also the characteristic nitrosate of delta-3-carene could not be prepared from Fraction 11.

Preparation of Fraction 11 nitrosochloride

For the preparation of the nitrosochloride of Fraction 11, 1 ml. of Fraction 11, 0.85 ml. of glacial acetic acid, and 1.0 ml. of amyl nitrite were mixed and cooled in an ice-salt bath. To the well-cooled mixture was added dropwise 0.5 ml. of 32% hydrochloric acid. The solution turned blue on addition of the first drops of acid and the blue color persisted throughout the rest of the addition. The solution was allowed to stand for a few minutes in the ice-bath during which time a white, crystalline precipitate formed.
The solid derivative was filtered by suction, washed with cold methanol, and air-dried. It was recrystallized by dissolving in a small amount of chloroform and re-precipitating with the addition of methanol. The air-dried solid melted at 101-101.5°. The product was recrystallized a second time and melted at 102.5-103°. There was not sufficient material left for further recrystallizations.

**Attempted preparation of Fraction 11 tetrabromide**

In order to characterize this fraction, an attempt was made to prepare the tetrabromide. One ml. of the fraction, 4 ml. of amyl alcohol, and 4 ml. of dry ether were mixed and added dropwise to an ice-cooled solution of 0.6 ml. of dry bromine in 3 ml. of dry ether. Another ml. of Fraction 11 was required in order to just decolorize the bromine solution. The terpene-bromine solution was placed in the refrigerator and the solvent allowed to evaporate. After several days no crystals had formed which indicated either that the compound had only one double bond and formed a liquid dibromide or that its tetrabromide, if it had two double bonds, was not a solid. This conclusion is substantiated by the fact that an authentic sample of limonene treated in the same manner easily yielded a solid tetrabromide.

**Attempted preparation of Fraction 11 nitrosate**

An attempt was made to prepare the nitrosate of Fraction 11 according to Simonsen (46) by mixing 1.0 ml. of the fraction
with 0.5 ml. of glacial acetic acid and 1 ml. of amyl nitrite, cooling the solution in an ice-salt bath, and adding 1.0 ml. of nitric acid (sp. gr. 1.395) dropwise with shaking. The color of the solution throughout the addition of the acid was a light yellow-green. No solid product formed on standing in the cold for two weeks. It was concluded that Fraction 11 is not delta-3-carene which will form the nitrosate even if present in small concentration.

**Attempted Identification of Fraction 14 of Oil of Haplopappus laricifolius**

The characterization of the main constituent of Fraction 14 as a carbonyl compound was accomplished by the preparation of the 2,4-dinitrophenylhydrazone; m.p. 214-215°. The compound could not be identified by name since nowhere in the literature could there be found a 2,4-dinitrophenylhydrazone, melting at this temperature, which was derived from a compound with the same properties as Fraction 14. Attempts to prepare the semicarbazone by various methods were unsuccessful, thereby making the identification of this compound even more difficult, since the semicarbazone is the classical carbonyl derivative used in essential oil research for identification purposes.

The physical properties of Fraction 14 suggest the occurrence of a cyclic, unsaturated aldehyde or ketone. The apparent non-reactivity of the fraction in ether solution toward sodium
bisulfite indicates the presence of a ketone, and from the maximum extinction at 236 millimicrons in the absorption spectrum exhibited by Fraction 14 it would appear to be an alpha-beta-unsaturated ketone. On the basis of the nitrogen analysis of the 2,4-dinitrophenylhydrazone the empirical formula is $C_{10}H_{16}O$ or $C_{10}H_{18}O$, or perhaps $C_{10}H_{14}O$ if the compound is doubly unsaturated. This last possibility is rather doubtful, however, since both the refractive index and the density of Fraction 14 appear to be too low for this type of structure. It should also be mentioned that the compound regenerated from its 2,4-dinitrophenylhydrazone has an odor similar to that of cuminaldehyde, as does phellandral; however, there was not recovered a sufficient quantity of the pure oil to determine its physical properties nor could the semicarbazone be prepared from the amount available.

Preparation of the 2,4-dinitrophenylhydrazone of Fraction 14

The preparation of the 2,4-dinitrophenylhydrazone of Fraction 14 of *Haplopappus loricifolius* was carried out as follows: 1 ml. of Fraction 14 was added to 2-3 ml. of hot 2,4-dinitrophenylhydrazine solution. There was an immediate formation of a heavy, orange precipitate. This was filtered by suction, washed with cold methanol, and air-dried. It was recrystallized from a 1:1 chloroform-ethanol solution and was found to melt at 204°. Subsequent recrystallizations raised the melting point to 214-215°. The derivative
crystallized in the form of orange platelets. This evidence points to the presence of a carbonyl compound whose exact identity has not been reported in the literature.

**Attempted Preparation of the Semicarbazone of Fraction 14**

Four attempts to prepare the semicarbazone were made. In the first trial, 0.5 ml. of the oil was added to 1 g. of semicarbazide hydrochloride and 0.5 ml. of pyridine in 5 ml. of alcohol. The mixture was refluxed for 30 minutes on the steam bath, the solvent distilled in vacuo, and the residue cooled and diluted with 5 ml. of water. The mixture was stirred in an ice bath, but the gummy, yellow oil did not crystallize. In the second attempt, 1 g. of semicarbazide hydrochloride and 1.5 g. of sodium acetate were rubbed together in a little ethanol. The alcoholic solution was filtered from the sodium chloride and was added to 1 ml. of Fraction 14. The solution was refluxed for one hour and treated as before. Again no solid product resulted. In the third trial, 1 ml. of Fraction 14 was dissolved in 10 ml. of ether and extracted three times with a saturated solution of sodium bisulfite. No solid product was observed in the aqueous phase. The aqueous bisulfite solution was treated with a few ml. of 6 N sodium hydroxide and steam-distilled. No oil phase was observed in the distillate nor did the distillate have the odor of Fraction 14, so it was not extracted with ether. The ether solution of unreacted oil was dried over
sodium sulfate, the ether removed, the oil dried again on a small amount of sodium sulfate and decanted. Approximately 0.8 ml. of oil remained. To this was added 0.8 g. of semi-carbazide hydrochloride, 1 ml. of pyridine, and 10 ml. of ethanol. The mixture was refluxed for one hour on the steam bath and treated as before. Again only a yellow, viscous oil was obtained. The mixture was made acid with 6 N sulfuric acid and was steam-distilled, yielding an oil with an odor similar to that of cuminaldehyde. The oil layer was decanted, dissolved in ethanol, and hot 2,4-dinitrophenylhydrazine reagent added. After two days red rosettes formed which melted at 214-215°. In the fourth attempt, the ketone was regenerated from the 2,4-dinitrophenylhydrazone obtained above by treatment with sulfuric acid and steam distillation. The steam distillate was extracted with ether, the ether solution dried over anhydrous sodium sulfate, and the ether removed by evaporation leaving 5 or 6 drops of colorless oil. The oil was taken up in alcohol and treated with semi-carbazide hydrochloride and pyridine as in the first trial. Again no solid product was obtained.

Apparently in the first three cases, the semicarbazone could not be crystallized because of impurities present in the fraction. In the last case, the purified compound was not of sufficient quantity to allow isolation of the solid derivative.
Attempted preparation of the p-nitrophenylhydrazone of Fraction 14

An attempt was made to prepare the p-nitrophenylhydrazone according to Cheronis and Entrikin (15). Approximately 0.5 ml. of Fraction 14 was added to a solution of 200-300 mg. of p-nitrophenylhydrazine hydrochloride and 300-400 mg. of sodium acetate in 10 ml. of methanol. The solution was boiled for one minute, one drop of glacial acetic acid was added, and the solution was boiled gently for 5 minutes. Water was added dropwise to the hot solution until it became just turbid, and the solution was boiled a few seconds until clear and allowed to cool to room temperature. On cooling an orange-yellow turbidity developed, but no large crystals were observed. More water was added to the solution, and it was boiled and placed in the cold; however, no crystals formed. This does not necessarily argue against the possible presence of a carbonyl compound since impurities may well have interfered with the crystallization.

Attempted Identification of Fraction 15 of Oil of Haplopappus larinifolius

On the basis of a positive test for aldehydes and ketones with 2,4-dinitrophenylhydrazine reagent, the 2,4-dinitrophenylhydrazone of Fraction 15 was prepared. From the manner in which this derivative behaved on recrystallization, it was apparent that the fraction contained a mixture of two or possibly three aldehydes or ketones. During four recrystallizations
the melting point of the derivative was raised from about 130° to 210°, but the product had the appearance of impure, orange-colored particles containing smaller, red particles embedded within. It melted over a temperature range of 195-210°.

A portion of the fraction was extracted with sodium bisulfite solution in order to effect a separation of the carbonyl compounds, and as described under the identification of Fraction 18, decomposition of the bisulfite complex yielded an aldehyde which was identified as phellandral by means of its 2,4-dinitrophenylhydrazone. However, the residual oil from the bisulfite extraction, when converted to the 2,4-dinitrophenylhydrazone, still seemed to consist of a mixture. An attempt was made to purify the product by chromatographic adsorption, but no separation was effected. Undoubtedly with more experimentation using different conditions of solvent, adsorbent, and concentration, a separation or purification could be obtained.

Although it could not be confirmed, the presence in Fraction 15 of Haplopappus laricifolius oil of cryptone (4-isopropyl-2-cyclohexen-1-one) was suspected on the basis of the work of Berry, MacBeth, and Swanson (8) in which they identified beta-phellandrene, phellandral, and cryptone occurring in the essential oil of Phellandrium aquaticum L. in a sort of biogenetic relationship. Cryptone forms a
2,4-dinitrophenylhydrazone melting at 136°, which may account for the wide spread in melting-point ranges during the recrystallization of Fraction 15 2,4-dinitrophenylhydrazone. Since phellandral forms a solid bisulfite addition complex and cryptone forms a soluble product, the latter could possibly be isolated from a much larger sample of the oil of *Haplopappus laricifolius* than was used in the present investigation by a careful extraction with sodium bisulfite solution.

**Preparation of the 2,4-dinitrophenylhydrazone of Fraction 15**

The 2,4-dinitrophenylhydrazone was prepared according to the method of Shriner and Fuson (43) by treating 1 ml. of Fraction 15 of *Haplopappus laricifolius* oil in ethanol with 2,4-dinitrophenylhydrazine reagent. A red precipitate formed immediately which, after filtering and drying, melted at 130-160°. This derivative was recrystallized four times from a 1:1 ethanol-ethyl acetate solution. Following the fourth recrystallization, the orange, amorphous-looking crystals, which contained smaller particles of a dark red color, melted from 195-210°. This product was used in the chromatographic experiment, together with the product obtained as next described.

About 2 ml. of Fraction 15 and 4 ml. of a 35% solution of sodium bisulfite were shaken on a mechanical shaker for 5 hours. The mixture was allowed to stand for two days in the refrigerator whereupon some white, crystalline solid separated. The layer of unreacted oil was removed by extraction with
ether, and the bisulfite addition compound and bisulfite solution were treated as described under the identification of phellandral in Fraction 18. The ether solution of the unreacted oil was dried over anhydrous sodium sulfate, and the ether removed by evaporation leaving a small amount of brown oil with a camphoraceous odor. This oil was dried over a little sodium sulfate, dissolved in a small amount of ethanol, and treated with 2,4-dinitrophenylhydrazone reagent whereupon a precipitate formed. After having been filtered and air-dried, the product was a dark orange powder, melting at 155-170°. After two recrystallizations from a 1:1 chloroform-ethanol solution, the product was a light orange powder melting at 195-205°. This solid was combined with the 2,4-dinitrophenylhydrazone of Fraction 15 described above and used in the chromatographic experiment.

**Attempted Chromatographic purification of the 2,4-dinitrophenylhydrazone of Fraction 14**

The method used was similar to that of Roberts (58). The column employed, as shown in Plate XVI, was 1 cm. by 4.5 cm. long and was packed with a 2:1 mixture of silicic acid and Super Cel. The 2,4-dinitrophenylhydrazones from the two preparations described above were combined and dissolved in 10 ml. of a 1:2 benzene-ligroin solution, which was then poured into the column. A very gentle suction was applied to the column and the solvent was pulled through leaving a uniform yellow color the full length of the column. No
Plate XVI. Semimicro Chromatographic Adsorption Column.
individual zones of color were apparent. The column was rinsed clean with more solvent which was then concentrated down in a current of air to one-half its original volume and the process was repeated with the same result. The procedure was repeated once again on a column 1 cm. by 15 cm., but the result was still the same—no separation or purification could be effected.

**Attempted preparation of the semicarbazone of Fraction 15**

Two preparations of the semicarbazone according to the methods of Shriner and Fuson (43) and McElvain (31) were attempted. In each case a gummy oil was obtained which could not be crystallized, probably due to the mixture of carbonyl compounds present and to the terpene impurities in the fraction.
Identification of Phellandral

A positive test with 2,4-dinitrophenylhydrazine reagent indicated the presence of a carbonyl compound in Fraction 18, confirming the classification of this fraction as an oxygenated terpene. Fractions 17 and 18 were assumed to contain the same major constituent since their physical properties were very similar. The positive test with dimethone given by Fraction 17 indicated that the carbonyl constituent of both fractions was an aldehyde since, as previously mentioned, dimethone does not react with ketones.

Recrystallization of the solid addition product obtained by action of this test reagent yielded a product which melted sharply at a temperature in close agreement with that reported for phellandral 2,4-dinitrophenylhydrazone. In addition, the physical constants of Fraction 18 resembled to a fair degree the constants for phellandral as recorded in the literature. By means of its bisulfite addition product a small amount of an aldehyde was isolated from Fraction 15.
From this aldehyde was prepared a 2,4-dinitrophenylhydrazone which, when mixed with the same derivative of Fraction 18, showed no depression in its melting point. This indicated the presence of a small amount of phellandral in Fraction 15.

The semicarbazone of Fraction 18 prepared in the usual manner could not be obtained in a solid state, probably due to the presence of impurities in the fraction. However, a solid semicarbazone of an aldehyde, presumably phellandral, was obtained by performing another extraction of Fraction 15 with bisulfite solution, regenerating the aldehyde with alkali, and treating it with semicarbazide reagent as before. The melting point of this derivative approached that of phellandral, but there was not sufficient quantity of the product to allow the necessary recrystallizations.

Preparation of Fraction 18 p-nitrophenylhydrazone was not successful. Since the preparation of this derivative of pure, known compounds was accomplished with no difficulty, it was surmised that the failure of Fraction 18 to react favorably was caused by impurities.

The ultraviolet absorption spectrum of a solution of Fraction 18 in ethanol, shown in Figure 2, exhibits a maximum extinction at 231 millimicrons, which closely corresponds to the value of 232 reported by Evans and Gillam (18) for phellandral in ethanol. On the basis of the fractionation data the oil of *Haplopappus laricifolius* is estimated to contain about 1% 1-phellandral.
Preparation of the 2,4-dinitrophenylhydrazone of Fraction 18

The 2,4-dinitrophenylhydrazone of Fraction 18 was prepared in the following manner: 0.5 g. of the fraction was dissolved in 10 ml. of ethanol. To this solution was added about 10 ml. of hot 2,4-dinitrophenylhydrazine reagent prepared according to the method of Shriner and Fuson (43). Granular, red crystals formed immediately. The product was filtered by suction, washed with a little absolute ethanol, and recrystallized from a 1:1 chloroform-ethanol solution. After two recrystallizations the derivative separated in the form of reddish-orange prisms which melted at 201-202°.

Phellandral was also isolated from Fraction 15 by means of its sparingly soluble bisulfite addition product. Two ml. of Fraction 15 and 4 ml. of 35% sodium bisulfite solution were shaken for 5 hours in a shaking machine. An oily layer separated on standing, and after several days in the cold some white crystalline material appeared. The solid was filtered by suction and after drying was in the form of a white
powder. To this solid was added about 3 ml. of N/2 sodium hydroxide; the addition of a few drops of alkali caused the solid substance to dissolve completely. Immediately an odor similar to that of cuminaldehyde was apparent. The oil was steam-distilled, the distillate extracted with ether, the ether solution dried over anhydrous sodium sulfate, and the ether removed in a current of air, leaving two or three drops of colorless oil which had the strong odor of cuminaldehyde. The oil was taken up in 2 ml. of ethanol and to it was added 1-2 ml. of 2,4-dinitrophenylhydrazine reagent. A heavy orange-colored flocculate immediately formed. This was filtered by suction, washed with cold ethanol, and air-dried to an orange-colored powder. It was recrystallized from a 1:1 chloroform-ethanol solution in the form of fine, orange-red needles melting at 201.5-202°. A second recrystallization yielded orange prisms which melted at 202.5-203°. A mixture of this product and the same derivative obtained from Fraction 18 melted at 202-202.5°.

**Attempted preparation of Fraction 18 semicarbazone**

An attempt was made to prepare the semicarbazone of Fraction 18. About 0.5 ml. of Fraction 18, 0.2 g. of semicarbazide hydrochloride, and 0.3 ml. of pyridine were added to 5 ml. of ethanol. The mixture was refluxed on a steam bath for 30 minutes, the alcohol was removed by distillation.
under reduced pressure, and the residue allowed to cool.
Five ml. of water was added to the residue, and it was stirred
while immersed in an ice bath. After 20 minutes only a
yellow viscous oil resulted. Purification of the product
was attempted by decanting the water, dissolving the oily
residue in ethanol, adding water to the point of turbidity,
immersing the flask in an ice-water bath, and scratching the
sides of the flask. Since this procedure failed to give a
solid product, the solution was heated on a steam bath and
by the addition of ethanol and water was brought just to the
point of turbidity while still hot. The solution was then
allowed to cool very slowly and was finally placed in the
refrigerator for several days but no solid product formed.
It was concluded that the presence of sesquiterpene impurities
from the higher-boiling fractions prevented the crystalliza-
tion of the semicarbazone.
A semicarbazone was prepared from a sample of the alde-
hyde isolated from Fraction 15 by means of the bisulfite
addition product obtained as described in the preceding
paragraph. The semicarbazone was prepared by dissolving a
few drops of the aldehyde in 5 ml. of ethanol, adding to the
solution 0.2 g. of semicarbazide hydrochloride and 0.25 ml.
of pyridine, and refluxing the mixture on a steam bath for
40 minutes. The ethanol was removed by distillation under
reduced pressure. A white, oily substance remained which
was then added to 5 ml. of water; the flask was immersed in
an ice bath while the sides of the flask were scratched with a glass rod to promote crystallization. After 10 minutes a white solid formed which was filtered and dissolved in a little hot ethanol. Water was added to the point of turbidity and the solution placed in the refrigerator for 24 hours, whereupon a few scintillating crystals were observed. On filtering and drying, these melted at 196–197°. The product was recrystallized once more from an ethanol-water solution, m.p. 197–198°. The quantity of product was insufficient for further recrystallization. According to Berry, MacBeth, and Swanson (8) phellandral semicarbazone melts at 203–204°.

The experimental data proving phellandral to be the main constituent of Fraction 18 are shown in Table 13.

<table>
<thead>
<tr>
<th>Property</th>
<th>Pure Phellandral</th>
<th>Fraction 18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boiling point (°C.)</td>
<td>75/1.5 mm. (16)</td>
<td>68/0.25 mm.</td>
</tr>
<tr>
<td>Density</td>
<td>d^20 0.9412 (36)</td>
<td>d^30 0.9509</td>
</tr>
<tr>
<td>Refractive index</td>
<td>n_D^20 1.4912 (36)</td>
<td>n_D^20 1.4920</td>
</tr>
<tr>
<td>2,4-dinitrophenylhydrazone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melting point (°C.)</td>
<td>203–204°</td>
<td>201–202°</td>
</tr>
<tr>
<td>% Nitrogen</td>
<td>16.73 (calcd.)</td>
<td>17.00</td>
</tr>
</tbody>
</table>
Attempted Identification of the 
Sesquiterpene Fractions

The physical properties of Fractions 20 through 28 indicated that they contained sesquiterpene hydrocarbons. The small quantities of these fractions obtained did not permit making the detailed analyses necessary to identify the constituents present; however, attempts were made to prepare certain derivatives as noted below.

The ultraviolet absorption spectra previously mentioned proved to be continuous for all of the fractions and reveal nothing concerning their structure. The spectrum of Fraction 23 showed a slight arrest at 235 millimicrons which may have been due to a conjugated double bond system.

In order to prove that these fractions contained no higher-boiling oxygenated C_{10} terpenes, qualitative tests were made as described in the next section.

From the results obtained no definite conclusions could be reached concerning the identity of the sesquiterpene fractions, which amounted to about 4% of the oil extract.

Qualitative tests on the sesquiterpene fractions

The ester test (15) consisted of adding to 5 drops of Fraction 25 in a test tube. 0.5 ml. of a N solution of hydroxylamine hydrochloride in methanol. Sufficient 2 N potassium hydroxide in methanol (about 3 drops) was added to make the solution alkaline to litmus. The mixture was heated just
enough to bring about a reaction as evidenced by the rapid evolution of small bubbles after the heat was removed. The solution was allowed to cool, acidified with dilute hydrochloric acid, and 4 to 6 drops of a 1% aqueous solution of ferric chloride added. A red color in the solution would have indicated a positive ester test, but in this case the color was yellow. A test on Fraction 28 yielded the same negative result.

The test for carbonyl compounds (43) involved the addition of 3 ml. of 2,4-dinitrophenylhydrazine reagent (0.4% in alcoholic solution) to 2 drops of Fraction 25. The solution was shaken and allowed to stand for 15 minutes, but no precipitate formed, indicating a negative test for carbonyl compounds.

Fraction 25 was tested for alcohol content (15) by adding a pellet of potassium hydroxide to 5 drops of the fraction and heating the mixture. The pellet did not dissolve as it would have in an alcohol by formation of the potassium alcoholate; instead, the oil polymerized to a dark brown mass.

**Attempted Preparation of the Picrate of Fraction 26**

An attempt was made to prepare the picrate of Fraction 26. About 0.5 ml. of the fraction was dissolved in 3 ml. of alcohol. The solution was warmed and to it was added 2 ml. of a hot, saturated, alcoholic solution of picric acid. The mixture was allowed to cool to room temperature. No crystallization occurred, nor was any solid derivative observed after
the solution stood for two weeks in the cold.

**Attempted Preparation of the Hydrochloride of Fraction 27**

About 0.5 g. of Fraction 27 was diluted with 5 ml. of anhydrous ethyl ether and cooled in an ice-salt bath. Dry hydrogen chloride was bubbled through the ether solution for 20 minutes. The solution was then kept at a temperature of -20° C. and the ether allowed to evaporate. No solid product was observed during the evaporation and the residue was a brown oil. It is very possible that the sesquiterpene occurring in this fraction does not form a solid hydrochloride.

**Attempted Preparation of the Nitrosochloride of Fraction 28**

About 0.5 ml. of the fraction, 0.4 ml. of acetic acid, and 0.5 ml. of amyl nitrite were mixed and cooled in an ice bath. During the dropwise addition of 0.25 ml. of 32% hydrochloric acid, a green color appeared in the solution. On standing for 15 minutes at -20° C., amber-colored oil droplets separated. After the solution had been maintained at this temperature for two weeks, a yellow layer of oil formed below the green aqueous layer, but no solid derivative was obtained.
PART III. INVESTIGATION OF THE OIL OF 
Tagetes lemmoni

Fractionation of the Bulk Extract

About 140 g. of the volatile oil of Tagetes lemmoni, which had been freshly steam-distilled and dried over anhydrous sodium sulfate, was fractionated by means of the fractionation column shown in Plate XV. The results of the preliminary fractionation are listed in Table 14.

Table 14. Preliminary Fractionation of Oil of Tagetes lemmoni

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Pressure</th>
<th>Distillation Temperature</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mm. Hg</td>
<td>°C.</td>
<td>g.</td>
</tr>
<tr>
<td>1</td>
<td>15</td>
<td>46-56</td>
<td>0.4</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>56-70</td>
<td>2.1</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>70-75</td>
<td>32.3</td>
</tr>
<tr>
<td>4</td>
<td>0.5</td>
<td>40-50</td>
<td>27.5</td>
</tr>
<tr>
<td>5</td>
<td>0.5</td>
<td>50-55</td>
<td>39.7</td>
</tr>
<tr>
<td>6</td>
<td>0.5</td>
<td>55-60</td>
<td>17.9</td>
</tr>
<tr>
<td>Residue</td>
<td></td>
<td></td>
<td>19.5</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>139.4</td>
</tr>
</tbody>
</table>
The above fractions, with the exception of Fraction 6, were then refractionated into two-degree fractions. Since the yellow color of Fraction 6 was appreciably darker than that of the other fractions, indicating polymerization or resinification, this fraction was not refractionated. The refractionation data are given in Table 15 and include the densities, refractive indices, and optical rotations of the various fractions. These data are shown graphically in Figure 3 in the form of a quantitative distribution chart and three physical measurement curves.
Table 15. Physical Constants and Quantitative Distribution of Various Fractions of Oil of Tagetes lemmoni

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Pressure mm. Hg</th>
<th>Distillation Temperature °C.</th>
<th>Weight g.</th>
<th>Weight %</th>
<th>n&lt;sub&gt;D&lt;/sub&gt;&lt;sup&gt;20&lt;/sup&gt;</th>
<th>d&lt;sub&gt;15&lt;/sub&gt;&lt;sup&gt;28&lt;/sup&gt;</th>
<th>[η]&lt;sub&gt;D&lt;/sub&gt;&lt;sup&gt;20&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18</td>
<td>54-70</td>
<td>1.8</td>
<td>1.29</td>
<td>---*</td>
<td>---*</td>
<td>---*</td>
</tr>
<tr>
<td>2</td>
<td>18</td>
<td>70-72</td>
<td>1.3</td>
<td>0.93</td>
<td>---*</td>
<td>---*</td>
<td>---*</td>
</tr>
<tr>
<td>3</td>
<td>18</td>
<td>72-74</td>
<td>4.0</td>
<td>2.86</td>
<td>1.4708</td>
<td>0.8179</td>
<td>+ 6.5</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>74-76</td>
<td>12.1</td>
<td>8.64</td>
<td>1.4788**</td>
<td>0.8724**</td>
<td>+ 4.4</td>
</tr>
<tr>
<td>5</td>
<td>18</td>
<td>76-78</td>
<td>8.2</td>
<td>5.86</td>
<td>1.4597</td>
<td>0.8178</td>
<td>+ 6.2</td>
</tr>
<tr>
<td>6</td>
<td>18</td>
<td>78-80</td>
<td>12.2</td>
<td>8.71</td>
<td>1.4545</td>
<td>0.8319</td>
<td>+ 7.3</td>
</tr>
<tr>
<td>7</td>
<td>0.5</td>
<td>30-34</td>
<td>3.9</td>
<td>2.78</td>
<td>1.4642</td>
<td>---*</td>
<td>---*</td>
</tr>
<tr>
<td>8</td>
<td>0.5</td>
<td>34-36</td>
<td>1.1</td>
<td>0.79</td>
<td>1.4558</td>
<td>0.8483</td>
<td>+ 6.5</td>
</tr>
<tr>
<td>9</td>
<td>0.5</td>
<td>36-38</td>
<td>6.1</td>
<td>4.36</td>
<td>1.4491</td>
<td>0.8327</td>
<td>+ 7.9</td>
</tr>
<tr>
<td>10</td>
<td>0.5</td>
<td>38-40</td>
<td>3.3</td>
<td>2.36</td>
<td>1.4493</td>
<td>0.8428</td>
<td>+ 7.8</td>
</tr>
<tr>
<td>11</td>
<td>0.5</td>
<td>40-42</td>
<td>1.9</td>
<td>1.36</td>
<td>1.4489</td>
<td>0.8896</td>
<td>+ 7.4</td>
</tr>
<tr>
<td>12</td>
<td>0.5</td>
<td>42-44</td>
<td>7.5</td>
<td>5.35</td>
<td>1.4500</td>
<td>0.8794</td>
<td>+ 7.7</td>
</tr>
<tr>
<td>13</td>
<td>0.5</td>
<td>44-46</td>
<td>5.4</td>
<td>3.86</td>
<td>1.4541</td>
<td>0.8529</td>
<td>+ 7.1</td>
</tr>
<tr>
<td>14</td>
<td>0.5</td>
<td>46-48</td>
<td>0.2</td>
<td>0.14</td>
<td>---*</td>
<td>---*</td>
<td>---*</td>
</tr>
<tr>
<td>15</td>
<td>0.5</td>
<td>55-60</td>
<td>17.9</td>
<td>12.79</td>
<td>1.4908</td>
<td>0.8872</td>
<td>+ 0.4</td>
</tr>
<tr>
<td>Residue</td>
<td></td>
<td></td>
<td>52.1</td>
<td>37.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>139.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Fraction polymerized before the value could be determined.
** Slight resinification occurred before value was determined.
Figure 3. Quantitative distribution and physical properties of the essential oil fractions of Tagetes lemmoni.
The quantitative distribution chart indicates the presence of seven "peak" fractions, namely Fractions 1, 4, 6, 9, 12, and 15. According to the classification of types of terpenes listed on page 67, the boiling points of the fractions indicate that Fraction 1 was a terpene hydrocarbon while the other fractions were oxygenated terpenes. Actually Fraction 1 seemed to be a low-boiling ester according to a positive ferric hydroxamate test. This hypothesis could not be confirmed by a comparison of the physical constants with the literature since both Fractions 1 and 2 polymerized, probably because of some terpene impurity present in the fractions. Also Fractions 3 through 6 must contain terpene hydrocarbons rather than oxygenated terpenes since, with the exception of Fraction 4, they form well-defined nitrosochlorides. It is not to be inferred that oxygenated terpenes do not form solid nitrosochlorides, for such was not the case; however, a nitrogen determination on the Fraction 6 nitrosochloride showed it to be a derivative of a C\textsubscript{10}H\textsubscript{16} hydrocarbon. Comparison of the boiling points of Fraction 3 to 6 with the boiling points listed in the literature for some of the higher-boiling terpene hydrocarbons indicates that the boiling range for this class of terpene should be revised upward to 75° or 80° as the upper limit. Under these conditions the volatile oil of \textit{Tagetes lemmoni} would appear to consist of about 1% of a low-boiling ester, 30% terpene hydrocarbons, 30% oxygenated terpenes, and 37-40% of residue.
In connection with the identification of the major constituents in this oil extract, it was of interest to note the work done by Jones and Smith (29) on the volatile oil of Tagetes glandulifera. These authors fractionated about 3800 ml. of this volatile oil, obtaining four fractions, with a loss of 675 ml. by resinification (18% of the oil extract).

On refractionation they obtained three main fractions, the physical constants of which are listed below.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Boiling range (°C)</th>
<th>Volume (ml)</th>
<th>d^15.5</th>
<th>n_D</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a)</td>
<td>72-75/24 mm.</td>
<td>800</td>
<td>0.8143</td>
<td>1.4695</td>
</tr>
<tr>
<td>(b)</td>
<td>85-90/24 mm.</td>
<td>150</td>
<td>0.8420</td>
<td>1.4523</td>
</tr>
<tr>
<td>(c)</td>
<td>55-65/3 mm.</td>
<td>1300</td>
<td>0.8804</td>
<td>1.4890</td>
</tr>
</tbody>
</table>

Loss by resinification on preliminary fractionation: 675 ml.

The authors state that the fraction (a) was mainly terpenic, containing limonene and ocimene; fraction (b) contained ketones as indicated by its low refractive index, the primary ketone being identified as 2,6-dimethyl-7-octen-4-one; and fraction (c) consisting of the primary constituent of the oil extract also contained a ketone which was shown to be 2-methyl-6-methylene-7-octen-4-one and which the authors named "tagetone". There is a striking similarity between the physical properties of fractions (a), (b), and (c) as tabulated above and the properties listed in Table 15 for Fractions 3, 9, and 15 respectively. This suggests a chemical composition for the
oil of *Tagetes lemmoni* very similar to that of the oil of *Tagetes glandulifera*. On the basis of this similarity, the present investigation was carried out.

**Determination of the Ultraviolet Absorption Spectra**

The absorption spectra of the fractions of the oil extract of *Tagetes lemmoni* were determined on a Model DU Beckman Spectrophotometer in the same manner as before, the concentration being about 0.05% in ethanol. The spectra are plotted in Figure 4 in which the ordinate units are again taken arbitrarily in order to compare the maxima of the various fractions. The spectra for Fractions 1, 2, and 14 are not included since those fractions had polymerized before their spectra could be determined.

Fractions 3 through 6 show a maximum extinction at 236 millimicrons and Fractions 7 and 8 show one at 234, all indicative of a conjugated double-bond system. Fraction 4 also exhibits a maximum at 268 which could indicate a mono-substituted, conjugated system of three double bonds according to Braude (13). Fraction 9, later identified as 2,6-dimethyl-7-octen-4-one, exhibits a maximum at 238, also indicative of a conjugated double-bond system. This is probably due to contamination by some of the lower-boiling fractions, since dimethyloctenone contains no conjugated double bonds. The ketone is a gamma-delta-unsaturated ketone and as such should exhibit a maximum between 278 and 285 millimicrons according
Figure 4. Ultraviolet absorption spectra of the essential oil fractions of Tagetes lemmoni.
to Braude (13). Fraction 9 exhibits a slight arrest at 280 or 282 millimicrons. Jones and Lahey (28) reported a maximum for dimethylcetone at 280 millimicrons. Fraction 15 shows a maximum at 270 millimicrons which indicates a mono-substituted, conjugated system of three double bonds, according to Braude (13), or a mono-substituted, alpha-beta-, gamma-delta-unsaturated ketone according to Evans and Gillam (18). The enolic form of tagetone, which according to Jones and Smith (29) is responsible for the ease of resinification of tagetone, fulfills the requirements of the structure having three conjugated double bonds.

This is also in accord with the maximum extinction exhibited by tagetone at 279 millimicrons reported by Jones and Lahey (28).
Determination of Degree of Unsaturation by Means of Iodine Absorption

As an aid in identifying the primary constituents in the oil of Tagetes lemmoni it was desired to determine the number of double bonds occurring in those constituents. The method used was that prescribed by the Association of Official Agricultural Chemists (3) for the determination of the degree of unsaturation of fatty acids and oils. The method consists of adding 25 ml. of Hanus IBr solution to a weighed sample of the fraction being investigated, allowing the solution to stand for 30 minutes with intermittent shaking, and titrating the excess iodine with standard thiosulfate solution. A blank determination is made using the same volume of iodine solution but omitting the sample. The difference between the volume of thiosulfate solution required in the blank determination and the volume required by the excess iodine in the sample determination is equivalent to the iodine absorbed by the sample. The result is obtained in terms of the number of double bonds in a gram molecular weight of the sample by means of the following equation:

\[
\text{No. of double bonds} = \frac{(\text{ml. } S_2O_3 \text{ for blank} - \text{vol. for sample}) \times \text{Sample weight} \times 1000}{\text{normality of } S_2O_3 \times \text{Mol. Wt. of sample}}
\]

Trial determinations of the degree of unsaturation were made on samples of pinene, limonene, and alpha-phellandrene,
which contain one, two, and two double bonds respectively.

The results obtained are presented in Table 16.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Time of Contact</th>
<th>Method of Shaking</th>
<th>Number of Double Bonds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pinene</td>
<td>30 min.</td>
<td>Intermittent</td>
<td>1</td>
</tr>
<tr>
<td>Pinene</td>
<td>30 min.</td>
<td>Continuous</td>
<td>1</td>
</tr>
<tr>
<td>Limonene</td>
<td>30 min.</td>
<td>Intermittent</td>
<td>2</td>
</tr>
<tr>
<td>Limonene</td>
<td>30 min.</td>
<td>Continuous</td>
<td>2</td>
</tr>
<tr>
<td>Limonene</td>
<td>45 min.</td>
<td>Continuous</td>
<td>2</td>
</tr>
<tr>
<td>Limonene</td>
<td>15 min.</td>
<td>Continuous</td>
<td>2</td>
</tr>
<tr>
<td>Phellandrene</td>
<td>30 min.</td>
<td>Continuous</td>
<td>2</td>
</tr>
</tbody>
</table>

The results obtained are in accord with those cited by Parry (35) who states that the iodine value as a measure of unsaturation in essential oils is notoriously unreliable. In explanation he says:

"These substances, in the first place, consist of such widely different classes of substances, that the reactions taking place are different in almost every group. Hence the iodine value ceases to have the precise meaning that it has in the case of fatty oils. Secondly, with many of the substances found in essential oils, the reactions are so energetic that it is impossible to control them quantitatively. Thirdly, the reactions are such that"
the slightest variation in temperature, exposure to
light, time of reaction, and—which is most important--
the excess of iodine used over that which can
possibly enter into action, so influences the result
that the same worker can but rarely obtain con-
cordant values with the same sample."

Thus it is apparent from the data in Table 16 that the
iodine absorption technique cannot be used reliably as a
measure of the degree of unsaturation of the various essen-
tial oil fractions.

Indication of the Ester Content of Fraction 1

The very fragrant odor of Fractions 1 and 2 suggested
the presence of one or more esters. Both fractions gave a
positive hydroxamate test with the hydroxylamine-ferric
chloride reagent. As mentioned previously, almost complete
polymerization or resinification to a syrupy consistency had
taken place in both fractions before their physical constants
could be determined, so the presence of an ester could not be
confirmed in that manner. Two preparations were attempted
in order to characterize the ester constituent of Fraction 1.
The first was the benzylamide of the acid part of the ester,
and the second was the 3,5-dinitrobenzoate of the alcohol
part.

Attempted Preparation of the N-Benzylamide of Fraction 1

About 0.5 ml. of Fraction 1, 1.5 ml. of benzylamine, and
0.1 g. of ammonium chloride were mixed and refluxed for 1
hour. The reaction mixture was allowed to cool, washed with a little water, immersed in an ice-water bath, and scratched to promote crystallization. When no crystals formed, two drops of dilute hydrochloric acid were added to induce crystallization. Again no crystals developed. No odor of unreacted ester was apparent, however. A little more water was added and the mixture boiled for a few minutes to volatilize any unreacted ester which might interfere with the crystallization of the amide. The mixture was then cooled in an ice bath and scratched, and then allowed to stand in the refrigerator for several weeks, but no crystals were observed. It is possible that the polymerized product in the fraction prevented the formation of the N-benzylamide.

Attempted preparation of the 3,5-dinitrobenzoate of Fraction 1

About 0.5 ml. of the fraction was mixed with 0.3-0.4 g. of 3,5-dinitrobenzoic acid and 2 drops of concentrated sulfuric acid was added. The mixture was warmed very gently over a low flame. No refluxing was possible since there was an immediate formation of a brown, solid mass. The solid was allowed to cool, a few ml. of absolute ether was added, and the mixture was extracted with small portions of 5% sodium carbonate solution. The ether layer was washed with a few ml. of water and evaporated in a stream of air, leaving a few drops of water and no product which probably would have been in the form of an oil. Apparently instead of the occurrence of an ester exchange, the sulfuric acid caused
a more complete polymerization of the constituent or constituents of Fraction 1.

**Indication of the Probable Presence of Ocimene in Fraction 4**

Although the presence of ocimene in the oil of *Tagetes lemmoni* is indicated by the observations discussed below, it could not be definitely proved by the preparation of solid derivatives. According to Guenther (25, II, p. 11) ocimene itself forms no solid derivatives and must be converted by hydrogenation, hydration, or oxidation into compounds that can be characterized by the preparation of solid derivatives. Two such conversions were tried and are described below.

The presence of ocimene in Fraction 4 is indicated by its physical properties. The boiling point and refractive index of Fraction 4 compare favorably with those properties of ocimene cited by van Romburgh (51):
The formation of a yellow, resinous crust at the surface of Fraction 4 after a short exposure to air is in accord with the behavior of ocimene as stated by Simonsen (47) as is the lack of formation of a solid nitrosochloride.

The conversion of Fraction 4 to an alcohol, ocimenol, by hydration with 50% sulfuric acid in glacial acetic acid solution was probably successful, based on the ester-like odor of the product (ocimenyl acetate would probably be formed in acetic acid solution). However, the amount of product formed on hydrolysis of the ester was so small that no solid phenylurethane could be isolated after treatment of the hydrolytic product with phenyl isocyanate.

Hydrogenation of Fraction 4 with sodium and ethanol in order to prepare dihydromyrcene was not successful, based on the determined absorption spectrum of the product which exhibited maxima at 234, 274, 284, 295, and 310 millimicrons. On the basis of Braude's (13) work, dihydromyrcene, which is a 2,3-6,7-unsaturated diene (two isolated chromophores) would be expected to show a maximum only at 175-180 millimicrons. Therefore, it was not surprising that the product failed to yield a solid tetrabromide. According to Enklaar (17)
dihydromyrcene forms a tetrabromide melting at 88°.

**Attempted reduction of Fraction 4 of oil of Tagetes lemmoni**

About 2.5 ml. of Fraction 4 was diluted with 35 ml. of 95% ethanol. To the solution was added about 1.5 g. of sodium in small pieces and over a period of 30 minutes with no external cooling. The mixture was at first orange-colored, changing to red-brown, with the occurrence of some refluxing. The mixture was allowed to stand overnight and 50 ml. of water was then added. A dark brown, oily layer separated immediately on the surface of the water and was decanted. The aqueous layer was then added to a large volume of water to separate any more oil. The oil layer was washed with several portions of 1:1 hydrochloric acid and then with water. The dark brown, oily product was steam-distilled yielding about 0.5 ml. of a light-yellow, mobile liquid. The oil was separated from the steam distillate by extraction with ether and dried over sodium sulfate, and the ether was removed by evaporation.

The dried oil (about 0.5 ml.) was added to 2 ml. of dry amyl alcohol and 4 ml. of anhydrous ether, and this solution was added dropwise to an ice-cooled solution of 0.3 ml. of dry bromine in anhydrous ether. The bromine solution was almost completely decolorized when the addition of the terpene solution was complete. The mixture was placed at a temperature of -20° C. and the ether allowed to evaporate slowly. After four weeks, no solid tetrabromide was observed.
The residue was a yellow-brown oil.

**Attempted hydration of Fraction 4**

Hydration of Fraction 4 of oil of *Tagetes lemmoni* was tried according to the method of Bertram and Walbaum (9) by adding to 2 ml. of the fraction 5 ml. of glacial acetic acid and 0.2 ml. of 50% sulfuric acid. The solution immediately turned dark red. It was placed in an oven at 50-60° for 3 hours and was shaken at frequent intervals. The acid layer was then separated, and the terpene layer was washed three times with water. The dark oil was then steam-distilled yielding about 1 ml. of a viscous, light-brown oil of a rather pleasant, ester-like odor. The steam distillate was extracted with ether and dried over sodium sulfate, the ether having been removed by evaporation. To the residual oil was added 2 ml. of 20% alcoholic solution of potassium hydroxide and 5 ml. of ethanol. This was refluxed for 1 hour. The alcohol was removed by distillation under reduced pressure, and the residue was taken up in water and extracted with ether. The ether solution of ocimenol was dried over sodium sulfate and the ether removed by evaporation, leaving about 5 drops of a brown oil with a pleasant odor. The oil was dried with a very small amount of potassium carbonate, added to an equal volume of phenyl isocyanate, heated in a steam bath for 5 minutes, and cooled in an ice bath. No solid product was observed, even after the reaction mixture stood for several days at -20° C.
Attempted preparation of the nitrosochloride of Fraction 4

About 0.5 ml. of the fraction, 0.5 ml. of amyl nitrite, and 0.4 ml. of glacial acetic acid were mixed and cooled in an ice-salt bath. To this solution was added dropwise 0.25 ml. of 32% hydrochloric acid. The color of the solution remained yellow throughout the addition of the acid and no solid product was obtained even after 24 hours at -20° C.

If Fraction 4 is actually ocimene, this result would be expected since no solid derivatives of ocimene have been prepared directly.

Attempted identification of Fraction 6 of oil of Tagetes lemmoni

The physical constants, formation of a solid nitrosochloride, and the nitrogen analysis of the nitrosochloride indicate the main constituent of Fraction 6 to be a \( \text{C}_{10}\text{H}_{16} \) or \( \text{C}_{10}\text{H}_{18} \) hydrocarbon. The constituent could not be identified by name since no compound could be found in the literature with physical constants approximating those of Fraction 6 and yielding a nitrosochloride of this particular melting point. The tetrabromide of this fraction could not be prepared, probably because of impurities present in the fraction, although it is quite possible that this constituent does not form a solid tetrabromide if it is monocyclic, or a dibromide if it is bicyclic. The possibility of limonene occurring here, as it does in the oil of Tagetes glandulifera, is eliminated since the odor of the fraction does not resemble that of limonene, the nitrosochloride melts 12 degrees high,
and the fraction does not form a solid tetrabromide, which limonene does with ease.

Preparation of the nitrosochloride of Fraction 6

One ml. of Fraction 6 was treated in the usual way. During the addition of the acid, the solution had a yellow-green color. A few minutes after the addition of the acid was complete, fine white crystals were observed forming. The reaction mixture was placed at a temperature of -20° C. overnight, during which time the yield of crystals seemed to have increased. The product was filtered and air-dried. It melted at 103.5-105°. After five recrystallizations, the product melted at 115.5-116°. The melting point of the product resulting from addition of nitrosyl chloride does not conform to any specific compound thus far reported in the literature. One can only conclude that it must be a compound whose identity is not yet known. Proof of its structure would have involved much additional work beyond the projected limits of this investigation.

Attempted preparation of the tetrabromide of Fraction 6

About 1.5 ml. of Fraction 6 was treated in the usual manner by diluting with ether and amyl alcohol and adding it to a cold, ethereal solution of bromine. The calculated amount of bromine was not quite decolorized by the terpene solution. Evaporation of the solvent resulted in no solid product.
This finding is in accord with the well-known and often observed phenomenon in the bromination of unsaturated cyclic compounds. Notwithstanding the presence of double bonds where bromine normally would add with great ease, it appears that slight amounts of impurity inhibit the formation of crystals. The frequent result is the formation of an oily product. The writer found that the tetrabromide of pure limonene, for example, crystallizes with little or no difficulty.
Identification of 2,6-Dimethyl-7-octen-4-one in Fraction 9 of Oil of Tagetes lemmoni

Because of the similarity between the physical constants of Fraction 9 and those of the intermediate fraction of the oil of Tagetes glandulifera obtained by Jones and Smith (29), which has already been discussed, Fraction 9 was investigated for its possible content of 2,6-dimethyl-7-octen-4-one. A semicarbazone was prepared, the melting point of which closely agrees with that recorded for dimethyloctenone semicarbazone. Also the fraction was hydrogenated using a palladium-on-charcoal catalyst, which resulted in the formation of the saturated ketone, 2,6-dimethyloctan-4-one. The semicarbazone of this ketone was prepared.

According to Jones and Lahey (28) dimethyloctenone has an absorption spectrum exhibiting a maximum at 280 millimicrons in ethanol. Fraction 9 possesses a spectrum with a slight arrest at 280 or 282 in ethanol.

The semicarbazones were prepared from samples of Fractions
12 and 13. The main constituent of both fractions is apparently 2,6-dimethyl-7-octen-4-one since semicarbazones were prepared from both which melted at 91.5-92° and which in admixture with Fraction 9 semicarbazone did not depress its melting point.

Based on the fractionation data in Table 15 the oil of Tagetes lemmoni contains about 17% of 2,6-dimethyl-7-octen-4-one.

Preparation of the semicarbazone of Fraction 9

The semicarbazone was prepared from Fraction 9 of oil of Tagetes lemmoni according to the method of Haagen-Smit (25, II, p. 818) as previously described. A white, crystalline product was obtained which after recrystallizing from dilute ethanol melted at 89-90°. After three additional recrystallizations, the product melted at 92°.
Catalytic hydrogenation of Fraction 9 of oil of Tagetes lemmoni

The preparation of the saturated ketone, 2,6-dimethyl-octan-4-one, by the hydrogenation of Fraction 9 was accomplished by use of a semimicro apparatus kindly placed at the writer’s disposal by Dr. G. R. Lappin of the Department of Chemistry. The apparatus is shown in Plate XVII.

About 50 mg. of the catalyst, palladium on charcoal, was placed in the hydrogenation flask along with 0.20 ml. (0.16 g.) of Fraction 9 and 5 ml. of anhydrous ethanol. With the stopcock on the hydrogenation flask open to the air the system was flushed with hydrogen ten times. The stopcock was closed, the system filled with hydrogen under pressure, and mechanical shaking of the hydrogenation flask commenced. The sample absorbed 36.7 cc. of hydrogen under standard conditions of pressure and temperature in 15 minutes, after which the gas buret reading was constant. This preparation was not carried out strictly quantitatively, but the calculated volume the sample should have absorbed was about 24.1 cc. of hydrogen at S.T.P. The sample was removed from the hydrogenation flask,
Plate XVII. Semimicro Hydrogenation Apparatus.
filtered from the catalyst, and used in the alcoholic solution for the preparation of the semicarbazone.

Preparation of dimethyloctanone semicarbazone

\[
\begin{align*}
\text{H}_2\text{C} & \quad \text{CH} & \quad \text{CH}_2 \\
\text{H}_3\text{C} & \quad \text{CH} & \quad \text{CH}_3
\end{align*}
\]

\[\xrightarrow{\text{NH}_2\text{-CO-NH-NH}_2}\]

\[
\begin{align*}
\text{H}_2\text{C} & \quad \text{CH} & \quad \text{CH}_2 & \quad \text{CH}_3 \\
\text{H}_3\text{C} & \quad \text{C} & \quad \text{N-NH-CO-NH}_2 & \quad \text{CH}_3
\end{align*}
\]

To the alcoholic solution of the saturated ketone resulting from the hydrogenation of Fraction 9 were added 0.1 g. of semicarbazide hydrochloride and 0.5 ml. of pyridine. The mixture was refluxed on a steam bath for 30 minutes and treated in the usual manner. A white, crystalline product was easily obtained which was recrystallized from dilute alcohol and melted at 86.5-87°.

In Table 17 below is presented the evidence for the occurrence of 2,6-dimethyl-7-octen-4-one in the oil of Tagetes lemmoni.
Table 17. Identification of 2,6-Dimethyl-7-octen-4-one in Oil of Tagetes lemmoni

<table>
<thead>
<tr>
<th>Property</th>
<th>Pure Dimethyloctenone</th>
<th>Fraction 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boiling point (°C.)</td>
<td>185-6°</td>
<td>182/700 mm. Hg</td>
</tr>
<tr>
<td></td>
<td>85-90/24 mm. Hg</td>
<td>36-38/0.5 mm. Hg</td>
</tr>
<tr>
<td>Specific gravity</td>
<td>d₁5.₅ 0.8354</td>
<td>d₂₅ 0.8327</td>
</tr>
<tr>
<td>Refractive index</td>
<td>nₒ ₁.4295</td>
<td>nₒ 1.4491</td>
</tr>
<tr>
<td>Semicarbazone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melting point (°C.)</td>
<td>92.5</td>
<td>92.0</td>
</tr>
<tr>
<td>% Nitrogen</td>
<td>19.89 (calc.)</td>
<td>20.12</td>
</tr>
<tr>
<td>Dimethyloctanone Semicarbazone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melting point (°C.)</td>
<td>91.5-92.5</td>
<td>86.5-87.0</td>
</tr>
</tbody>
</table>
Identification of Tagetone in Fraction 15 of Oil of Tagetes lemmoni

The presence of tagetone in Fraction 15 of the oil of Tagetes lemmoni was suspected from the similarity between the physical constants of Fraction 15 and those of the high-boiling fraction obtained by Jones and Smith (29) from the oil of Tagetes glandulifera, which they found to contain tagetone. Hence in the course of this investigation, Fraction 15 was analyzed for its possible content of tagetone.

The semicarbazone of Fraction 15 came out in the form of a resin, and the oxime resulted in an oil, both confirming the results of the investigation of tagetone by Jones and Smith. Tagetone was identified in Fraction 15 by permanganate oxidation to isovaleric acid and preparation of the acid anilide, and by catalytic hydrogenation of the fraction to form the saturated ketone 2,6-dimethyloctan-4-one and the preparation of its semicarbazone.

The ultraviolet absorption spectrum of Fraction 15 exhibits a maximum in ethanol at 270 millimicrons, confirming...
the presence of tagetone, which as found by Jones and Lahey (28) exhibits a maximum at 269 in ethanol.

According to the fractionation data in Table 15, the oil of Tagetes lemmoni consists of about 13% tagetone; however, there is probably a fair amount of this constituent lost to the residue by resinification.

Preparation of the semicarbazone of Fraction 15

The semicarbazone of Fraction 15 of oil of Tagetes lemmoni was prepared by the method of McElvain (31); however, the product obtained was in the form of a brown resin.

According to Jones and Smith (29), tagetone forms a resinous semicarbazone.

Preparation of the oxime of Fraction 15 of oil of Tagetes lemmoni

The oxime was prepared according to McElvain (31) by refluxing 1 ml. of Fraction 15, 2 g. of hydroxylamine hydrochloride, 2 ml. of pyridine, and 10 ml. of ethanol on the steam bath for 1 hour. The alcohol was removed by distillation
under reduced pressure. Five ml. of water was added to the residue and the mixture stirred in an ice bath. A few globules of amber-colored oil resulted. The mixture was extracted with ether and dried over anhydrous sodium sulfate. The ether was removed in a stream of air after which a few drops of dark brown oil remained. According to Jones and Smith (29), tagetone oxime is an oil, but in the present investigation an insufficient quantity was obtained to determine the physical properties for comparative purposes.

Oxidation of Fraction 15 to isovaleric acid

About 3 ml. of Fraction 15 was cooled in an ice bath and 100 ml. of a saturated, aqueous solution of potassium permanganate containing 0.5 ml. of 6 N sodium hydroxide was added gradually with stirring. A brown precipitate of hydrated manganese dioxide formed immediately. The brown solid was removed by filtration and washed with water, then with sodium hydroxide solution, and finally with water. The filtrate was filtered repeatedly until a clear solution was obtained. The filtrate was made just acid with 50% sulfuric acid solution and steam-distilled. The odor of the distillate was suggestive of valeric or isovaleric acid. The distillate was made alkaline with sodium hydroxide to pH 10 and evaporated to dryness. About 1 to 1.5 g. of the residue was added to 5 ml. of thionyl chloride, upon which an immediate reaction took place. After the reaction had subsided, the mixture was
heated on a steam bath for a few minutes. The product was allowed to cool and 2 g. of aniline in 30 ml. of benzene was added. After warming the mixture for two minutes on the steam bath, the benzene solution was decanted from the solid residue and was washed with 5-ml. portions of water, 5% hydrochloric acid, 5% sodium hydroxide, and then with 2 ml. of water. The benzene was evaporated in a stream of air leaving a reddish-brown residue. This was dissolved in hot ethanol, filtered, and the filtrate allowed to cool. Fine, white needles formed which melted at 108-109°. Recrystallization from alcohol yielded a product melting at 109-110°. According to Jones and Smith (29), alkaline permanganate oxidation of tagetone yields oxalic and isovaleric acids, of which the latter is identified by formation of its anilide, m.p. 111°. Shriner and Fuson (43) list the melting point of isovaleric acid anilide at 109°.

Catalytic hydrogenation of Fraction 15.
The hydrogenation of Fraction 15 to form 2,6-dimethyl-octan-4-one was carried out in the same manner as that of dimethyloctenone. About 0.25 ml. (0.222 g.) of Fraction 15 absorbed 62 cc. of hydrogen at standard conditions of temperature and pressure in 25 minutes. The theoretical value of hydrogen absorbed by the targetone molecule containing two double bonds, and with a molecular weight of 154.2, is calculated to be 61.5 cc. Thus the experimental value is in good agreement with the theoretical value and indicates the presence of two double bonds in the constituent in Fraction 15.

The sample was removed from the catalyst by filtration and used in the preparation of the semicarbazone.

Preparation of dimethyloctanone semicarbazone

The alcoholic solution of the saturated ketone obtained above was treated with semicarbazide hydrochloride and pyridine as described previously under dimethyloctenone. The product formed as a white, waxy, semi-solid mass. The water was decanted and the solid taken up in alcohol. To the alcoholic solution, water was added dropwise until a slight turbidity developed. The solution was placed in the cold for 24 hours, after which time fluffy, white crystals had settled out. On recrystallization, the product melted at 86.5-87°. A mixed melting point of this product and the semicarbazone prepared from the hydrogenated Fraction 9 (m.p. 86.5-87°) showed no depression, even though neither product melted as high as that reported by Jones and Smith (29), m.p. 91.5°.
The evidences for the occurrence of tagetone in the oil of Tagetes lemmoni are listed below in Table 18.

**Table 18. Identification of Tagetone in Oil of Tagetes lemmoni**

<table>
<thead>
<tr>
<th>Property</th>
<th>Pure Tagetone</th>
<th>Fraction 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boiling point (°C.)</td>
<td>62/3-4 mm. Hg</td>
<td>55-60/0.5 mm. Hg</td>
</tr>
<tr>
<td>Specific gravity</td>
<td>d&lt;sub&gt;15.5&lt;/sub&gt; 0.8803</td>
<td>d&lt;sub&gt;15&lt;/sub&gt; 0.8872</td>
</tr>
<tr>
<td>Refractive index</td>
<td>n&lt;sub&gt;D&lt;/sub&gt; 1.4895 (29)</td>
<td>n&lt;sub&gt;D&lt;/sub&gt; 1.4908</td>
</tr>
<tr>
<td>Iso-valeric acid anilide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melting point (°C.)</td>
<td>110-111° (29)</td>
<td>109-110°</td>
</tr>
<tr>
<td>Dimethyloctanone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Semicarbazone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melting point (°C.)</td>
<td>91.5-92.5°</td>
<td>86.5-87°</td>
</tr>
</tbody>
</table>
Fractionation of the Oil Extract

Because of the very pleasant, fruity odor of the oil, it was desired to fractionate the volatile extract of *Choisya dumosa* in order to gain some knowledge as to the percentage distribution of its constituents and of the physical properties of the various fractions. Since only 20 g. of the extract was available for fractionation, it was necessary to use a high-boiling diluent which would not materially alter the results of the fractionation. For this purpose benzyl benzoate, which boils at 323°/760 mm. Hg, was chosen. About 200 ml. of the ester was added to the *Choisya* extract and the mixture fractionated under reduced pressure in the macro fractionating apparatus pictured in Plate XVIII. Since the fractions obtained were quite small, refractionation was not possible. In Table 19 are listed the fractionation data, physical properties, and quantitative distribution of the various fractions.
Plate XVIII. Macro fractional distillation apparatus.
<table>
<thead>
<tr>
<th>Fraction</th>
<th>Pressure</th>
<th>Distillation Temperature</th>
<th>Weight</th>
<th>Weight</th>
<th>$n_D^{20}$</th>
<th>$d_{25}^{\circ}$</th>
<th>$[\alpha]^\circ_{\text{D}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>40-62</td>
<td>3.5</td>
<td>26.5</td>
<td>1.4413</td>
<td>0.8685</td>
<td>-18.7</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>62-65</td>
<td>1.4</td>
<td>6.9</td>
<td>1.4645</td>
<td>0.8584</td>
<td>-9.6</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>65-68</td>
<td>2.1</td>
<td>10.4</td>
<td>1.4668</td>
<td>0.8576</td>
<td>+3.5</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>40-60</td>
<td>1.9</td>
<td>(added to Fraction 1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>35-70</td>
<td>0.2</td>
<td>1.0</td>
<td>4.661</td>
<td>0.9169</td>
<td>----</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>70-80</td>
<td>0.5</td>
<td>2.5</td>
<td>1.4649</td>
<td>0.8943</td>
<td>-0.6</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>80-90</td>
<td>1.0</td>
<td>4.9</td>
<td>1.4683</td>
<td>0.9294</td>
<td>-8.4</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>90-95</td>
<td>0.7</td>
<td>3.4</td>
<td>1.4803</td>
<td>0.9496</td>
<td>-11.7</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>115-120</td>
<td>0.8</td>
<td>3.9</td>
<td>1.5021</td>
<td>0.9792</td>
<td>-6.6</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>120-130</td>
<td>2.2</td>
<td>10.8</td>
<td>1.5187</td>
<td>0.9988</td>
<td>-1.6</td>
</tr>
<tr>
<td>11</td>
<td>1</td>
<td>130-140</td>
<td>2.9</td>
<td>14.3</td>
<td>1.5400</td>
<td>1.0043</td>
<td>+2.2</td>
</tr>
<tr>
<td>12</td>
<td>1</td>
<td>140-145</td>
<td>0.9</td>
<td>4.4</td>
<td>1.5595</td>
<td>1.0101</td>
<td>+1.3</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>18.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 19. Physical Constants and Quantitative Distribution of Various Fractions of Oil of *Choisy a dumosa*
The physical properties listed in Table 19 are shown graphically in Figure 5 together with a chart representing the quantitative distribution of the various fractions. According to this last chart the "peak" fractions of the oil appeared to be Fractions 1, 3, 7, and 11; however, based on the optical purity as indicated by the specific rotation Fraction 8 rather than Fraction 7 may be considered to be a "peak" fraction.

On the basis of the boiling point, refractive index, and density, Fraction 1 would seem to be a terpene hydrocarbon, probably bicyclic in nature similar to the fenchones, thujane, or sabinene. The fact that Fraction 1 forms a nitrosochloride does not eliminate the possibility of the presence of thujane or sabinene (these compounds form no crystalline addition products) in the fraction, since the yield of nitrosochloride was small enough to have been formed from higher-boiling impurity, such as the constituent in Fraction 3.

The physical properties of Fraction 3 suggest the presence of another bicyclic type of terpene hydrocarbon, such as delta-3-carene.

The physical constants for Fraction 8 indicate a content of an oxygenated terpene, but the negative test with 2,4-dinitrophenylhydrazine eliminates aldehydes or ketones.

The physical data for Fraction 11 suggest the presence of a benzene ring which could be due to the presence of the benzyl benzoate diluent in the fraction.
Figure 5. Quantitative distribution and physical properties of the essential oil fractions of *Choisya dumosa* var. *mollis*.
Determination of the Ultraviolet Absorption Spectra

The ultraviolet absorption spectra of the various fractions of the oil of *Choisya dumosa* were determined in the same manner as were those of the two previous oil extracts studied and are shown in Figure 6. The lower-boiling fractions (1 through 5) exhibited no characteristic maxima, which would support the hypothesis of the bicyclic constituents of Fractions 1 and 3. Fraction 6 exhibited a maximum at 272 millimicrons indicative of either three conjugated double bonds, or of an alpha-beta, gamma-delta-unsaturated ketone of aldehyde, if the constituent is an oxygenated terpene derivative. The absorption spectra of Fractions 11 and 12 resemble very closely the spectrum of benzyl benzoate, which is included in Figure 6, and thus indicate the presence of the diluent in these higher-boiling fractions. The maxima at 228 to 230 in Fractions 7 through 10 could be due to conjugated unsaturation in the "peak" fraction, Fraction 7; but more probably they are due to the conjugated unsaturation of the small amount of diluent which undoubtedly distilled along with the "peak" fraction.

Attempted Isolation of the Principal Odor Constituent from the Flowers of *Choisya dumosa*

During the collection of the leaf material for steam distillation of the volatile oil of this plant, it was noted by the writer that the flowers had a distinct odor of anis-aldehyde. In order to confirm the presence of this compound.
Figure 6. Ultraviolet absorption spectra of the essential oil fractions of *Choisya dumosa* var. *mollis*.
in the flower oil of Choisy dumosa, a collection was made of about 50 g. of the small, white, fragrant flowers, including no leaf material. After as short a delay as possible, the flower material was extracted four to six times with petroleum ether in a soxhlet extractor, and a yellow extract was obtained. The petroleum ether was removed by distillation at reduced pressure leaving an orange, waxy residue. This was dissolved in 95% ethanol, brought just to refluxing temperature over a free flame, and allowed to cool to room temperature. The solution was placed in the refrigerator overnight whereupon a small amount of waxy material settled out. This was removed by filtration, and the clear, yellow alcoholic solution was concentrated to a volume of 3 to 5 ml. under reduced pressure, at which time more waxy solid settled out. The solution was again filtered and a portion of the filtrate tested with 2,4-dinitrophenylhydrazine reagent. A very faint, red turbidity formed indicating the presence of an aldehyde or ketone in very low concentration. However, no macro crystals formed after standing in the refrigerator for several months. It was not possible to make a larger collection of flowers in order to isolate a sufficient quantity of the odoriferous constituent to permit its identification.
Tests on Fraction 1

The ferric hydroxamate test for esters when performed on Fraction 1 yielded a negative result, as did the xanthate test for alcohols, and the 2,4-dinitrophenylhydrazine test for carbonyl compounds.

Preparation of the nitrosochloride of Fraction 1

One ml. of Fraction 1 was treated in the usual manner with nitrite and hydrochloric acid. After the solution stood for 15 minutes in the ice bath, fine, white crystals were observed to form. The solid was filtered, washed with cold methanol, and air-dried, m. p. 93-95°. Before the product could be recrystallized, it decomposed to a red-brown oil.

Tests on Fraction 3

The fraction gave a negative result when tested for carbonyl compounds with 2,4-dinitrophenylhydrazine reagent. The spot test for delta-3-carene was negative.

Tests on Fraction 8

The 2,4-dinitrophenylhydrazine test reagent showed carbonyl compounds to be absent from Fraction 8. The xanthate test for alcohols was negative.

Tests on Fraction 11

The test for carbonyl compounds in Fraction 11 with 2,4-dinitrophenylhydrazine reagent was negative.
### Test for Carbonyl Compounds of the Various Oils

The following volatile oil extracts were tested for carbonyl content with 2,4-dinitrophenylhydrazine reagent with the results noted:

<table>
<thead>
<tr>
<th>Extract</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dalea lumholtzii</td>
<td>Very faint orange turbidity; no solid.</td>
</tr>
<tr>
<td>Lippia wrightii</td>
<td>Very faint orange turbidity; no solid.</td>
</tr>
<tr>
<td>Hyptis-emoryi</td>
<td>Very faint orange turbidity; no solid.</td>
</tr>
<tr>
<td>Encelia farinosa</td>
<td>Immediate red-orange turbidity; no solid but a red oil separated.</td>
</tr>
</tbody>
</table>

From the above results it was concluded that all of the oils listed contain carbonyl compounds but in such small concentrations; except perhaps for Encelia oil, that fractionation of a large amount of the oil would be necessary in order to isolate and identify the carbonyl compound.

#### Preparation of a Semicarbazone from Encelia farinosa Oil

One ml. of the oil extract was treated with semicarbazide in the usual manner. After removal of the alcohol by distillation under reduced pressure, a gummy, yellow oil resulted. The addition of water and stirring the mixture in an ice-water bath did not bring about crystallization. The water
was decanted, the gummy residue taken up in 95% ethanol, and water added to the point of turbidity. The solution was kept at a temperature of $-20^\circ$C. for several weeks. After this period of time, some solid seemed to have formed. The material was rapidly filtered by suction, but it became converted to an oil in a matter of seconds. The lack of crystallization was probably due to the amount of other constituents present in the reaction mixture during the formation of the semicarbazone.

The temperature and pH of the oil extract were measured in the same manner that the oil extract in the previous experiment. The oil of Dalea humboldtii, which seemed to have the Content of Dalea humboldtii.

**Attempted Isolation of the Aldehyde and Ketone**

The odor and physical constants of the oil extract strongly suggested the presence of citral in the extract. In order to isolate this constituent, 5 ml. of the oil extract was treated with 10 ml. of 35% sodium bisulfite solution and chipped ice. The mixture was shaken on a mechanical shaker for 5 hours, and was placed in the refrigerator. After several weeks, no crystalline product had settled out. The unreacted oil layer was separated, and the aqueous layer was treated with sodium carbonate and steam-distilled. The distillate contained no citral. Two occurrences could account for this observation. During the prolonged shaking the reaction mixture could possibly have warmed up enough to cause the citral bisulfite complex to be converted to the labile dihydrosulfonic acid derivative, from which citral can be regenerated only with
alkali hydroxide, not with sodium carbonate. Also it is possible that during the steam distillation of the citral bisulfite complex in the presence of sodium carbonate, the stable di-hydroxysulfonic derivative could have been formed, from which citral cannot be regenerated.

Absorption Spectra of the Various Oil Extracts

The absorption spectra of some of the whole oil extracts were measured in the same manner as were the oil fractions in the previous sections. The oil of Dalea lumholtzii, which seemed to have a high content of citral as mentioned above, exhibited a maximum extinction at 234 millimicrons. According to Cooke and MacBeth (16) and Van-Os and Dykstra (50), citral exhibits a maximum at 235 millimicrons. The present writer obtained a value of 238 for pure citral.

The oil extracts of Lippia wrightii, Choisyia dumosa, and Hyptis emoryi all gave absorption spectra in the ultraviolet containing no maxima. The oil of Artemisia carruthii var. wrightii, however, showed an arrest at 240 millimicrons and a maximum at 285, which according to Evans and Gillam (18) could be due to the presence of a di-substituted, conjugated dienone or dienal.

Since the oil of Artemisia carruthii also contained the dark blue constituent, it was of interest to determine its visible absorption spectrum. This was accomplished by use of
a tungsten lamp in place of the hydrogen tube in the spectrophotometer. The spectrum obtained, shown in Figure 7, exhibited maxima at 610 millimicrons and two arrests at 660 and 735. Also shown in Figure 7 is the absorption spectrum for S-guajazulene, according to Haagen-Smit and Siu (26), which shows a maximum at 603 and two arrests at 661 and 732. The remarkable similarity between the two spectra indicates that the structure of the azulenlic constituent of the oil of *Artemisia carruthii* is very similar to, if not identical with, S-guajazulene, whose structure is shown below.

![Chemical structure](image)

*Photomicrographs of leaf sections of some essential oil plants*

In Plates XIX and XX are shown photomicrographs* of transverse leaf sections of several of the plants which were examined for their essential oil content. The large, round, sometimes open structures are apparently oil glands in which the essential oil could conceivably have been stored in the living plant. The section of the bud of *Choisya dumosa* is interesting for it shows the occurrence of an oil gland in the very young leaf.

*The leaf tissue slices were prepared by Mr. E. B. Kurtz of the Department of Botany, University of Arizona.*
Figure 7. Visible absorption spectrum of volatile oil of *Artemisia carruthii* compared with spectrum of S-guajazulene.
Plate XIX. Photomicrographs of leaf sections.

*Choisya dumosa leaf (200x)*

*Choisya dumosa bud (200x)*

*Dalea lumholtzii, low power (100x)*

*Dalea lumholtzii, high power (200x)*
Plate XX. Photomicrographs of leaf sections.
DISCUSSION

In this investigation plants of thirty-five species growing in desert areas and mountain ranges of southern Arizona were examined for their content of volatile oil by means of steam distillation. It was found that in general the more odoriferous plants were members of plant families high on the phylogenetic scale—families of plants whose ability to synthesize the complex essential oil constituents was in accord with their more advanced stage of development in the evolutionary scheme. This is attested by the fact that of the thirty-five plants studied, twenty belonged to the Compositae, one to the Labiatae, and one to the Verbenaceae, the three highest families in Bessey's (59) phylogenetic classification of flowering plants.

It is not implied that these thirty-five species exhaust the supply of plants available in southern Arizona for this type of study, for such is not the case. In addition to the plants listed on page 9 the writer knew of the following but could not locate them in the field:

Anaphalis margaritacea  Gnaphalium leucocephalum
Anagallis arvensis  Hedeoma dentatum
Buddleia sessiliflora  Matricaria matricarioides
Choisya arizonica  Salvia alba
Erodium cicutarium  Salvia lemmonii
Galium triflorum  Salvia mohavensis

And, in addition, according to Kearney and Peebles (30), there
are 594 species in the families Compositae, Verbenaceae, and Labiatae, disregarding twenty or so other families, any of which might well be examined.

Regarding the fractionation apparatus used in this investigation, in the light of past experience it is now apparent that the time involved constructing and modifying and re-modifying the fractionation equipment would have been more profitably used in the collection and steam distillation of ten, twenty, or thirty times the amount of plant material that was collected. In this manner a standard fractionation apparatus of much higher efficiency could have been employed in the separation of the volatile oil constituents in much larger quantities and in a purer state, thus immensely diminishing the problems encountered in the identification procedures.

In the analysis of the oil of Hanlopappus laricifolius the constituents 1-beta-phellandrene and 1-phellandral were found. This was most interesting in view of the fact that Berry, MacBeth, and Swanson (8) identified 1-beta-phellandrene and 1-phellandral in the oil of Eucalyptus cneorifolia, and somewhat later the same authors in an investigation of the oil of water fennel (Phellandrium aquaticum) found both d-beta-phellandrene and d-phellandral. These authors imply that there is a biogenetic relationship between these compounds
since the d-isomer of each was found to exist in one oil and
the l-isomer of each was found in the other oil. The present
investigation of the oil of Haplopappus laricifolius confirms
these findings. In the same two oils mentioned, Berry and
his co-workers also identified cryptone (4-isopropyl-2-cyclo-
hexen-1-one) as the l-isomer in the eucalyptus oil and as the
d-isomer in the oil of water fennel. This ketone was obtained
by Wallach (60) by the aerobic oxidation of beta-phellandrene
in the presence of sunlight, and the same process can conceiv­
ably occur in the living plant. This facet of the biogenetic
relationship existing among these three compounds, beta-phell-
andrene, cryptone, and phellandral, was not confirmed in the
present investigation since cryptone could not be identified.
It is very possible that a small amount of cryptone did occur
in Fraction 15 and that its 2,4-dinitrophenylhydrazone was
lost through solubility during the recrystallizations of the
2,4-dinitrophenylhydrazone of Fraction 15. It may also be
hazarded that the unidentified aldehyde or ketone of Fraction
14 is a product resulting from the oxidation of phellandrene
or the isomerization of phellandral.

It was also of interest to note, as mentioned previously,
the similarity in composition between the oil of Tagetes
glandulifera analyzed by Jones and Smith (29) and the oil of
Tagetes lemmoni reported in the present work since 2,6-di-
methyl-7-octen-4-one and tagetone have been found in both
volatile oils. The presence of ocimene reported by Jones
and Smith could not be proved to occur in the Tagetes lemmoni
oil but strong indications were presented. Unlike the
occurrence of limonene in Tagetes glandulifera, it was shown
rather definitely to be absent from the extract analyzed in
this investigation.

The fractionation of the oil of Choisya dumosa var.
mollis was carried out chiefly to determine whether a small
amount of an oil extract could be fractionated by use of a
carrier or diluent. The procedure appeared to be successful
except that the two or three highest-boiling fractions were
apparently contaminated with the diluent, as shown by their
absorption spectra in comparison to the spectrum of the diluent.
However, the volume of the fractions obtained was too small to
do more than perform a few qualitative tests. On the basis
of the odor and physical constants of the oil, it would seem
well worthwhile to steam distill a large quantity for the
purpose of fractionation and identification of the constituents.

The occurrence of the blue constituent in the oil of
Artemisia carruthii var. wrightii also provided some interest,
since this constituent is not one of the more common in the
plant volatile oils. This constituent was shown by means of its visible absorption spectrum to be an azulene similar in structure to S-guajazulene. From the very dark blue color of the oil it would seem to consist almost entirely of this azulenic compound.

It is not contended that this investigation is complete for much remains that could be accomplished, given the time and available material. The problem of identifying the major constituents of the oils of Haplopappus laricifolius and Tagetes lemmoni has been in the main successfully completed, but a more complete elucidation should some day be undertaken.
SUMMARY

1. Thirty-five plants of the desert and mountains of southwestern Arizona were steam-distilled in order to extract the volatile oil content.

2. The physical and chemical properties of some of these oil extracts were determined.

3. The essential oil of *Haplopappus laricifolius* was analyzed and found to contain 15-20% alpha-pinene, 30-35% l-beta-phellandrene, and 1% phellandral.

4. The findings concerning the composition of the oil of *Haplopappus laricifolius* presents additional evidence toward confirming the hypothesis of Berry, MacBeth and Swanson that beta-phellandrene and phellandral exist in a biogenetic relationship.

5. The essential oil of *Tagetes lemmoni* was analyzed and found to contain about 17% 2,6-dimethyl-7-octen-4-one and about 13% tagetone.

6. The results of the analysis of the oil of *Tagetes lemmoni* support Jones and Smith's observation that tagetone is a characteristic constituent in many *Tagetes* species oils.

7. The essential oil of *Choisya dumosa* var. *mollis* was fractionated by means of a diluent, but none of the constituents were identified since the quantity of oil obtained was too small.
BIBLIOGRAPHY


11. Bradley, C. E.  
1949 Oral communication.


13. Braude, E. A.  


15. Cheronis, N. D. and Entrikin, J. B.  


17. Enklaar, C. J.  

18. Evans, L. K. and Gillam, A. E.  

19. Fester, G. A.  

20. Finlayson, H. H.  

21. Francesconi, L. and Sernagiotto, E.  

22. Gaponenkov, T. K.  
23. Gildemeister, E. and Hoffmann, F.  
1922 The volatile oils. Volumes I-III. Second edition  
translated by Edward Kremers. John Wiley and  

24. Goodway, N. F. and West, T. F.  
1938 Addition reactions to conjugated systems. Beta-  
2028-31.

25. Guenther, E.  
1950 The essential oils. Volumes I-IV. D. Van Nostrand  

26. Haagen-Smit, A. J. and Siu, R.  
1944 Chemical investigations in guayule. I. Essential  
oil of guayule, Parthenium argentatum, Gray.  

27. Hardy, E.  
1946 New perfume plants from the East. Perfumery and  
Essential Oil Record 37:170-1.

28. Jones, T. G. H. and Lahey, F. N.  
1942 Ultraviolet absorption spectra of tagetone and  
related ketones. Univ. Queensland Papers,  
Dep't. Chem. 1:26-8.

29. Jones, T. G. H. and Smith, F. B.  
1925 Olefinic terpene ketones from the volatile oil  
of flowering Tagetes glandulifera. Part I.  
129:2767-70 (1926).

1942 Flowering plants and ferns of Arizona. U. S.  

31. McElvain, S. M.  
1945 The characterization of organic compounds.  

32. Narayan, K. and Dutt, S.  
1945 Characteristics of the oils of Bursera delpechiana  
(Original not seen).

33. Naves, Y. R. and Mazuyer, G.  
1947 Natural perfume materials. Translated by Edward  
34. Parry, E. J.  

35. Parry, E. J.  

36. Penfold, A. R.  

37. Penfold, A. R. and Simonsen, J. L.  

38. Pregl, F.  


40. Sanderson, R. T.  

41. Shapter, R. E.  
1941 A preliminary investigation into the yield and composition of the oil distilled from Chenopodium ambrosioides (Linn.) var. anthelminticum (Gray). J. Council for Sci. and Ind. Research, 14, No. 3.

42. Shrader, S. A. and Ritzer, J. E.  

43. Shriner, R. L. and Fuson, R. C.  

44. Sievers, A. F. and Marshall, C. G.  
45. Siggia, S.

46. Simonsen, J. L.

47. Simonsen, J. L.

48. Smith, G. E. and West, T. F.

49. Thurber, F. H. and Thielke, R. C.

50. Van Os, D. and Dykstra, K.

51. Van Romburgh, K.

52. Von Rechenberg, C.

53. Wallach, O.

54. Wallach, O. and Gildemeister, E.

55. West, T. F.
1939 The addition of nitrosyl chloride to beta-phellandrene and the occurrence of phellandrene in some essential oils. J. Soc. Chem. Ind. 58:122-5 T.
56. Whitmore, F. C. and Lux, A. R.

57. Woodward, R. B.

58. Roberts, J. D. and Green, Charlotte

59. Bessey, C. E.

60. Wallach, O.