Essential Oil of *Salvia dorisiana* (Standley)

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Analysis of the steam-distilled oil of *Salvia dorisiana* (Standley) revealed perillyl acetate, methyl perillate, myrtenyl acetate, caryophyllene, and limonene to be the major constituents. In addition, 8 monoterpene hydrocarbons, 10 oxygenated monoterpenes, 12 sesquiterpene hydrocarbons, nerolidol, α-cadinol, *n*-pentyl *n*-butyrate, 2-octanol, and butyric acid were identified. The structure of a new diterpene hydrocarbon was elucidated as 2,6-dimethyl-10-(*p*-tolyl)undeca-2,6-diene. Infrared, gas chromatographic retention time, and, where necessary and possible, mass and NMR spectroscopy and chemical means were used for identification.

The genus *Salvia,* family Labiatae, comprises numerous species which produce essential oils. Some of these oils have commercial importance in the food and cosmetic industries. For example, Dalmation sage oil obtained from *S. officinalis* is one of the most important flavoring oils and is used mainly for flavoring table sauces, canned and packed food, soups, meats, and especially sausages (Guenther, 1949). Clary sage oil obtained from *S. sclarea* is used principally in perfumery and sometimes in flavors, especially in wine bouquets (Guenther, 1949).

S. dorisiana (Standley) is a herbaceous plant indigenous to Honduras (Standley, 1950) and probably other places in Central America. This species produces an essential oil possessing a very pleasant aroma. A review of the literature does not show any attempts to analyze the oil, which is somewhat surprising as the plant produces a large amount of oil and is easy to grow. The present investigation is, as far as the authors are aware, the first report on the composition of the leaf oil of *S. dorisiana*.

EXPERIMENTAL SECTION

Separation of the main oil fraction was accomplished by means of an Aerograph Model A-700 Autoprep gas chromatograph equipped with a thermal conductivity detector. Subfractions were further purified or separated using a Hewlett-Packard Model 6720A gas chromatograph equipped with an FID (flame ionization detector) and an effluent splitter with a 1/20 split ratio. The temperatures for the injector and detector in both instruments were adjusted at 150° in the case of the monoterpene hydrocarbon fraction and at 240° for all other fractions. The He flow rates were 25-30 ml/min for the ⅛ in. o.d. and 10% loaded columns and 40-60 ml/min for the ¼ in. o.d. and 15% or more loaded columns. The liquid phases employed and other GC parameters are listed in Table I.

Isolated components were trapped from the exit port of the chromatograph by condensation in Dry Ice cooled capillary tubes. Purity of the isolated individual component was demonstrated by rechromatographing on a column of different polarity as will be mentioned under the specific fractions. Retention time (t_R) values were measured on at least two different columns using an authentic reference sample as an internal standard. The instrument used for these analyses was a Hewlett-Packard Model 6720A GC instrument equipped with an effluent splitter with a 1:1 split ratio. Infrared (ir) spectra were recorded on a Perkin-Elmer Model 137 Infracord as a thin film between two KBr pellets (Halim and Collins, 1970). The NMR spectrum was recorded on a Hitachi-Perkin-Elmer Model R24 using CCl_4 as a solvent and Me_4Si as an internal standard. Mass spectra (MS) were run using a combined GC-MS. The GC was a Hewlett-Packard Model 5750B equipped with an FID and a splitter having a 1:10 split ratio. Different columns with variable parameters depending on the compound under investigation were used. The GC effluents were passed through a stainless steel capillary (2.5 ft × 0.013 in.) to a Biemann all-glass molecular separator. The latter was attached to an AEI-MS 902 mass spectrometer operating at an ionization energy of 70 eV. The temperatures for the capillary line, molecular separator, and the ion source were all kept at 225°.

Table I. GC Columns Used in the Analysis of *S. dorisiana* Oil[a]

Column	Liquid phase	Solid support	Length (ft) × diameter (in.)
a	Carbowax 20M (10%)	High performance Chromosorb S, 80–100 mesh	8 × 1/8
b	W98 (10%)	Chromosorb W, 80–100 mesh	6 × 1/8
c	SAIB (10%)	Chromosorb W, 80–100 mesh	6 × 1/8
d	Carbowax 20M (10%) + PDEAS (1%)	Chromosorb W, 80–100 mesh	8 × 1/8
e	Carbowax 20M (15%)	Chromosorb W, 60–80 mesh	5 × 1/4
f	SAIB (15%)	High performance Chromosorb W, 80–100 mesh	8 × 1/4
g	Apiezon L (25%)	Glass beads, 80–120 mesh	5 × 1/4

[a] Columns a, b, c, and d were used with the Hewlett-Packard instruments, while columns e, f, and g were used with the Aerograph Model A-700 instrument. The injector and detector temperatures were 250 and 235°, respectively.

Reduction of the major esters to the corresponding alcohols was accomplished by means of $LiAlH_4$. The ester (20 mg) was dissolved in 2 ml of anhydrous ether and added slowly to 100 mg of $LiAlH_4$ in 7 ml of ether. The reaction was left for 30 min at room temperature and then excess reagent was destroyed by cold water. The ether layer was separated and dried with Na_2SO_4 and the solvent was removed. The products were identified by comparing their ir and T_r with those of a reference sample.

The plants used in this study were originally provided by Dr. Howard Pfeifer of the University of Connecticut. The small plants were transferred from the greenhouse to a field plot provided by the University, in early June. The leaves were harvested in early September and subjected immediately to steam distillation at atmospheric pressure. The water and oil extract was collected in large carboys. The oil was concentrated by redistilling the water-

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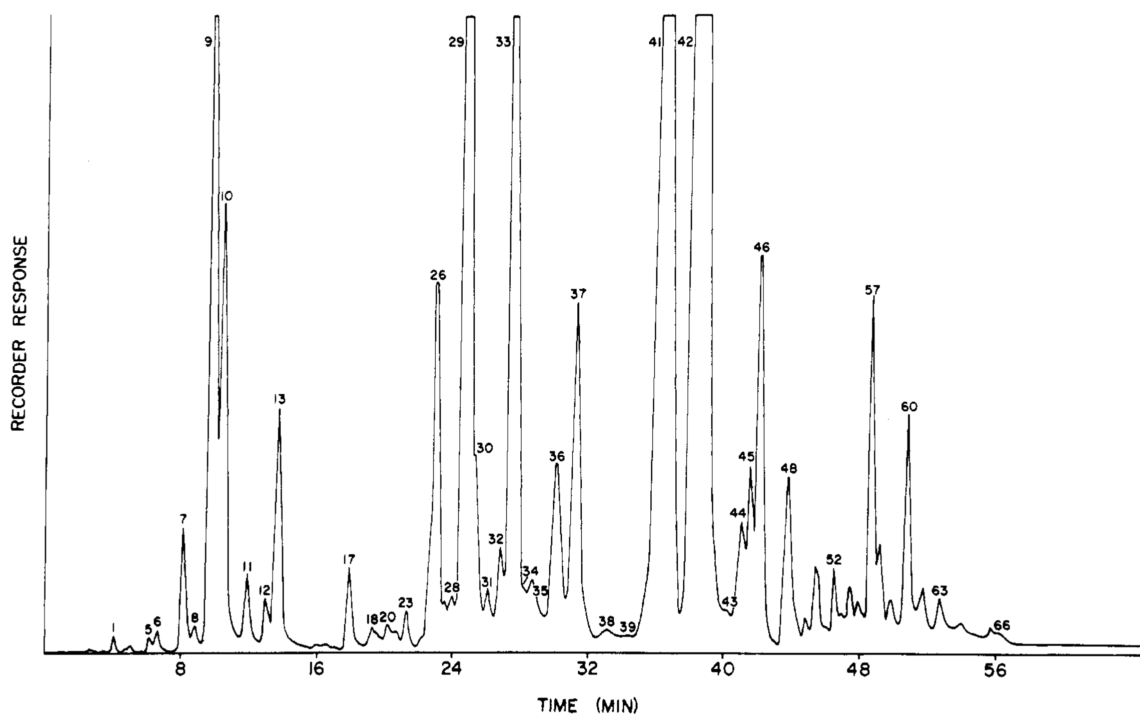

Figure 1. Gas chromatogram of the essential oil of *S. dorisiana* using column a; starting temperature, 60° for 6 min, programming 60–140° at 4°/min, hold at 140° for 10 min, programming 140–220° at 4°/min, and hold at 220° until the end of the run.

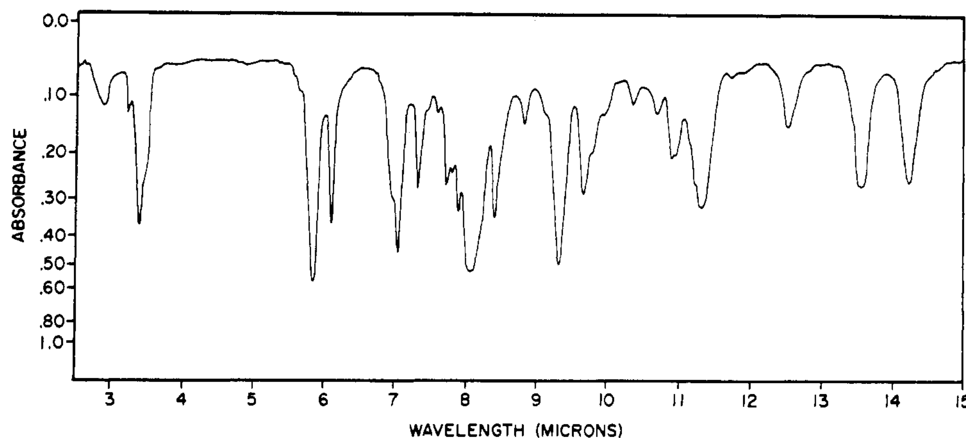

Figure 2. Infrared spectrum of the compound identified as methyl perillate (peak 41).

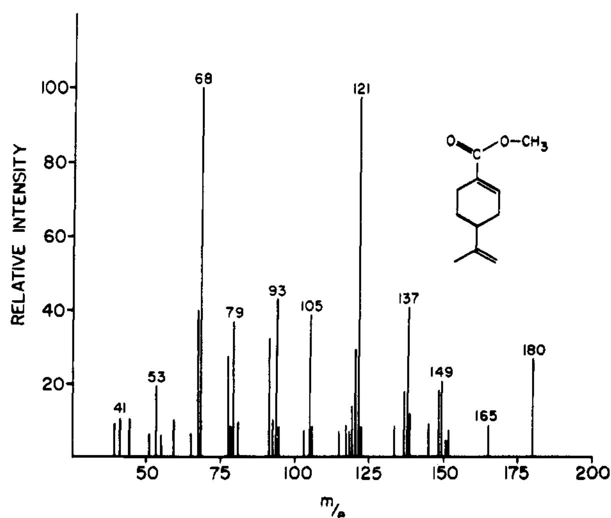

Figure 3. Mass spectrum of the compound identified as methyl perillate (peak 41).

oil extract in a cohobation still. The concentrated oil was collected, dried, and then analyzed.

Prefractionation. The leaf oil (6.5 g) was chromatographed on deactivated silicic acid (60 g). Elution with petroleum ether (bp 30–60°) (250 ml) gave a hydrocarbon fraction (A) (1.1 g), elution with petroleum ether + 10% ether (250 ml) gave a mid-fraction (B) (1.8 g), and elution with pure ether gave a polar fraction (C) (3.6 g).

Hydrocarbons. Fraction A was prefractionated on column e (Table I) into a mono- and sesquiterpene fraction. The monoterpenes were separated on column a and each isolated peak was checked for purity or repurified, if necessary, on column b.

The sesquiterpene fraction was further subfractioned by means of TLC using layers of silica gel G impregnated with 30% $AgNO_3$ and acetone–benzene (2:98) as the developing solvent. Several bands were revealed. Each band was separately extracted with CH_2Cl_2 and its sesquiterpene content was resolved by GC using either column b or d.

Mid-fraction. The mid-fraction B was subfractioned on

Table II. Composition of the Essential Leaf Oil of *S. dorisiana*

Peak no.	Compd identified	Peak size[a]	% composition	Means of ident.[b]
1	α-Pinene	T	T	T_r
6	β-Pinene	T	T	T_r
7	Myrcene	S	0.6	T_r; ir
8	α-Terpinene	ES	0.1	T_r
9	Limonene	L	4.5	T_r; ir
10	Cineole	M	3.0	T_r; ir
11	γ-Terpinene	XS	0.5	T_r; ir
12	*p*-Cymene	XS	0.3	T_r; ir
	Terpinolene	S	1.4	T_r; ir
13	*n*-Pentyl *n*-butyrate	S		ir
17	2-Octanol	XS	0.4	T_r; ir
20	α-Cubebene	XS	0.1	T_r
23	Copaene	XS	0.2	T_r
	α-Gurjunene	XS	2.2	T_r
26	Linalool	M		T_r; ir
28	α-*trans*-Bergamotene	XS	0.2	T_r; ir
	Isocaryophyllene	XS		ir
29	Terpinen-4-ol	S	12.5	T_r; ir
	Caryophyllene	L		T_r; ir
	Butyric acid	XS	0.7	T_r; ir
30	Aromadendrene	XS		T_r; ir
	α-Humulene	ES		T_r; ir
	α-Terpineol	S	9.2	T_r; ir
33	γ-Muurolene	ES		ir
	Myrtenyl acetate	L		ir; chem.
35	α-Muurolene	XS	0.3	ir
	Citronellol	T		T_r
36	δ-Cadinene	S	1.2	T_r; ir
	γ-Cadinene	XS		T_r; ir
	Myrtenol	M	2.5	T_r; ir
37	Perillaldehyde	S		T_r; ir
38	Calamenene	XS	0.1	T_r; ir
39	Geraniol	T		T_r
41	Methyl perillate	XL	21.2	ir; MS; NMR
42	Perillyl acetate	XL	27.7	ir; chem.
45	Perillyl butyrate	S	0.9	ir
46	Perillyl alcohol	M	2.4	T_r; ir
48	Nerolidol	S	1.0	T_r; ir
57	α-Cadinol	M	1.7	T_r; ir
60	2,6-Dimethyl-10-(*p*-tolyl)-undeca-2,6-diene	S	1.0	ir; MS; NMR

[a] Peak area was measured by the height × ½ width method and was considered to be "extra large" (XL) when the relative percent composition was above 15, "large" (L) between 4.5 and 15, "medium" (M) between 1.5 and 4.5, "small" (S) between 0.5 and 1.5, "extra small" (XS) between 0.1 and 0.5, and "trace" (T) below 0.1. [b] T_r, retention time; ir, infrared; MS, mass spectrometry; NMR, nuclear magnetic resonance; chem., chemical means.

column g and ten peaks were collected. The peaks were purified on either column a, b, or c.

Polar Fraction. Fraction C (3 g) was subjected to fractional distillation using a Nester-Faust stainless steel semimicro spinning band still. The distillation was controlled by varying the bath temperature between 130 and 180°, maintaining the vacuum at 2 mmHg, and holding the reflux ratio at 10:1. The various fractions (seven) collected in the receiver vials, as well as the residue remaining in the pot, were analyzed on column a and those fractions which revealed nearly identical composition were combined. The combined fractions were then separated in column f and each isolated peak was checked for purity and refractionated, if necessary, on columns a or c.

RESULTS AND DISCUSSION

A typical gas chromatogram of the volatile leaf oil of *S. dorisiana* as obtained on column a is shown in Figure 1. A list of the identified compounds, their relative percentage, and means of identification is given in Table II.

Peak 41 possessed a very pleasant fruity odor. Its ir and MS spectra are given in Figures 2 and 3. The NMR signals (δ) occurred at 1.7 (3 H, s), 1.95–2.5 (7 H), 3.61 (3 H, s), 4.68 (2 H, s), and 6.78–7 (1 H). The spectral data agreed with the structure of methyl perillate (1-cyclohexene, 4-isopropenyl, 1-carboxylic acid methyl ester). For further confirmation the compound was subjected to $LiAlH_4$ reduction and the product was identified as perillyl alcohol (ir, T_r). Although perillic acid has been known for some time as an oxidative product of perillaldehyde, its highly odoriferous methyl ester has rarely been found as a natural product.

Peak 60 possessed a faint uncharacteristic odor. Its ir (Figure 4) did not reveal any absorption bands attributable to any functional groups other than C and H. Strong absorption bands at 3.29, 5.54, 6.64, and 12.15 μ indicated a para-disubstituted benzene. The ir of this compound has a striking similarity to that of *ar*-curcumene (Wenninger et al., 1967). However, the emergence of peak 60 at a considerably longer retention time than *ar*-curcumene, which should emerge near 30 min (refer to Figure 1), immediately precluded this compound. On the other hand, the MS of peak 60 (Figure 5) revealed major ions at *m/e* 145, 132, 119, 105, 91, 69, and 41 which are also the prominent ions in the fragmentation pattern in a published spectrum for *ar*-curcumene (Asakawa et al., 1971). A parent ion at *m/e* 270 indicates a molecular formula $C_{20}H_{30}$ or a C_5H_8 mass unit more than *ar*-curcumene. This extra mass is most likely due to a fourth isoprene unit attached head-to-tail to the terminal isopentenyl group of the *ar*-curcumene skeleton. This conclusion was also supported by other examples where two structurally very related terpenoids that differ merely in their masses by one isoprene unit have their ir and MS revealing numerous features in common, e.g., linalool and nerolidol (Russell and Jennings, 1969), myrcene (Mitzner et al., 1965) and B-farnesene (Wenninger et al., 1967), and geranylacetone (Buttery and Seifert, 1968) and farnesylacetone (Buttery et al., 1969). Consequently, the compound represented by peak 60 was characterized as 2,6-dimethyl-10-(*p*-tolyl)undeca-2,6-diene. An interpretation of its fragmentation pattern is also included in Figure 5.

The proposed structure was further confirmed by the NMR spectrum as shown in Figure 6, where the δ values assigned to the different protons are also included. There are two positions of stereochemical interest, namely the configuration at the ethylenic double bond, C-6, and at the asymmetric center, C-10. Bates et al. (1963) examined the NMR of four isometric farnesols and concluded that a trans methyl group has its proton less shielded (δ 1.66) than those of a cis isomer (δ 1.59). Consequently, as the signal at δ 1.59 integrates for six protons while at δ 1.66 integrates for three protons (Figure 6), it may be concluded that the ethylenic double bond at C-6 has a trans configuration. Unfortunately, lack of material makes it impossible to examine the asymmetric center at C-10. To the authors' knowledge, this compound has not been previously reported as a constituent of a natural product.

In general, the oil composition in *S. dorisiana* is quite unique for this species. The GC patterns of some fractions are rather complicated and, while 41 components were identified in the present work, more than 90 peaks could be counted. It is interesting to notice that many of the major constituents are presumably derived from 1,8-*p*-menthadiene possibly through a stepwise oxidation

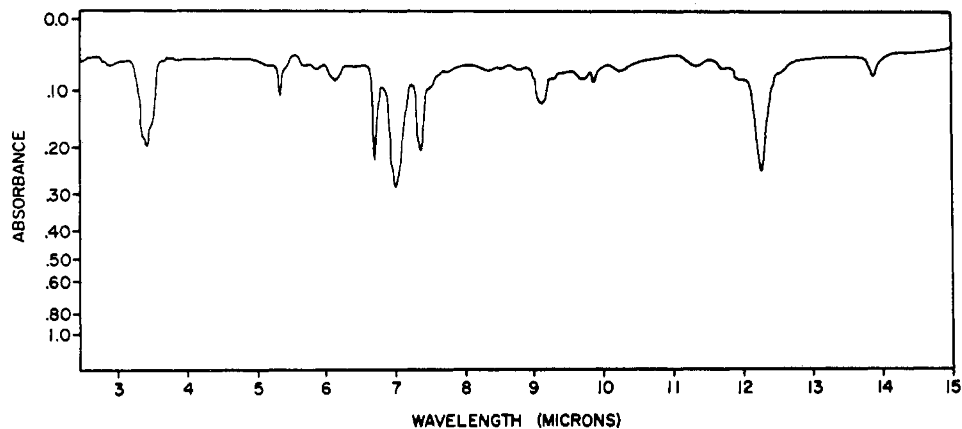

Figure 4. Infrared spectrum of the compound characterized as 2,6-dimethyl-10-(*p*-tolyl)undeca-2,6-diene (peak 60).

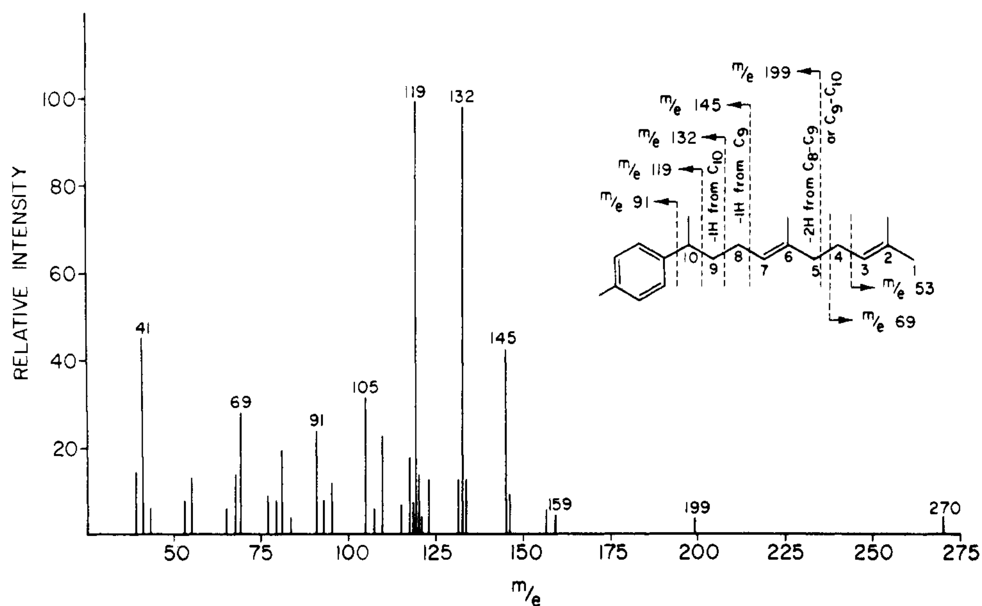

Figure 5. Mass spectrum of the compound characterized as 2,6-dimethyl-10-(*p*-tolyl)undeca-2,6-diene (peak 60).

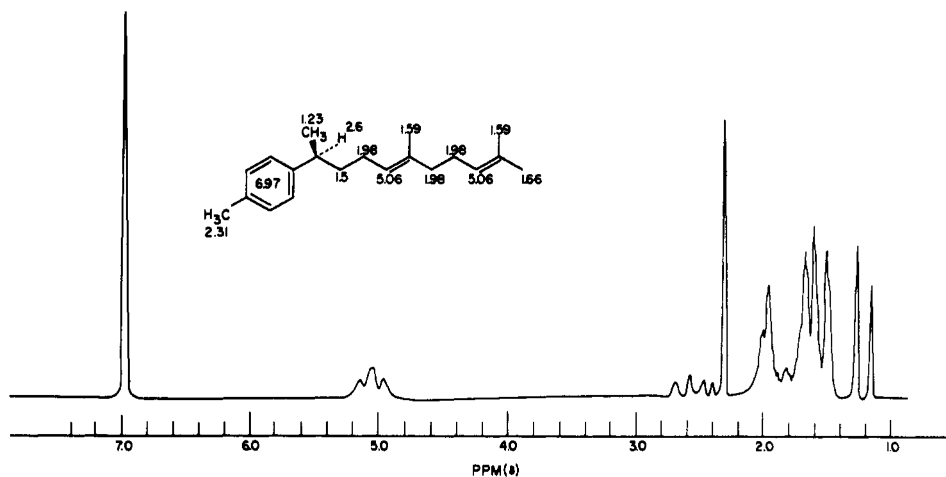

Figure 6. Nuclear magnetic resonance spectrum of the compound characterized as 2,6-dimethyl-10-(*p*-tolyl)undeca-2,6-diene (peak 60).

of the C_7 methyl group as illustrated.

In the monoterpene hydrocarbon fraction, limonene is the main compound while α- and β-pinenes are present in trace amounts. Other species of *Salvia* usually produce a significant amount of α-pinene, camphene, and β-pinene (Emboden and Lewis, 1967; Guenther, 1949; Lawrence et al., 1970, 1971).

The sesquiterpene hydrocarbon fraction was found to be too complex (21 components) to be resolved by GC alone. TLC using silica gel G impregnated with $AgNO_3$ was es-

sential for an efficient separation. Six bands designated 1–6 in the decreasing order of their R_f values were developed. Band 1 contained several components of which α-muurolene, δ-cadinene, and calamenene were identified (ir, T_r), while α-cubenene, copaene, and α-gurjunene were tentatively identified (T_r). α-*trans*-Bergamotene was identified in band 2, aromadendrene, γ-muurolene, and γ-cadiene in band 3, isocaryophyllene in band 4, caryophyllene in band 5, and α-humelene in band 6.

The mid-fraction (B) contained several esters (ir) of which *n*-pentyl *n*-butyrate, myrtenyl acetate, methyl perillate, perillyl acetate, and perillyl butyrate were identified. In addition, the last peak to exit from the GC in this fraction was the diterpene hydrocarbon (peak 60) characterized above. This compound was polar enough so that it was clearly separated from the hydrocarbon fraction A.

The rest of the oxygenated compounds (Table II) were identified in the polar fraction C. The residue remaining in the pot after fractional distillation was found to be rich in oxygenated sesquiterpenoids. However, only nerolidol and α-cadinol could be positively identified in this mixture.

While the pleasant fruity odor of the oil in the authors' judgment is undoubtedly due to methyl perillate and perillyl acetate, its camphoraceous by-note is mainly due to myrtenyl acetate and to a lesser extent to cineole. On the other hand, camphor, cineole, thujone, and, to a lesser extent, borneol and its acetate ester are responsible for the important camphoraceous odor in Dalmation sage oil, its substituents, or adultrants (Guenther, 1949; Lawrence et al., 1970, 1971). As no standard sample of myrtenol or its ir spectrum was found in our acquisition, myrtenyl acetate (peak 33) was reduced with $LiAlH_4$ and the ir and T_r of the produced myrtenol were identical with those of peak 37.

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Cycloalkapyrazines in Coffee Aroma

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The volatile components of roasted coffee were separated into basic and neutral components and analyzed on a 185 m × 0.31 mm i.d. glass capillary column coupled to a mass spectrometer. Seventeen alkylated five- and six-membered alicyclic pyrazines are reported for the first time in roasted coffee. The identities of bicyclic pyrazines were confirmed by direct comparison of their mass spectral and gas chromatographic retention data with those of authentic samples and with spectra given in the literature. Possible precursors of the bicyclic pyrazine compounds are discussed. The principal mass spectrometric fragmentation pathways of cyclopentapyrazines and tetrahydroquinoxalines are demonstrated.

Although extensive work has been done on the volatile constituents of coffee aroma, not a single carrier compound which could be responsible for coffee flavor has yet been elucidated. In order to simulate the natural product effectively one must first have an accurate knowledge of its chemical composition. However, in recent years there has been a tremendous increase in the volume of research effort devoted to the separation and identification of organoleptically important constituents of roasted coffee.

Walter and Weidemann (1969), in their comprehensive review of coffee constituents, list a large number of compounds which have been isolated from coffee aroma. The majority of the heterocyclic substances identified were furans, pyrroles, thiophenes, pyrazines, and pyridines.

During the last few years, evidence has been accumulated that heterocyclic nitrogen-containing compounds contribute directly to coffee aroma. Therefore, we studied some relationships between the basic fraction of roasted coffee and coffee flavor and identified 86 heterocycles in the basic coffee fraction of steam volatiles: oxazoles, thiazoles, pyrroles, acetylpyridines, acetyl- and furylpyrazines, quinoxalines, indoles, and quinolines (Vitzthum and Werkhoff, 1974a,b).

Pyrazines are important flavor constituents of a variety of roasted foods (Maga and Sizer, 1973). A considerable number of pyrazines have also been found in roasted coffee (Bondarovich et al., 1967; Goldman et al., 1967; Stoffelsma et al., 1968; Friedel et al., 1971). In addition to the compounds described by these investigators we wish to report here on 17 new volatile bicyclic pyrazine components occurring in coffee aroma. At present, cycloalkapyrazines have been identified in roasted food products such as peanuts (Walradt et al., 1971), filberts (Kinlin et al., 1972), cooked beef (Mussinan et al., 1973), roasted green tea (Yamanishi et al., 1973), and roasted sesame seed (Manley et al., 1974).

EXPERIMENTAL SECTION

Preparation of Aroma Sample. The method of isola-

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