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ESSENTIAL OILS AND THEIR CONSTITUENTS

III. SOME NEW TRACE CONSTITUENTS IN THE ESSENTIAL OIL OF *SALVIA LAVANDULAEFOLIA*, VAHL.

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SUMMARY

The essential oil of *Salvia lavandulaefolia*, Vahl. was investigated by a combination of the usual techniques of fractional distillation, column chromatography, capillary and preparative gas chromatography. With the aid of a home-made pressure programmer, capillary chromatographic analysis was carried out more effectively. In the analysis, a number of hitherto unreported trace constituents were isolated and identified.

INTRODUCTION

The oil of *Salvia lavandulaefolia*, Vahl. (Spanish sage) is obtained from the distillation of a wild growing plant found in the Spanish provinces of Almeria, Granada, Jaën and Murcia, Granada being the main area producing the oil[1]. According to ARCTANDER[2], the annual production of the oil varies from 3 to 10 metric tons but accurate information is scanty as the oil is subjected to adulteration especially in conjunction with the oil of *Rosmarinus officinalis* L. (rosemary) and *Lavandula latifolia* Vill. (spike lavender).

During the last war, when the oil of Dalmatian sage (*Salvia officinalis*) became scarce, a number of companies in the food industry used the oil of Spanish sage together with other essential oils as a replacement for Dalmatian sage oil. There is still some usage (although very small) of Spanish sage oil in the food industry at this time.

The composition of the essential oil of *Salvia lavandulaefolia*, Vahl. has been the subject of a few investigations. Some of the compounds reported are as follows: α-thujene[6], α-pinene[3–6], tricyclene[5], camphene[6], β-pinene[4–6], myrcene[5,6], α-phellandrene[4–6], limonene[3–5], δ-3-carene[4], terpinolene[5,6], *p*-cymene[5,6], γ-terpinene[6], cineol[3–6], α-thujone[6], β-thujone[6], α-pinene epoxide[5], linalool[3–6], linalyl acetate[3–5], linalyl isobutyrate[3], camphor[3–6], isoborneol[4–6], isobornyl acetate[4,5], borneol[4–6], bornyl acetate[4–6], α-terpineol[4–6], α-terpinyl acetate[4–6], β-terpineol[5], carvotan acetone[5], carvone[4,6], caryophyllene[4–6], farnesene[5], bisabolene[6], humulene[5], viridiflorol[5] and geraniol[6].

It is the purpose of this paper to describe the detection and identification of a number of previously unreported trace constituents which we have found present in the oil of *Salvia lavandulaefolia*, Vahl.

EXPERIMENTAL

The Spanish sage oil used in this study was stated to be authentic and was obtained from G. Lueders, Toronto.

In the analysis, all of the compounds were characterized by carefully comparing their IR spectra with standard IR spectra which were obtained from reliable commercial sources or from previously published spectra.

Fractionation of the oil

Spanish sage oil (430 g) was fractionally distilled under vacuum through a twelve theoretical plate column. During the distillation, the pot temperature was maintained at 130–150° and the reflux ratio was held at 3:1 with an average throughput of 4 ml/min. Some problem was encountered with the camphor crystallizing in the condensor and trap, but this was alleviated by the careful application of external heat. The distillation was controlled by altering the vacuum from 11.5 to 0.1 cm of Hg and varying the pot temperature within the limits previously mentioned.

Twenty-six fractions were collected, their amount being composition dependent. The residue (45.8 g) was further distilled through a Podbielniak Spinning Band column and fractions 27–30 were collected. The final residue (29.7 g) was by this time a dark, viscous, polymeric mass.

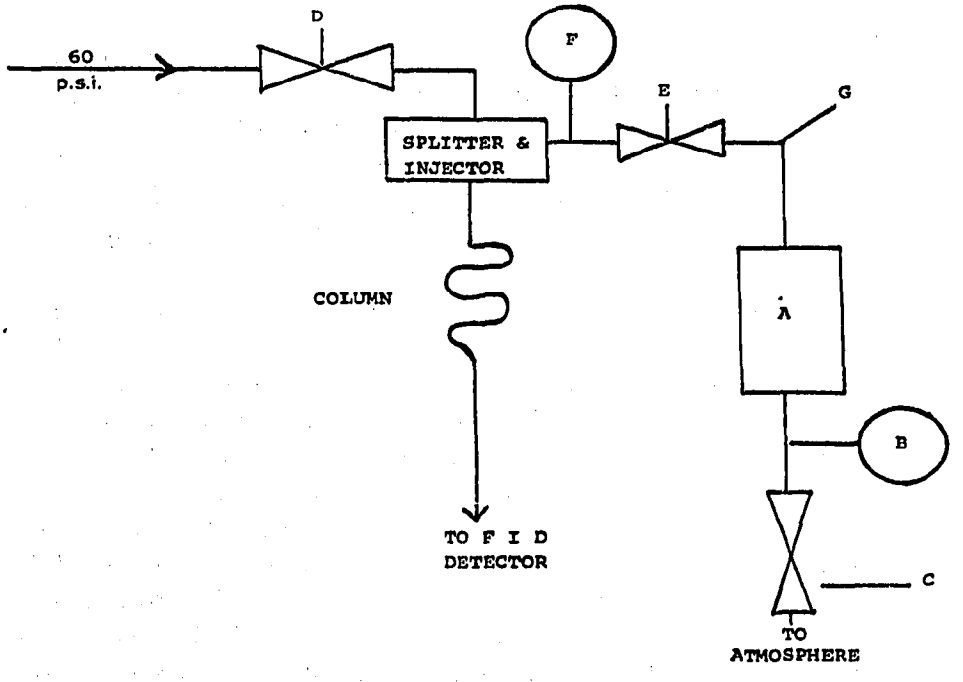

Fig. 1. A diagrammatic representation of the pressure programmer. A = Reservoir; B = pressure gauge; C = exhaust valve; D = carrier gas control valve; E = split control valve; F = pressure gauge; G = "normal" split outlet.

Chromatography

Because of the complexity of the distillation fractions, certain of them were subjected to alumina and silver nitrate alumina column chromatography[7]. The subfractions from each column chromatographic separation were examined gas chromatographically on Carbowax 20M capillary columns so that the amount collected in each fraction was composition dependent. As time was a limiting factor in monitoring each column chromatographic separation, a pressure programmer was designed so that the GLC runs could be carried out in less time. The pressure programming system used is shown diagrammatically in Fig. 1.

Pressure programmer

A Varian-Aerograph Model 1200 gas chromatograph which has been adapted for capillary work was used in this study. The inlet carrier gas was regulated by the

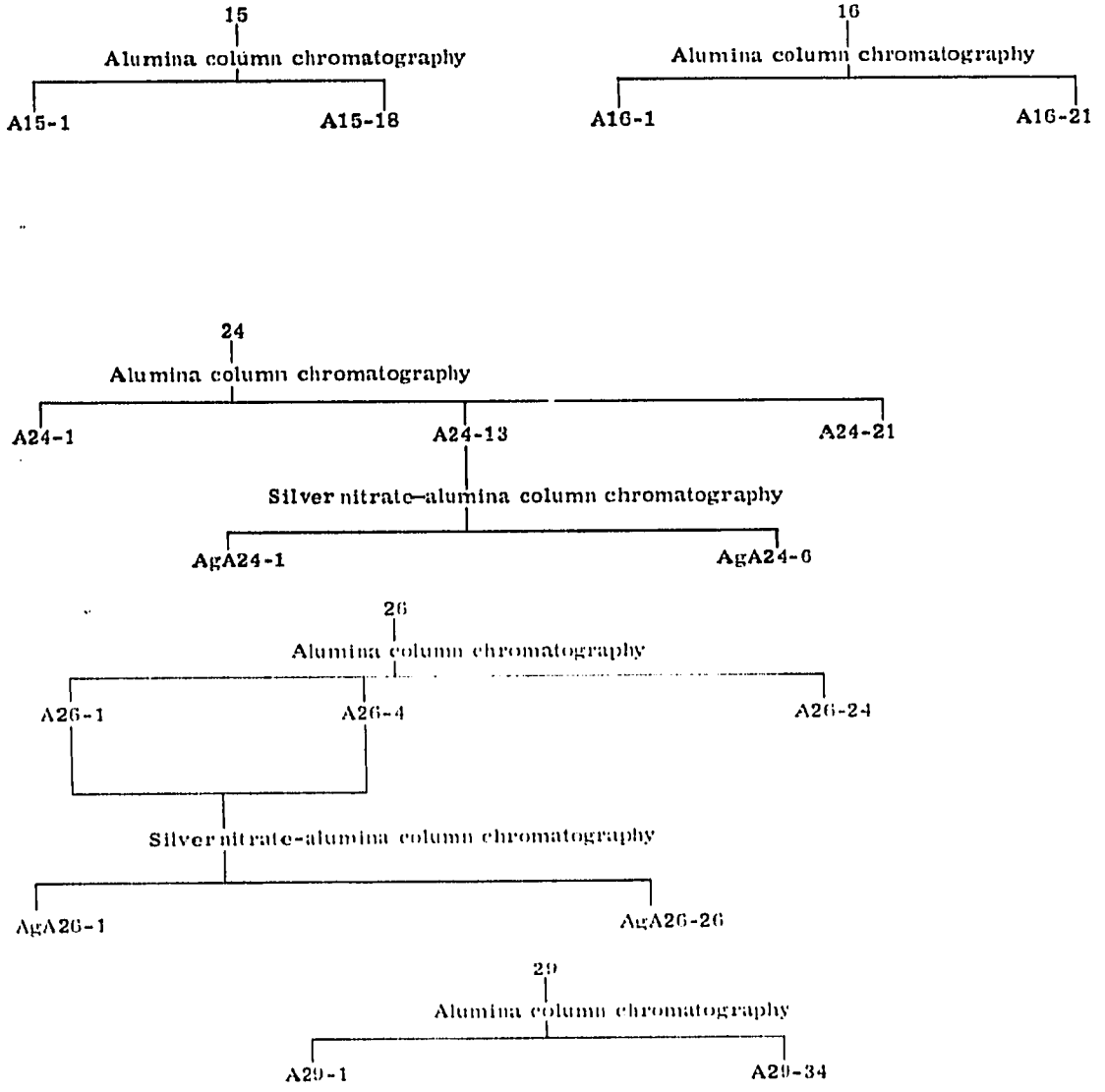

Fig. 2. Flow charts for subfractionation of distillation fractions on both alumina and silver nitrate–alumina columns.

needle valve D on the chromatograph and the flow of gas through the column was adjusted by the inlet splitter. The reservoir (A) (13 ft. × ¾ in. O.D. copper tube) equipped with a pressure gauge (B) and an exhaust valve (C) was connected to the split gas exhaust port (G) which is normally vented to the atmosphere. Initially, a column flow of 1.5 ml/min and a split flow of 90 ml/min was set. Upon injection of sample into the gas chromatograph, the exhaust valve (C) was closed. This caused an increase in reservoir pressure, which could be followed on the pressure gauge (B), and in turn caused the column inlet pressure to increase with time. At the end of a chromatographic run the exhaust valve (C) was opened and the reservoir was allowed to reach atmospheric pressure.

RESULTS

In the following compilation of analytical results, the figures in parentheses given after each compound designate the fraction or subfraction origin of that compound which can be followed on the flow charts (see Fig. 2).

TABLE I

COMPOSITION OF THE VOLATILE OIL OF *Salvia lavandulaefolia*, VAHL.

Peak No.	*Compound*	*Percentage composition*
1	α-Thujene	0.2
2	α-Pinene	8.6
3	Camphene	7.0
4	β-Pinene	4.5
5	Sabinene	1.3
6	Myrcene	3.2
7	Limonene	4.5
8	1,8-Cineöl and *cis*-ocimene	23.2
9	*trans*-Ocimene	0.4
10	γ-Terpinene	tr
11	*p*-Cymene	1.9
12	Terpinolene	tr
13	*cis-allo*-Ocimene	0.1
15	α-*p*-Dimethylstyrene	0.3
16	*trans*-Sabinene hydrate	0.5
17	α-Cubebene	tr
18	Copaene	tr
19	Camphor	20.1
20	Linalool and α-gurjunene	3.6
21	Linalyl acetate and *cis*-α-bergamotene	
22	Bornyl acetate and *trans*-α-bergamotene	1.6
23	Terpinen-4-ol	1.0
24	Caryophyllene and aromadendrene	
25	δ-Terpineol and *allo*-aromadendrene	0.1
26	Sabinyl acetate	3.6
27	Sabinol, α-humulene and borneol	0.3
28	α-Terpineol	tr
29	α-Terpinyl acetate	11.2
32	δ-Cadinene	0.4
33	Aromatic curcumene	0.4
34	Nerol	0.1
35	Geraniol	0.4
Unidentified constituents		1.1

Monoterpene hydrocarbons (12)

α-Thujene (1), α-pinene (1), camphene (1), β-pinene (1), sabinene (1), myrcene (1), limonene (A15-3), γ-terpinene (A15-3), terpinolene (A15-3), *cis*-ocimene (A15-13), *trans*-ocimene (A15-13) and *cis-allo*-ocimene (A15-14).

Aromatic hydrocarbons (2)

p-Cymene (A15-13) and α-*p*-dimethylstyrene (A15-16).

Oxygenated terpenes (12)

1,8-Cineol (1), linalool (A15-18), *cis*-sabinene hydrate (A16-21), linalyl acetate (A24-11), bornyl acetate (A24-11), α-terpinyl acetate (A24-11), camphor (A24-16), terpinen-4-ol (A24-20), δ-terpineol (A24-20), geraniol (A24-21), nerol (A24-21) and α-terpineol (AgA24-5).

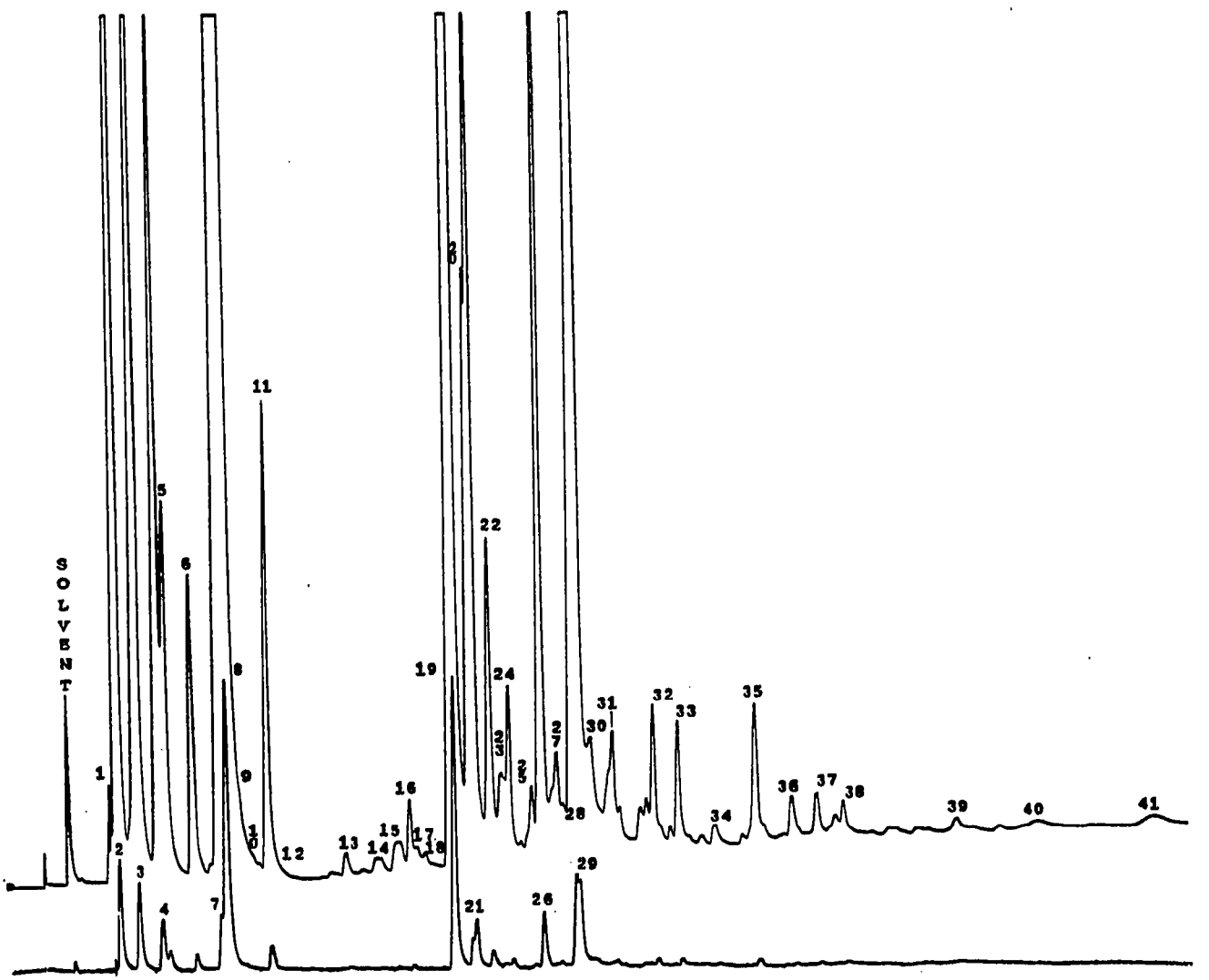

Fig. 3. A temperature and pressure programmed capillary GLC analysis of the volatile oil of *Salvia lavandulaefolia*, Vahl. using a 100 ft. × 0.01 in. I.D. stainless steel capillary column coated with F.F.A.P.

Sesquiterpene hydrocarbons (11)

α-Cubebene (A24-2), copaene (A24-2), α-gurjunene (A24-2), caryophyllene (A24-5), α-humulene (A24-5), *cis*-α-bergamotene (AgA26-18), *trans*-α-bergamotene (AgA26-18), aromadendrene (AgA26-20), *allo*-aromadendrene (AgA26-20), δ-cadinene (A29-2) and aromatic curcumene (A29-15).

A list of the above compounds along with their area percentages calculated from disc integration measurements, obtained using a flame ionization detector, is given in Table I. Also, the position of these same compounds on a chromatogram of the original oil can be seen in Fig. 3.

DISCUSSION

With the aid of the previously described home-made pressure programmer, which required no calibration once optimum conditions were established, the analysis was undertaken more effectively. Occasionally, the use of a dual-channel recorder was used for the same capillary GLC run. This procedure enabled both the major and minor peaks which were present in the oil to be monitored without having to continually attenuate (an example of this can be seen in Fig. 3). This technique was found to be invaluable when the isolated compounds were positioned on a chromatogram of the original oil. The conditions used for both preparative and capillary GLC are given in Table II. In the analysis reported, 22 of the 35 previously reported constituents were identified in addition to a further 18 constituents previously unreported.

TABLE II

CONDITIONS FOR GAS CHROMATOGRAPHY

Information	*Capillary*	*Preparative*
Instrument	Varian-Aerograph Model 1200	Varian-Aerograph Model 700
Columns	100 ft. × 0.01 in. stainless steel coil	20 ft. × 1/4 in. aluminum coil
Stationary phases	(1) SF-96 (2) Carbowax 20M (3) F.F.A.P.	(1) Carbowax 20M (2) Apiezon L
Flow rate	1–3.0 ml N_2/min	60 ml H_2/min
Split ratio	100:1	—
Pressure program	16 → 60 p.s.i.	—
Column temperature	70–180°	80–200°
Temperature program rate	mostly 4°/min	ballistically
Injector temperature	220°	250°
Detector temperature	220°	250°
Detector	Flame	Thermal conductivity
Recorder	Mosley	Leeds & Northrup
Chart speed	0.25 in./min	6 in./h
Sample size	0.2 μl	1–100 μl solutions

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