A GAS CHROMATOGRAPHIC STUDY OF OILS FROM SOME *AGATHOSMA* SPECIES (family RUTACEAE)

Thesis submitted to Rhodes University
in Partial Fulfilment of the Requirements for
the Degree of Master of Science.

by

PETER HENRY ROBERT PERSICANER

Department of Pharmaceutical Sciences,
Rhodes University,
Grahamstown.
Republic of South Africa.

December 1971.

ACKNOWLEDGEMENTS

The major part of this investigation was carried out in the Department of Zoology and Entomology, Rhodes University, Grahamstown. The author wishes to express his appreciation to Professor B.R. Allanson, for making the necessary facilities available.

Particular gratitude is expressed to:

Dr. V.C. Moran, for his guidance and assistance at all times throughout this study.

Dr. D.E.A. Rivett, for his supervision and encouragement at all times.

Professor C.H. Price, Dean of the Faculty of Science and Head of the Department of Pharmaceutical Sciences, for his co-operation.

Mr. J. Lee and Mr. K.L. Pringle for technical assistance.

Mrs. T. Walker for typing the final draft.

The following companies for making chemicals available for this study.

Fritzsche-D & O

Schuchardt-München

Dragoco Chemicals

Naarden Chemicals

Maschmeijer Aromatics

Fluka A.G.

Hopkin and Williams

CONTENTS

PART I

	Pages
Introduction	1 - 3
1. A review of the chemical composition of essential oils of plant members of the genus *Agathosma*.	4 - 26
2. Discussion of Results.	27 - 44
3. Experimental.	45 - 53
Compounds employed in the qualitative GLC investigation.	54 - 57
Bibliography.	58 - 61

PART II

Tables and Chromatograms.	62 - 105

PART I

INTRODUCTION

INTRODUCTION

Buchu leaf is a very widely used household medicine in South Africa, and is usually administered in the form of a brandy tincture or a vinegar, known as "buchu brandy" and "buchu vinegar" respectively. These preparations have a great reputation in curing diseases of the kidney and urinary tract, and in addition are employed as local applications to bruises, and for the relief of rheumatic pains. We owe its introduction into medicine to the Hottentot, who gave the name "buchu" or "bookoo" to any aromatic herb or shrub which they found suitable for use as a dusting powder.[1]

The buchu of commerce is derived from the following species:

(a) Barosma betulina Bartl. and Wendl.
(otherwise known as short buchu)

(b) Barosma crenulata Hook.
(otherwise known as oval buchu)

(c) Agathosma crenulata Pillans
(known as long buchu)[1]

Botanical taxonomists have suggested however, that Agathosma and Barosma (both belonging to the family Rutaceae) should be united into one genus. The chief characters which are used in separating these genera, are in the arrangement of the leaves and the position of the flowers. However, these characters are not always constantly associated.

For instance, the arrangement of the leaves in a species may have all the possibilities existing in both genera, with the result that some species could not be satisfactorily placed in either genus. Accordingly, the two genera have been united, preference being given to Agathosma, (which is much the larger genus) so as to minimise changes in nomenclature.[2] This nomenclature will be used throughout this thesis.

The genus Agathosma is endemic in South Africa, and is confined to areas of the Cape Flora in the Cape Province, Natal and Basutoland (now known as Lesotho). The greatest concentration of species occurs in the south-western part of the Cape Province, where the distribution extends from the coast to the tops of the highest mountains. Very few extend eastward into the region of summer rainfall. Many species are restricted to certain soils, altitudes, aspects and conditions of dryness and moisture.[3]

One of the main characters of this genus, is the fact that many species secrete an essential oil, which is usually concentrated in special glands in the leaf. In many cases these oil glands can be readily observed with the naked eye, and usually occur on the undersurface of the leaf. In many cases, the oil is characterised by a pungent odour, which can be detected from some distance away, and

which leads the collector to its habitat.

In this thesis, the main interest concerns a chemical investigation of the essential oils extracted from different species of Agathosma.

CHAPTER 1

A review of the chemical composition of essential oils of plant members of the genus *Agathosma*.

CHAPTER 1

A REVIEW OF THE CHEMICAL COMPOSITION OF ESSENTIAL OILS OF PLANT MEMBERS OF THE GENUS AGATHOSMA (FAMILY RUTACEAE).

Although "buchu leaves" play an important part in folk medicine, little interest has been shown in the genus from a chemical point of view, the only exception being Agathosma betulina (Thunb.) Pillans, which is exported in great quantities to both the United States and United Kingdom.

The recent analysis by Klein and Rojahn using gas-liquid chromatography,[4] although far more comprehensive than earlier reports regarding the chemistry of the oil, lacks details of positive proof of the identification of the constituents.

J.L.B. Smith, at this university, pioneered the chemical investigation into the constituents of Agathosma oils. His discovery of sulphur-containing compounds in A. apiculata[5] and A. puberula[6] is possibly the most interesting observation from a chemo-taxonomic point of view, and it was proposed that such plants be regrouped into a new genus known as Thiosma.[6]

Relatively few of the approximately 130 species of Agathosma have been investigated, and these will now be reviewed. It is clear that the identity of

many constituents of these oils rests on very flimsy evidence and most, if not all, should be re-investigated using modern techniques (such as gas-liquid chromatography), which is bound to reveal the presence of many unsuspected components.

(1) Agathosma apiculata Meyer[1,5]

This species is commonly referred to as "sea buchu", and is found along the coastal belt of the south-eastern Cape.

The fresh leaf was found to yield 0.32% of oil, which is noteworthy for its high sulphur content (up to 13%), this being no doubt responsible for its nauseous and pungent odour. The composition of the oil was not found to be constant, since the leaves collected in the spring yielded a greater proportion of volatile components than the leaves collected in autumn.

Qualitative tests on the oil indicated the presence of sulphur, but the absence of phenols, aromatic acids, aldehydes and ketones.

The oil was subjected to fractional distillation under reduced pressure, four fractions being obtained in addition to a gummy residue.

Fraction 1 was shown to consist mainly of β-pinene, by carefully controlled oxidation with

alkaline permanganate to nopinic acid, and also by conversion to the crystalline terpin-hydrate on shaking with dilute sulphuric acid.

Sulphur-containing compound(s) were concentrated in Fraction 3A. These compound(s) were suspected of being disulphides, which can be fissioned to mercaptides in the following way:

$$R\text{-}S\text{-}S\text{-}R' \xrightarrow[\text{sodium}]{\text{metallic}} RSNa + R'SNa$$

Mercaptides can then be converted back tc the disulphides with iodine as follows:

$$RSNa \xrightarrow{\text{iodine}} R\text{-}S\text{-}S\text{-}R + NaI$$

Accordingly, fraction 3A (dissolved in dry ether) was reacted with metallic sodium. The insoluble product was washed repeatedly with dry ether and then treated with iodine. A small amount of a dark oil, analysing for $C_9H_{18}S_2$, was obtained. From the method of formation it must clearly be a mixture of R-S-S-R, R'-S-S-R' and R-S-S-R', where $R' + R = C_9H_{18}$.

Oxidation of this dark oil gave a mixture of n-butyric, oxalic and sulphuric acids, as well as carbon dioxide. The compound was thus considered to be

butyl-1-pentenyl disulphide

$$CH_3.CH_2.CH_2.CH_2.S.S.CH = CH.CH_2.CH_2.CH_3$$

Since $C_9H_{18}S_2$ contains 33.7% sulphur, and Fraction 3A had only 17.1% sulphur, the disulphide must be accompanied by other compound(s). Analysis of this fraction (Carbon 64%; Hydrogen 9.5%; Oxygen 9.4% and sulphur 17.1%) suggested that it could be a mixture of 51% $C_9H_{18}S_2$ and 49% $C_{10}H_{16}O_2$.

On the basis of the foregoing, the composition of the essential oil of A. apiculata was summed up as follows:

(a) About 25% of β-pinene.

(b) About 8% of a terpene of boiling point 171°-3°C (at 730mm.).

(c) About 30% of a compound $C_{10}H_{16}O_2$.

(d) About 30% of butyl-1-pentenyl disulphide.

(e) About 7% of resinous matter.

(2) Agathosma betulina (Thunb.) Pillans

This species yields the "buchu" of commerce, and used to be exported in large quantities to both the United Kingdom and to the United States. Clevenger described the analysis of such shipments of buchu to New York in the 1930's, quoting such physical and chemical characteristics of the oil as the specific gravity, the optical rotation, the refractive index, the acid number and the ester number, however making no mention of the chemical composition of the oil.[7]

The oil was first examined chemically by

Kondakov, who recognised that the main constituent of the oil was diosphenol ($C_{10}H_{16}O_2$), also known as "barosma camphor" or "buchu camphor". According to Kondakov, diosphenol occurred in the oil partly free, and partly esterified with an acid.[8]

According to van der Riet, the diosphenol is best purified by freezing the oil and recrystallising from acetone. The colourless crystals obtained had a melting point of 82° - 84°C, and in general the findings of van der Riet agreed very well with those of Kondakov.[9]

Diosphenol was found to be present in the oil to the extent of 15-30%. Later reports regarding the presence of diosphenol in the oil by Klein and Rojahn, indicate in fact that it is doubtful whether diosphenol itself can be regarded as a genuine constituent of the leaf oil, since piperitone epoxide (a constituent of the oil) can be rearranged into diosphenol under acid conditions, which were thought to be fulfilled in the steam distillation of the plant material.[4]

Kondakov also detected the presence of the following compounds in the oil:[8]

(a) d-limonene, identified as the tetrabromide. (melting point 104°).

(b) Dipentene, present in small quantities and characterised as the tetrabromide. (Melting point 119°).

(c) l-menthone, characterised by the preparation of a hydrazone (melting point 80°), and of two semicarbazones (having melting points of 123° and 180°).

In the most recent paper dealing with the chemical composition of the oil, the constituents were separated using gas-liquid chromatography.[4] Unfortunately the authors do not go into any detail regarding their means of identification, stating merely that the compounds were isolated by a combination of fractionated distillation and preparative gas chromatography, and identified both spectroscopically and by gas chromatographic comparison with authentic standards. No retention data, nor the nature of the columns used, nor the method whereby the percentages of the individual compounds were determined, are given.

The oil was shown to contain the following constituents:[4]

water	1.5%
α-pinene	0.5%
camphene	trace
β-pinene	trace
myrcene	1.0%
α-terpinene	trace
(+)-limonene	10.0%
p-cymene	0.5%
γ-terpinene	trace

(+) menthone	9.0%
(-) isomenthone	35.0%
(+) isopulegone	3.0%
(-) iso-isopulegone	3.0%
terpinenol-4	0.5%
(-) pulegone	11.0%
(±) piperitone epoxide	9.5%
diosphenol	12.0%

As opposed to Kondakov, the authors did not find l-menthone in the oil.

(3) Agathosma cerefolium Bartl. and Wendl.[10] (formerly known as Agathosma microphylla G.F.W. Mey.[2])

A. cerefolium or "Stembuck buchu" is a short, stunted shrub which grows in patches on the seaward side of the coastal hills, being found fairly extensively near Knysna and in the neighbouring districts. The shrub gives off a strong aniseed-like odour, which is very noticeable in the valleys where it flourishes.

The dried leaves have a large oil content which varies with the season, the leaves collected in summer yielding 2-3% of volatile oil, whereas the samples collected in winter contain as much as 5%.

The oil was shown to contain a small amount of eugenol by extraction with alkali.

Oxidation of the whole oil with permanganate gave a large yield of anisic (p-methoxy-benzoic) acid. Assuming that the methoxyl content (by Zeisel estimation) of the oil is due entirely to methylchavicol (p-$CH_3O.C_6H_4CH_2.CH = CH_2$), then this compound constitutes approximately 50% of the oil.

The oil was divided by fractional distillation at atmospheric pressure into eight fractions and a gummy residue.

Fraction 3 was found to contain approximately 20% of methylchavicol, the remainder consisting of alcohols (partly l-linalool). The latter were detected as follows: Titration with bromine in carbon tetrachloride indicated a molecular weight of about 150, with two ethylenic linkages in the molecule. Combustion analysis indicated a mixture of 20% methylchavicol ($C_{10}H_{12}O$) and 80% $C_{10}H_{18}O$. The odour of fraction 3, together with its physical constants suggested that l-linalool was present. On oxidation with chromic acid, fraction 3 yielded a pungent oil, the chief fraction of which appeared to be citral. Accordingly it was considered that fractions 3 and 4 (together comprising about 40% of the whole oil) contained at least 75% of alcohols of formula $C_{10}H_{18}O$, consisting in part of l-linalool.

Fraction 1 had a powerful odour resembling that of myrcene, but owing to the small quantity obtained

it was found impracticable to isolate pure myrcene.

The oil also possibly contains some anethole, since in the higher boiling fractions the odour of anisic aldehyde appeared immediately when the fractions were treated with permanganate solutions in the cold. However, due to the small amount of material available, the presence of anethole could not be definitely established.

The essential oil of Agathosma cerefolium was thus thought to be comprised of:

Terpene hydrocarbon: (probably myrcene)	3%
Alcohols: $C_{10}H_{18}O$, in part probably l-linalool	30%
Phenols: eugenol	1%
Phenol ethers: methylchavicol and probably anethole	50%
Esters: possibly $C_{10}H_{17}OCOCH_3$	4%
Residue, sesquiterpenes, loss etc.	12%

(4) Agathosma ciliata Link.[1]

The only reference to this plant in the literature states that it yields an almost colourless volatile oil, the fresh leaf yielding 0.4% and the air dried plant 1.2%. No further chemical investigation has been carried out on the plant.

(5) Agathosma clavisepela R.A. Dyer[1] is said to result in the tainting of milk and butter after being eaten by cows.

(6) Agathosma crenulata Pillans[1]

The leaf of this species rarely yields more than 1% of volatile oil, which unlike the oil from Agathosma betulina, contains only a trace of diosphenol, or none at all. The leaf however, contains diosmin, and the flower and fruit a resin and a volatile oil.

There appears to be a discrepancy in the literature regarding Barosma crenulata Hook. (now known as Agathosma crenulata Pillans). B. crenulata is referred to as "oval buchu", whereas A. crenulata is referred to as "long buchu".[1,2] However, according to present day taxonomists these plants are identical.

The leaf of Barosma crenulata was said to yield 1.32 - 2.19% of volatile oil, together with barosmin (or diosmin) which is a solid glucoside.

(7) Agathosma foetidissima Hortul. ex Steud.[1] (formerly known as Barosma foetidissima Bartl. and Wendl.[2])

This shrub has a foetid odour, and has been tested for the presence of antibacterial substances with negative results. No mention is made regarding the chemical composition of the leaf oil.

(8) Agathosma ovata (Thunb.) Pillans[1] (formerly

known as Barosma scoparia B. and Z.)[2]

The leaf of this species has been marketed under the name of "buchu", and apparently resembles Agathosma venusta. The leaves yield a volatile oil which was found to contain no diosphenol.

(9) Agathosma puberula (Steud.) Fourc.[1,6] (formerly known as Agathosma gnidioides Schltr.)[2]

This is a much branched woody shrub of 2 - 3 feet average height, occurring only in the south-eastern Cape Province. It was found that the proportions of the components of the volatile oil varied with the season, the fresh leaves yielding more oil in the winter than in the summer.

The oil of A. puberula contains sulphur, an average of 2.4% being present. Analysis also indicated a low proportion of oxygen in the oil, which is unique in containing up to 70% of terpenes. Qualitative tests showed that phenols, aldehydes and ketones were absent.

The oil was subjected to repeated fractional distillation under reduced pressure in a current of carbon dioxide. Seven fractions, as well as a gummy residue were obtained in this manner, fraction 1 being by far the largest, constituting 60% of the original oil.

Fraction 1 was shown by conversion with maleic

anhydride into crystalline 4-isohexenyl-*cis*- Δ^4-tetrahydrophthalic acid to be rich in myrcene. (Consideration of various data suggested that myrcene constituted about 60% of the crude oil). The optical activity and density of this fraction indicated the presence of other terpenes. Repeated refluxing of this fraction (in order to preferentially polymerise the myrcene present) yielded a volatile portion, which when heated with an excess of maleic anhydride and distilled, gave rise to four fractions (T_1 - T_4). Each of these was shown to contain *l*-β-pinene, since on cold alkaline permanganate oxidation, nopinic acid was isolated in quantity. Fraction T_4 had an odour reminiscent of limonene, and it was thought that dipentene was present as well. β-Pinene was estimated at about 5% of the original oil, and the proportion of limonene and dipentene together was thought not to exceed 4%.

Fraction 6 was shown as follows to contain *d*-linalyl isobutyrate:

A sample was refluxed with alcoholic potassium hydroxide, and the product subjected to steam distillation. The distillate was separated, and the residue in the saponification flask was treated with mercuric chloride to remove mercaptides, the separated solids being removed by filtration. The clear filtrate was evaporated to dryness, and the

solid residue acidified, whereupon an oily acidic product separated. This was extracted with ether, evaporation of which left a dark liquid with the odour of isobutyric acid. The oil was thus considered to contain an isobutyric ester, which is probably d-linalyl isobutyrate since d-linalool was also present in the fraction.

Fraction 6 was shown as follows to contain a tetrasulphide: After a sample of the fraction had been reacted with metallic sodium and refluxed, water was added dropwise with the resultant formation of a yellow precipitate, which was separated, dissolved in ice-water, and extracted with ether. The aqueous solution was filtered and treated with an excess of iodine solution. The resulting oily product was extracted with ether, on evaporation of which there remained a light brown oil having carbon 45.1%; hydrogen 6.9% and sulphur 47.8%, which was considered to be a tetrasulphide $C_{10}H_{18}S_4$, and not a disulphide $C_5H_9S_2$, because of its involatility (the boiling point being greater than 180° at 3mm. pressure). On very flimsy evidence a bis-(1-pentenyl-2) tetrasulphide structure was advanced for this compound.

$$(CH_3CH_2CH = CHCH_2)_2S_4$$

bis-(1-pentenyl-2) tetrasulphide

The essential oil of Agathosma puberula was thus proposed to have the following composition:

(a) myrcene (about 60%)

(b) l-β-Pinene (about 5%)

(c) l-limonene and dipentene (together about 4%)

(d) d-linalyl isobutyrate (about 25%)

(e) bis-(1-pentenyl-2) tetrasulphide (about 5%)

(f) isobutyric acid (a trace)

(g) traces of d-linalool, salicylic acid and methyl salicylate (not proved).

10. Agathosma pulchella Link.[1] (formerly known as Barosma pulchella Bartl. and Wendl.[2])

Schimmel and Co. found that the leaves of this species yielded 3% of a golden yellow oil with a decidedly unpleasant odour. The unpleasant smelling constituent was found by these workers to be a pungent narcotic base, having a boiling point of 130° - 140°/ 5mm. In addition to the latter, the oil was also said to contain a high proportion of citronellal, as well as methylheptanone.

11. Agathosma venusta (E. & Z.) Pillans[1] (formerly known as Barosma venusta E. & Z.[2]).

The leaf of this species was found to yield between 1.1 - 4.12% of a greenish-yellow volatile oil containing no diosphenol, but up to 43% of myrcene, 16% of chavicol, 15% of myrcenol and sesquiterpene alcohols, and 15% of methylchavicol and anethole. An alcoholic extract of the leaf was found to fluoresce in ultra-violet light.

There are some shrubs which, although not members of the genus Agathosma, are nevertheless referred to as "buchu". Examples of these are Pteronia onobromoides DC, Pteronia stricta Ait., Othonna auriculifolia Licht. ex Less., Empleurum serrulatum Ait. (commonly referred to as "berg buchu" or "false buchu"), Psoralea bracteata L., Psoralea obliqua E. Mey., Coleonema album B. & W. and Coleonema pulchrum Hook.

(12) Othonna auriculifolia Licht. ex Less. (family Compositae).[11]

This plant was found to be poisonous to stock, but is used by the Griquas as a cosmetic.

(13) Pteronia onobromoides DC (family Compositae).[11]

This shrub is much appreciated by the local Hottentot as a medicine and as a cosmetic. (It is used by them as a "buchu"). The oil is said to have a powerful odour.

(14) Pteronia stricta Ait. (family Compositae).[11,12]

The air dried leaves when steam distilled, afforded 1.8% of an essential oil of a deep yellow colour possessing a powerful "eucalyptus" odour.

Repeated fractionation of the oil yielded six fractions.

Fraction 1 was shown to contain sylvestrene,

since the characteristic blue colouration was produced with acetic anhydride and sulphuric acid. Cineole (eucalyptole) was also found to be present in this fraction.

When indole was added to a hot alcoholic solution of fraction 2, a yellow precipitate formed. This was washed and recrystallised from hot alcohol, whereupon the cineole - indole compound was obtained. By means of the phosphoric acid method of quantitative analysis, the original oil was found to contain 50% of cineole. The remainder of fraction 2 was thought to be mainly phellandrene, but attempts to establish this failed.

Fraction 3 was found to contain a large proportion of cineole, the remainder consisting of alcohols.

The original oil was found to contain 5% of esters (calculated as linalyl acetate) and 6% of alcohols (calculated as $C_{10}H_{17}OH$). It also contained 1% of phenols and acids extractable with alkali, and 1% of methoxyl groups, but the presence of anethole or of estragonole could not be definitely established.

The air dried leaves were found to contain 5.35% of ash, in which a considerable amount of manganese was found.

The composition of the oil was thus found to be as follows:[12]

Sylvestrene	2%
Phellandrene	17%
Cineole	50%
Phenols and acids	1%
Phenol ethers (estragonole)	5%
Esters	5%
Alcohols	6%
Residue, loss etc.	14%

(15) Empleurum serrulatum Ait.[13,14]

This species is found in the wooded coastal hills of the George-Knysna area, where it is known as "olifants" or "berg-buchu".

The air-dried leaves on steam distillation gave 1.15% of a yellow oil possessing a pungent clinging odour.

Repeated fractionation of the oil yielded seven fractions as well as a residue.

The odour of Fraction 1 indicated myrcene, and other terpene hydrocarbons were thought to be present in fraction 2, but this was not investigated further. Fraction 2 was also shown to contain cineole, since on the addition of indole to a hot alcoholic sample of the fraction, a precipitate of cineole-indole resulted.

The oil was found to contain 5.8% of esters

(calculated as the acetate of $C_{10}H_{17}OH$). The volatile portion of the acids obtained by saponification were considered to consist chiefly of acetic and n-butyric acids.

The oil contained 8.1% of alcohols (calculated as $C_{10}H_{17}OH$). The isolation of alcohols from the oil proved extremely difficult, but nevertheless a small amount of clear oil possessing the odour of nerol was obtained - although lack of material foiled complete identification.

Phenols and acids in the oil were extracted with alkali. Saturation of the aqueous extract with carbon dioxide and then with mineral acid liberated first the phenols (considered from their odour to be chiefly eugenol and guaiacol), and then the acids. The latter contained acetic and butyric acids, detectable by smell, and crystalline material considered to be a mixture of benzoic and salicylic acids.

The main constituent of the oil (which contains 26.3% methoxyl groups) is methyleugenol, which on oxidation with permanganate gave veratric acid in large yield.

The air-dried leaves were found to contain 4.4% of ash in which a considerable amount of manganese was present.

The composition of this oil was thus found to be as follows:[14]

Terpene Hydrocarbons:	(Myrcene)	1.2%
	(Other)	2.8%
Esters: as the acetate of $C_{10}H_{17}OH$		5.8%
Alcohols: Nerol		8.1%
Phenols: Eugenol: Guaiacol		0.4%
Phenol ethers: methyleugenol		70.0%
Acids: acetic, n-butyric, benzoic and salicylic		0.6%
Cineole		2.0%
Residue, loss etc.		9.1%

Although many species belonging to the genus Diosma have been reclassified as belonging to the genus Agathosma, many still remain classified as Diosmas. These include Diosma oppositifolia L. and Diosma vulgaris Schltr.

(16) Diosma oppositifolia L.[13]

This species has been marketed under the name of buchu, and researchers have isolated from the leaves, a small amount of a semi-solid volatile oil with a peppermint odour.

(17) Diosma vulgaris Schltr.[13,15]

This plant is commonly known as "bok buchu" and yields between 0.57% and 0.87% of a yellowish volatile oil.

Aldehydes and ketones (determined by Burgess method) were found to comprise 4.8% of the oil, sulphur 0.05% and methoxy groups 1.3%.

The chief constituents of the oil, determined by evaluation of physical data and by distillation under reduced pressure, were:

(1) A mixture of limonene and dipentene - 46%

(2) Alcohols (calculated as $C_{10}H_{18}O$) about 10.5%

(3) Aldehydes and ketones, about 5%.

(4) Esters (calculated as $C_{10}H_{17}OCOCH_3$), about 4%.

The remaining 35% was presumed to consist of sesquiterpenes, ethers, a trace of a phenolic compound, and a small proportion of sulphur compounds.[15]

N.B. According to the Index Kewensis (1893) and Flora Capensis, the two plants listed above should be in the same taxon, and since D. oppositifolia is the older name, this should be valid.

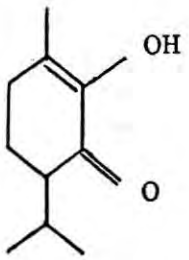
Hence D. oppositifolia L. (= D. vulgaris Schltr. and vars).

Buchu Leaf Oil:

Lamparsky and Schudel have recently reported the isolation and structure determination of two sulphur-containing monoterpenoids from commercial Buchu Leaf Oil (apparently obtained from one, or a mixture of Agathosma betulina (Thunb.) Pillans, Agathosma crenulata Pillans or Agathosma serratifolia Pillans).[16]

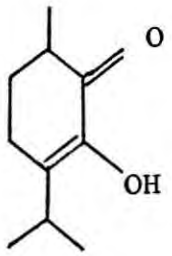
They were concerned with the isolation of the compound(s) responsible for the characteristic odour of the oil. Vacuum distillation gave a fraction (5% of the original oil) which was separated by chromatography on silica gel. The characteristic odour was concentrated in a fraction shown by GLC - MS coupling experiments to consist of 3 main components, diosphenol (1), ψ-diosphenol (2) and a sulphur-containing compound, $C_{10}H_{18}OS$, showing large fragment ion peaks at m/e 75 and 153.

It was thought that this structure could be derived from pulegone (3), isopulegone (4) or piperitone (5) by the addition of hydrogen sulphide to the C=C double bond. Addition of hydrogen sulphide to pulegone and isopulegone yielded (in both cases) a mixture of two stereoisomeric p-menthane-8-thiol-3-ones (6 and 7). These were separated by preparative GLC and subjected to IR, NMR, MS and optical rotation studies, whence it was decided that the trans configuration (6) should be assigned to the



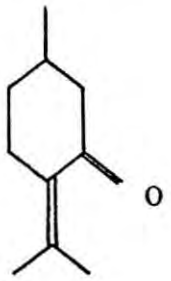
(1)

diosphenol



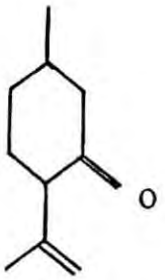
(2)

Ψ -diosphenol



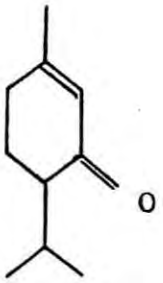
(3)

pulegone



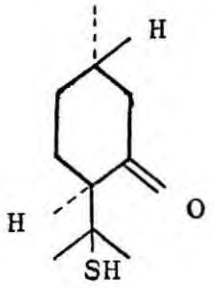
(4)

isopulegone

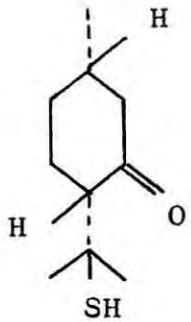


(5)

piperitone



(6)



(7)

more abundant, thermodynamically more stable isomer.

By processing a large amount of the oil, it was shown that p-menthane-8-thiol-3-one was present in the natural oil in both diastereomeric forms. The isolated natural diastereomeric mixture showed identical IR, NMR and MS spectra, as well as identical GLC retention times on two columns of opposite polarity as a synthetic mixture of the two diastereomers, thus proving their identity.

CHAPTER 2

Discussion of Results.

Agathosma apiculata G.F.W. Mey.

This shrub is also known as:

Diosma apiculata Spreng.

Barosma apiculata Eckl. and Zeyher

Agathosma aristata Presl.

Hartogia apiculata O.Kze.[17]

The leaves of this species are 2.5 - 8mm. long and bear a slightly recurved awn-like mucro. The latter enables a collector to distinguish this shrub from other species of Agathosma.[17]

The plant was collected at Rainbow Park on the west bank of the Kowie River beyond the golf course. The leaves were subjected to steam extraction, and the oil obtained was dried over anhydrous sodium sulphate and filtered. The yield of oil was found to be 0.32%. It has been found that the composition of the oil varies considerably according to the time of the year at which the plant is harvested.[5] In August, the more volatile components of the oil are present in considerably greater proportion than in March. When dried in air much of the oil is lost, especially the more volatile components.

The physical constants of the oil were found to be as follows:[5]

	Oil from fresh leaves collected in	
	March	August
d_{25}^{25}	0.890	0.888
N_D^{20}	1.4841	1.4837
$[\alpha]_D^{19}$	-5.1	-8.27
Sulphur content	11%	8.4%

Agathosma clavisepela R.A. Dyer

This plant bears leaves which are 3.5-5mm. long, convex or almost flat above, slightly recurved with conspicuous glands at the margin.[17]

The shrub was collected on a ridge overlooking the farm of Longford Grange in the Bushmans River Valley. The oil, obtained as before, was found to constitute 1.1% of the fresh leaves.

The oil was found to possess the following characteristics:

	Oil from fresh leaves collected in June 1970
d_{25}^{25}	0.872
N_D^{20}	1.478
$[\alpha]^D$	+ 8.831
Sulphur content	4.0%

Agathosma ovata (Thunb.) Pillans

Also known as:[17]

Diosma pulchella Houttuyn

D. ovata Thunb.

D. lanceolata Thunb.

D. oblonga Thunb.

Bucco ovata Wendl.

Diosma graveolens Licht. ex Roem.

D. punctata Licht. ex Roem.

D. linifolia Lodd.

D. dioica Spreng.

D. glandulosa Hort. ex Bartl. and Wendl.

D. orbicularis Hort. ex Bartl. and Wendl.

Barosma angustifolia Bart. and Wendl.

B. dioica Bartl. and Wendl.

B. oblonga Bartl. and Wendl.

B. ovata Bartl. and Wendl.

Bucco hamata Wendl. ex Bartl.

Diosma stenophylla Spreng.

D. spartiifolia Steud.

Barosma graveolens G.

B. scoparia Eckl. and Zeyher

B. ternata Eckl. and Zeyher

B. pauciflora Eckl. and Zeyher

B. Eckloniana Bartl.

B. Kraussiana Buch. ex Meissn.

B. setuliflora Delporte

B. acutata Sond.

B. lanceolata Sond.

B. Peglerae Dümmer

The leaves of this species are 0.5 - 1.5cm. long, flat or convex above, revolute at the margin or with the margin recurved to the nerve, and bearing large or small scattered oil glands on the lower surface.

This species was collected on the rim of the quarry below the old Port Elizabeth road, some 400 metres beyond the Leather Research Institute. The fresh leaves were found to yield between 0.45% (March) and 0.50% (November) of oil, which had the following characteristics:

	Oil from fresh leaves collected in	
	November 1968	March 1970
Yield	0.50%	0.45%
d_{25}^{25}	0.8529	0.8540
$[\alpha]^{D}$	$+8.27^{o}$	$+6.76^{o}$
N_{D}^{20}	1.4832	1.4832

No sulphur content.

Agathosma puberula Fourc.

Also known as:[17]

Diosma dubia Steud.

D. puberula Steud.

Agathosma gnidioides Schldl.

Barosma gnidioides Eckl. and Zeyher

B. mucronata Meissn.

B. puberula Buchinger ex Meissn.

Agathosma barosmoides Sond.

Hartogia barosmoides O.Kze.

H. puberula O.Kze.

The leaves of this shrub are 0.8 - 2cm. long, mucronate, somewhat thickened and gland crenate at the margin, with many glands in rows or scattered on the undersurface of the leaf.[17]

The leaves of this plant were collected on the farm "Upper Gletwyn" - some 8 miles out on the national road between Grahamstown and East London. On steam extraction the leaves yielded a clear oil comprising 0.72% of the plant material (in winter).

This oil has been found to have the following characteristics:[6]

	Oil from fresh leaves collected in		
	February	April	July
d_{15}^{15}	0.833	0.836	0.837
N_D^{20}	1.4758	1.4796	1.4759
$[\alpha]_D^{20}$	-0.46°	-0.07°	-0.87°
Sulphur	2.35%	2.56%	2.51%

GAS - CHROMATOGRAPHIC EXAMINATION OF THE ESSENTIAL OILS OF AGATHOSMA APICULATA, AGATHOSMA CLAVISEPELA, AGATHOSMA OVATA AND AGATHOSMA PUBERULA.

Each of the above-mentioned essential oils was examined using a gas chromatograph equipped with a hydrogen flame ionisation detector. Two different stationary liquid phases were employed in the investigation, one being of a polar nature (F.F.A.P.), and the other non-polar (Apiezon L). Liquid phases of opposite polarity are employed in qualitative work to allow the best estimate to be made of the nature and identity of the constituents.[18-32]

In order to identify the peaks appearing on the chromatogram, use was made of internal standards.[33-35] Initially it was thought that ether could serve as a suitable internal standard, but when calculations of adjusted retention times were made using ether as the "unretained solute" peak, the values obtained for peaks known to be the same (since they could be superimposed upon one another) differed markedly. Hence it was necessary to find another compound to serve as the "unretained solute".

After much experimentation it was decided to employ camphene (which always comes off at the beginning of a run) as the "unretained solute" peak, and to calculate the retention times of the various peaks relative to octanal and p-cresol (in the case

of F.F.A.P.), and to anethole and eugenol acetate (in the case of Apiezon L).

Compounds which serve as ideal internal standards should show the following characteristics:-

(1) They should be available in a high state of purity, thereby being represented by a single peak on the chromatogram.

(2) The compounds chosen as internal standards should not be present in the mixture being examined.

(3) The standards should be so selected that one of them comes off near the start of the run (and hence serves as the "unretained solute" peak), while the other two come off during the middle, and at the end of the run respectively.

(i) All of the compounds used as internal standards satisfy the first requirement.

(ii) It was found that the essential oils examined contained only a trace of camphene, p-cresol and anethole, but no octanal or eugenol acetate.

(iii) In the case of both columns, camphene was employed to serve as the "unretained solute" peak, while octanal (on F.F.A.P.) and anethole (on Apiezon L) come off during the middle of the run, and p-cresol (on F.F.A.P.) and eugenol acetate (on Apiezon L) at the end.

All of the oils were then injected together with the appropriate set of standards (depending on whether the column was polar or non-polar), and the adjusted relative retention times (distances) of the individual peaks were calculated as follows:

(1) With the F.F.A.P. column in operation:

$$r_{o.c} = \frac{t'_{R(i)}}{t'_{R(S)_o}} \Big/ \frac{t'_{R(i)}}{t'_{R(S)_c}}$$

where

$r_{o.c}$ = the adjusted retention time (distance) of the individual peak relative to octanal and p-cresol.

$t'_{R(i)}$ = the retention time (distance) from the "unretained solute" peak (camphene) to the individual peak under consideration.

$t'_{R(S)_o}$ = the retention time (distance) from the "unretained solute" peak to the first internal standard (octanal).

and $t'_{R(S)_c}$ = the retention time (distance) from the "unretained solute" peak to the second internal standard (p-cresol).

The results obtained are listed in Table 1.

(2) With the Apiezon L column in operation:

$$r_{a.e} = \frac{t'_{R(i)}}{t'_{R(S)_a}} \Big/ \frac{t'_{R(i)}}{t'_{R(S)_e}}$$

where

$r_{a.e}$ = the adjusted retention time (distance) of the individual peak relative to anethole and eugenol acetate.

$t'_{R(S)_a}$ = the retention time (distance) from the "unretained solute" peak (camphene) to the first internal standard (anethole).

and $t'_{R(S)_e}$ = the retention time (distance) from the "unretained solute" peak to the second internal standard (eugenol acetate).

The results obtained are listed in Table 2.

In both instances several peaks came off before the "unretained solute" peak, and values for these peaks are designated with a minus (-) sign. Although this (-) sign plays no important part, this nomenclature is adhered to throughout.

Once adjusted relative retention indices had been

affixed to all of the peaks on both series of chromatograms, the peaks of the constituents of the various oils had to be standardised i.e. the peaks of the same constituents in different oils on the same column had to bear the same number. Thus, for example, peak 5 of A. ovata (F.F.A.P.) is due to the same constituent as peak 5 of A. apiculata (F.F.A.P.), and similarly peak 8 of A. puberula (Apiezon L) arises from the same constituent as peak 8 of A. clavisepela (Apiezon L). However, peak 5 on a F.F.A.P. column will not correspond to peak 5 on an Apiezon L column. (See Tables 1 and 2.)

This standardisation of peaks was accomplished by consideration of the adjusted relative retention indices, and by comparing the chromatograms themselves on a light table.

Thus, all the peaks bearing the same number on a particular column will represent the same constituent.

Quantitative Analysis:[36, 37] This involved the cutting out of each peak from the chromatogram and weighing it. Although this method is subject to errors due to, for example, the lack of uniformity in the paper thickness, the results obtained were satisfactory since the percentages recorded in Table 5 are in close agreement. Geometrical methods were not employed since many of the peaks were very small, and in

addition a large number were asymmetrical.

Qualitative Analysis:[38] A very convenient method for the identification of peaks is to mix the particular essential oil under consideration successively with pure standards which are supposed to be among its components, and to analyse the new mixture. These analytical runs should also contain the appropriate set of internal standards so that the adjusted relative retention indices can be calculated.

If the substance which was added to the sample was already present in it, one of the peaks will be relatively larger than in the chromatogram obtained by analysing the original sample, thus indicating that the added substance has the same retention time as the component corresponding to the particular peak. This method of qualitative analysis is referred to as "peak enhancement".

The identification of peaks using this method presents one major difficulty. Even if the peak of the standard coincides with a peak of the original chromatogram, it is still possible that the original component is some other substance having the same retention time as the added standard on that particular column and under the given conditions. Hence, repetition of the analysis on another column employing a liquid phase of opposite polarity is always recommended, since if the two substances are not

identical, it is unlikely that they will overlap on both columns, e.g. when the A. ovata/internal standard mixture was injected together with eucalyptole on F.F.A.P., it appeared that peak 9 (comprising ± 40% of the original oil) could be eucalyptole. However, repetition of the analysis on Apiezon L, showed in fact that peak 9 on F.F.A.P. had not been resolved, since no peak representing 40% of the original oil could be detected on Apiezon L. Furthermore, it was found that eucalyptole was not even a constituent of the original oil, since the peak corresponding to it did not coincide with any other peak on Apiezon L.

N.B. It is very important that the purity of the standard to be added to the sample be first checked by gas-chromatographic analysis (under identical conditions) to avoid any misinterpretation due to the peaks of impurities.

The results obtained are shown in Tables 3 and 4.

The experimental conditions imposed when co-injecting standards were identical with those described alongside the particular chromatogram depicted at the end of this thesis. The retention indices of these standards are identical with those of the corresponding peaks in Tables 1 and 2.

Table 5 compares the percentages of the identified constituents on both columns. In all cases the lesser percentage can be taken to be the more accurate, since the presence of impurities would lead to a higher value.

Consideration of the relative retention indices and light table comparison of the relative chromotograms suggest that the sulphur-containing substances present in A. apiculata and A. puberula are in fact the same compound, and not different compounds as postulated in the literature.[5,6] In addition, this compound is identical with the sulphur-containing constituent of A. clavisepela, the oil of which has not previously been subjected to chemical investigation. Further gas-chromatographic analysis of the sulphur-containing fraction of A. apiculata, obtained by careful fractional distillation on a spinning band column, indicated that there may in fact be two peaks present, and not one as was at first thought. The two compounds could possibly be isomers or diastereomers as is the case with the sulphur-containing compounds in Buchu Leaf Oil.[16] Moreover, the percentages of the sulphur-containing compound(s) determined by earlier researchers using standard chemical methods,[5,6] agree very well with the quantitative results using gas-liquid chromatography. These workers found the sulphur-containing constituent to comprise 30% of A. apiculata[5] and 5% of A. puberula,[6] as opposed to 30% of A. apiculata and 5.3% of A. puberula found in

this investigation.

It is apparent from Table 5 that several compounds not previously reported in the oils of A. apiculata and A. puberula, have now been identified. These include

terpineol, which constitutes about 3% of A. clavisepela oil, as well as being present, albeit to a lesser degree, in all the other oils;

isopulegol, present in about 3% in A. clavisepela oil, and a minor constituent of the other oils;

caryophyllene, which provides one of the major peaks in A. apiculata and A. puberula; and

ocimene, which, except for A. ovata, constitutes a large proportion of all the oils examined.

Although earlier workers[6] found only a trace of linalool in A. puberula, it is in fact a major constituent (15%) of the oil. In addition, these researchers postulated that myrcene constitutes about 60% of the oil, whereas the present work shows the presence of only about 24%. Myrcene has also been found to be a major component of the other oils examined.

As well as agreeing qualitatively, the quantitative results in Table 5, obtained with columns of opposite polarity are in close agreement, the lower figures being probably more correct.

An attempt was made to separate the more volatile hydrocarbons in the oil of A. apiculata by low pressure fractionation on a spinning band column, followed by preparative GLC. Although the first fraction from the distillation contained only α-and β-pinene (as shown by analytical GLC), it was not possible to isolate either in the pure form by preparative GLC, since both rearranged to yield complicated mixtures. Attempts were then made to separate a synthetic mixture of α-and β-pinene, but these again rearranged, yielding chromatograms identical in every respect with those obtained previously.

Initially it was thought that the high temperature of the "spider" caused the compounds to rearrange, but the results were the same when the experiments were repeated using lower temperatures. Another possibility was that the liquid air used to condense the vapours was too drastic a process, but even though ice-water was employed, rearrangement still occurred to the same degree.

Similarly it was found impossible to isolate any of the other major constituents in a reasonable state of purity by preparative GLC. Recently Bernhard et. al.,[39] in attempting to identify the constituents of cardamom oil using preparative GLC, found that several terpenes, alcohols and aldehydes tended to rearrange on the column. They were therefore compelled to identify the peaks using relative retention times and peak enhancement.

CHAPTER 3

Experimental.

EXPERIMENTAL

1. Isolation of the essential oils.

All of the oils examined were extracted by steam distillation, the following experiment being typical:

"Three sacks full of the freshly picked leaves and tops of Agathosma ovata (collected on 26/2/70) were allowed to dry overnight. The plant material was then subjected to steam distillation (using a 5'x 10" galvanised iron still) in two batches (17 lbs and 11 lbs). Steam was passed through each batch for 3 hours, about 12 litres of distillate being collected. The oily layers from the two distillations were combined. The aqueous layers were saturated with salt and stored in a refridgerator overnight, but no more oil separated out. The oil was dried over anhydrous sodium sulphate, filtered through a No. 1 sintered glass funnel and stored in the deep freeze.

The weight of clear oil obtained was 56g. (0.45%)."

The other oils were extracted from the plants in a similar manner, the yields being as follows:

	collected on	
A. apiculata	8/7/1970	0.32%
A. clavisepela	26/5/1970	1.10%
A. puberula	18/4/1971	0.72%

2. Fractional distillation of A. ovata oil using the DT 520 Fractional Distillation Unit (a column 50 centimetres long and 2 centimetres in diameter, packed with glass helices).

140 g. of A. ovata oil were subjected to fractional distillation under reduced pressure, the following fractions being collected:

Fraction	Temperature at the top of the column	Bath temperature	Vacuum (mm)	Weight of the fraction
1	59-62°	101°	23 mm.	10.75 g.
2	62-70°	100°	23 mm.	25.3 g.
3	65-70°	118°	23 mm.	16.0 g.
4	70-74°	125°	23 mm.	31.50 g.
5	74-75°	130°	23 mm.	25.8 g.
6	75°	140°	23 mm.	6.5 g.
7	80-100°	140-180°	22 mm.	8.5 g.
8	75-80°	145°	0.5 mm.	2.7 g.
9	80-95°	170-190°	0.5 mm.	3.75 g.

Fractions 1-7 were redistilled at atmospheric pressure (Fraction 1 gave Fractions 1a and 1b, etc.)

Fraction	Temperature at the top of the column	Weight of the fraction
1(a)	150-154°	7.1 g.
1(b)	154-156°	1.8 g.
2(a)	152-156°	2.5 g.
2(b)	154-160°	2.5 g.

Fraction	Temperature at the top of the column	Weight of the fraction
2(c)	154-158°	3.25 g.
2(d)	156-158°	2.80 g.
2(e)	154-160°	3.6 g.
2(f)	156-160°	3.6 g.
2(g)	156-162°	3.3 g.
2(h)	160-164°	2.3 g.
2(i)	150-156°	0.5 g.
3(a)	140-158°	2.6 g.
3(b)	158-160°	2.5 g.
3(c)	158-162°	1.75 g.
3(d)	158-162°	1.5 g.
4(a)	150-164°	2.5 g.
4(b)	160-166°	1.8 g.
4(c)	160-168°	2.3 g.
4(d)	166-169°	2.7 g.
4(e)	150-172°	3.3 g.
4(f)	172-174°	3.5 g.
4(g)	172-173°	2.5 g.
4(h)	170-174°	2.1 g.
4(i)	160-164°	0.5 g.
5(a)	150-164°	1.3 g.
5(b)	166-172°	2.2 g.
5(c)	172-173°	2.8 g.

Fraction	Temperature at the top of the column	Weight of the fraction
5(d)	172-173°	3.0 g.
5(e)	173°	3.0 g.
5(f)	170-173°	2.7 g.
5(g)	172-174°	2.0 g.
6(a)	168°	1.3 g.
6(b)	160-166°	1.0 g.
6(c)	140-160°	0.4 g.
7(a)	170-176°	0.4 g.
7(b)	178-186°	2.3 g.
7(c)	188-190°	1.1 g.

These fractions were examined by GLC, employing the same conditions which appear alongside Figure 3 (F.F.A.P.). No fraction was found to consist chiefly of a single substituent, only peak enrichment being observed in rare cases. Since this method was obviously not successful in separating the constituents of A. ovata oil, the other oils were not subjected to similar distillation.

3. Test for the presence of diosphenol.

In order to test for the presence of diosphenol, one or two drops of aqueous ferric chloride solution were added to a solution of the oil in ethanol. In

no case did a colour form, showing that diosphenol was not present in any of the oils as a major constitutent.

4. Qualitative and quantitative tests for sulphur.

The qualitative tests for sulphur were performed using the Lassaigne Test.[40] All of the oils except that from A. ovata were found to contain sulphur.

The sulphur content of the oil of A. clavisepela was then determined as described in the literature,[41] the following results being obtained:-

(A) Weight of A. clavisepela oil used: 3.5638 g.

Weight of barium sulphate after drying for 1 hour at 130°: 1.038 g.

The factor for sulphur in barium sulphate is 0.1374.

Hence, 3.5638 g. of A. clavisepela oil contains 1.038 x 0.1374 g. sulphur.

∴ Percentage of sulphur in the oil

$$= \frac{0.1374 \times 1.038 \times 100}{3.5638}$$

$$= \underline{3.99}$$

Weight of barium sulphate after drying for 2 hours at 200°C: 1.016 g.

∴ Percentage of sulphur in the oil

$$= \frac{0.1374 \times 1.016 \times 100}{3.5638}$$

$$= \underline{3.92}$$

(B) Repetition of the experiment gave 4.02% of sulphur (after drying for an hour at 130°) and 4.00% sulphur (after drying for 2 hours at 200°C).

From these results it was concluded that 4.0% of sulphur was present in the oil of A. clavisepela.

5. Gas-liquid chromatographic analysis.

A Perkin-Elmer 900 Gas Chromatograph equipped with twin flame ionisation detectors was used.

The following columns (of stainless steel tubing) were employed in the investigation:

(1) A support-coated open tubular column (S.C.O.T. column) - 50' long and 0.020" in diameter. This column was used in the case of all of the F.F.A.P. runs, and yielded the chromatograms depicted in Figures 1-4. The conditions imposed during these runs are listed alongside the appropriate chromatogram.

(2) Two 12' x ⅛" columns connected in series. Except in the case of A. puberula, these columns were employed in all of the Apiezon L runs (Fig. 5-7). As above, the conditions imposed during the runs are listed alongside the appropriate chromatogram.

(3) A 20' x ⅛" Apiezon L column (prepacked), which was used in analytical investigations of A. puberula (Figure 8). Conditions imposed are listed alongside the chromatogram.

For preparative gas-liquid chromatography, the two 12' x $\frac{1}{8}$" Apiezon L columns were connected in series. Column conditions were identical to those employed in obtaining chromatograms 5-7.

Initially, when attempting to separate α- and β-pinenes, the temperature of the "spider" (of the preparative accessory) was maintained at 300°C, but once rearrangement had been detected using analytical GLC, the "spider" temperature was reduced to 200°C, and the temperature of the manifold to 150°C. Nevertheless, rearrangement still occurred to the same degree.

6. Fractionation of A. apiculata oil using a spinning band column.

40.0 g. of the oil were distilled in an atmosphere of nitrogen using a 1 metre long "Normag" spinning band column, the rate of distillation being 3-5ml. per hour.

Fraction	Distillation temperature	Bath temperature	Pressure	Weight of distillate
1	59.6-66.2°	106-109.5°	15 mm.	9.1 g.
2	71.6-79.0°	109-120°	15 mm.	3.0 g.
3	71.1-76.4°	115-120°	10 mm.	3.1 g.
4	78.2-86.8°	112-115°	10 mm.	2.0 g.
5	99.2-100.6°	124°	10 mm.	9.0 g.
6	100.5-100.7°	125-134°	10 mm.	7.4 g.
7	100°	135°	10 mm.	1.8 g.
			Total	35.4 g.

Analytical GLC showed that Fraction 1 contained only α- and β-pinenes, but all attempts to separate them by preparative GLC failed. Similarly, fractions 2, 3 and 4 were shown to contain the same mixture of components only in varying proportions. Fractions 5, 6 and 7 contained a large proportion of the sulphur-containing constituent. The latter three fractions were then combined and redistilled in an attempt to yield a pure product.

Fraction	Distillation temperature	Bath temperature	Pressure	Weight of fraction
1_2	90-97.6°	128-131°	10 mm.	2.0 g.
2_2	98.6-99.6°	120-130°	10 mm.	1.3 g.
3_2	99.6-100.2°	121-122°	10 mm.	$\pm$3.0 g.
4_2	100.1°	122-124°	10 mm.	$\pm$6.0 g.
5_2	100.1-100.2°	125-140°	10 mm.	3.1 g.

Analytical GLC showed that fractions 4_2 and 5_2 consisted of about 80% of the sulphur-containing constituent. However, in fractions 1_2 and 2_2 this peak was split, suggesting that the sulphur constituent is a mixture.

Fractions 1_2, 2_2 and 3_2 were combined and redistilled in order to obtain separation of the more volatile constituents.

Fraction	Distillation temperature	Bath temperature	Pressure
1_3	134-135.5°	±180°	393 mm.
2_3	136.4°	±180°	393 mm.
3_3	136.8-138.8°	±190°	393 mm.
4_3	139-137.4°	±190°	393 mm.
5_3	114-118°	200°	210 mm.
6_3	120-109°	200-260°	210 mm.

Analytical GLC investigation of these fractions indicated no separation of the constituents.

Compounds employed in the qualitative GLC investigation.

COMPOUNDS EMPLOYED IN THE QUALITATIVE GLC INVESTIGATION.

FRITZSCHE - D & O

Anethole

Cadinene

Citral

Citronellal

Citronellol (pure)

Citronellyl formate (pure)

Eugenol U.S.P. (extra)

Eugenol acetate

Indolique

Iso-octyl acetate

Linalool

Linalyl acetate

Methyl anthranilate (extra)

Neo-geraniol

Neo-geranyl acetate

Nerol

Neryl acetate

SCHUCHARDT MÜNCHEN

Anethol (p-Propenylanisol, 1-Methoxy-4-propenyl-benzol)

p-Anisaldehyde (p-Methoxybenzaldehyde, Aubèpine)

Benzaldehyde

Camphene

Caprylic acid (n-Octoic acid, octanoic acid)

Cresol

Decanal (Capraldehyde, Caprinaldehyde, Decylaldehyde)

1-Decanol (Decylalcohol)

Dipentene

1-Dodecanal (Dodecylaldehyde, Laurinaldehyde)

1-Dodecanol (Dodecylalcohol, Laurylalcohol)

Guajazulene

Indol (1-Benzazol)

Nerolidol

Nonanal (Pelargonaldehyde, Nonylaldehyde)

Nonanoic acid (Pelargonic acid, n-Nonoic acid)

1-Nonanol

Octanal (Caprylaldehyde, Octylaldehyde, n-Octaldehyde)

n-Octylacetate

Phenethylalcohol (Benzylcarbinol)

Pyrrole

Quinoline

DRAGOCO

Borneol

Citronellyl acetate

Citronellyl formate

Citronellyl butyrate

Coumarin

Diacetyl

Farnesol

Furfurol

Geraniol

Geranyl acetate

Hydroxy-citronellal

Hydroxy-citronellal dimethyl acetal

Linalool

Octanal

Terpinyl acetate

NAARDEN

Bornylacetate

Caryophyllene

Citral

Decylacetate

Ethylvalerianate

n-Hexanal

Isopulegol

Limonene

n-Methylanthranilate

Myrcene

Ocimene

n-Octylacetate

Terpinolene

n-Undecanal

MASCHMEIJER AROMATICS

Bornylacetate

Caryophyllene

Decylacetate

n-Decylaldehyde

Geranyl formate

Isopulegol

n-Methylanthranilate

n-Octylacetate

Phenylacetaldehyde

Terpinolene

n-Undecanal

FLUKA AG

Eucalyptole (Cineole)

(+) Fenchone

(+) Limonene

(-) Limonene

p-Menthan-3-one

1-Octanol

α-Phellandrene

α-Pinene

β-Pinene

HOPKIN & WILLIAMS

p-Cymene

β-Ionone

Bibliography

BIBLIOGRAPHY

(Abbreviations according to Chemical Abstracts.)

1. J.M. Watt and M.G. Breyer-Brandwijk, "The medicinal and poisonous plants of southern and eastern Africa", (E & S Livingstone Ltd.) (1962) pp. 908-912.
2. N.S. Pillans, J.S. African Bot., 1950, 16, 57.
3. N.S. Pillans, J.S. African Bot., 1950, 16, 55.
4. E. Klein and W. Rojahn, "Dragoco Report", 1968, 1, 3.
5. J.L.B. Smith and D.E.A. Rivett, Trans. Roy. Soc. S. Africa 1946, 31, 111.
6. J.L.B. Smith and D.G. Roux, Trans. Roy. Soc. S. Africa 1947, 31, 333.
7. J.F. Clevenger, J. Ass. off. agric. chem. Wash., 1943, 26, 396.
8. E. Guenther, "The Essential Oils. Vol. III" (D. van Nostrand Company, Inc.) (1964) p. 368.
9. S.J. van der Riet, Proc. Chem. Met. Mining Soc. S. Africa 1933, 34, 82.
10. J.L.B. Smith and K.A.D. Elliott, Trans. Roy. Soc. S. Africa 1928, 17, 23.
11. J.M. Watt and M.G. Breyer-Brandwijk, "The medicinal and poisonous plants of southern and eastern Africa", (E & S Livingstone Ltd.) (1962) p. 255.
12. J.L.B. Smith and M.L. Sapiro, Trans. Roy. Soc. S. Africa 1932, 21, 51.

13. J.M. Watt and M.G. Breyer-Brandwijk, "The medicinal and poisonous plants of southern and eastern Africa", (E & S Livingstone Ltd.) (1962) p. 918.

14. J.L.B. Smith and M.L. Sapiro, S. African J. Sci., 1932, 29, 281.

15. S.J. van der Riet, Proc. Chem. Met. Mining Soc. S. Africa 1933, 34, 80.

16. D. Lamparsky and P. Schudel, Tetrahedron Lett., 1971, 36, 3323-6.

17. N.S. Pillans, J.S. African Bot., 1950, 16.

18. R.A. Bernhard and J.R. Clark, Food Res., 1960, 25, 389-394.

19. R.A. Bernhard, J. Chromatog., 1960, 3, 471-6.

20. R.A. Bernhard, Food Res., 1960, 25, 531-7.

21. R.A. Bernhard and J.R. Clark, Food Res., 1960, 25, 731-8.

22. R.A. Bernhard and B. Scrubis, J. Chromatog., 1961, 5, 137-146.

23. R.A. Bernhard, J. Food Sci., 1961, 24, 401-411.

24. R.M. Ikeda, W.L. Stanley, L.A. Rolle and S.H. Vannier, J. Food Sci., 1962, 27, 593-6.

25. D.B. Kataque and E.R. Kirch, J. Pharm. Sci., 1963, 52, 252-8.

26. L.M.A. Rasquinho, J. Gas Chromatog., 1965, 340-4.

27. G.L.K. Hunter and W.B. Brogden, J. Food Sci., 1965, 30, 383-7.

28. I.C. Nigam and L. Levi, J. Chromatog., 1966, 23, 217-226.

29. T. Sakai, H. Maarse, R.E. Kepner, W.G. Jennings and W.M. Longhurst, J. Agr. Food Chem., 1967, 15, 1070-2.

30. S. Kamiyama, Agr. Biol. Chem., 1967, 31, 1091-6.

31. S.H.M. Ashoor and R.A. Bernhard, J. Agr. Food Chem., 1967, 15, 1044-7.

32. N.H. Anderson and D.D. Syrdal, Phytochem., 1970, 9, 1325-1340.

33. J. Tranchant, "Practical manual of gas chromatography", (Elsevier Publishing Company) (1969) p. 226.

34. B.J. Gudzinowicz, "Gas-chromatographic analysis of Drugs and Pesticides" (Edward Arnold Publishers Ltd.) (1967) p. 76.

35. L.S. Ettre and A. Zlatkis, "The practice of gas chromatography" (Interscience Fublishers) (1967) p. 404.

36. J. Tranchant, "Practical manual of gas chromatography", (Elsevier Publishing Company) (1969) p. 260.

37. L.S. Ettre and A. Zlatkis, "The practice of gas chromatography" (Interscience Publishers) (1967) p. 390.

38. L.S. Ettre and A. Zlatkis, "The practice of gas chromatography" (Interscience Publishers) (1967) p. 389.

39. R.A. Bernhard, R.O.B. Wijesekera and C.O. Chichester, Phytochem., 1971, 10, 177-184.

40. F.G. Mann and B.C. Saunders, "Practical Organic Chemistry", (Longmans) (1960) p. 319.

41. E. Gildemeister and F. Hoffmann, "The Volatile Oils. Vol. 3" (2nd edition) (Longmans) p. 368.

PART II

Tables and Chromatograms.

TABLE 1

ADJUSTED RELATIVE RETENTION INDICES ON F.F.A.P.

Peak Number	Agathosma ovata	Agathosma clavisepela	Agathosma apiculata	Agathosma puberula
1	-0.105 / -0.037	-0.107 / -0.038	-0.116 / -0.041	-0.133 / -0.048
1(a)	-0.048 / -0.018	-0.049 / -0.018	-0.054 / -0.019	-0.061 / -0.022
2	0.131 / 0.046	0.133 / 0.047	0.146 / 0.052	0.160 / 0.058
3	0.184 / 0.064	0.187 / 0.066	0.198 / 0.071	0.233 / 0.084
4	0.314 / 0.110	0.316 / 0.111	0.337 / 0.119	0.393 / 0.141
5	0.389 / 0.136	0.396 / 0.140	0.423 / 0.150	0.477 / 0.171
6				
7	0.473 / 0.165	0.484 / 0.170	0.508 / 0.180	0.557 / 0.200
8	0.574 / 0.200	0.578 / 0.203	0.603 / 0.213	0.637 / 0.229
9	0.628 / 0.219	0.625 / 0.220	0.645 / 0.228	0.673 / 0.242
10		0.683 / 0.241	0.700 / 0.248	0.720 / 0.259
11	0.786 / 0.274	0.779 / 0.274	0.791 / 0.280	0.800 / 0.288
12	0.814 / 0.284	0.819 / 0.289	0.828 / 0.293	0.840 / 0.302

Table 1 continued/

TABLE 1 (Continued)

Peak Number	Agathosma ovata	Agathosma clavisepela	Agathosma apiculata	Agathosma puberula
13	0.861 / 0.300	0.853 / 0.300	0.859 / 0.304	0.867 / 0.312
14	0.922 / 0.322	0.920 / 0.324	0.928 / 0.328	0.935 / 0.336
15				
16	0.977 / 0.341	0.972 / 0.342	0.977 / 0.346	0.977 / 0.351
17	1.000 / 0.349	1.000 / 0.352	1.000 / 0.354	1.000 / 0.360
18	1.093 / 0.381	1.093 / 0.385	1.088 / 0.385	1.075 / 0.386
19	1.137 / 0.396	1.132 / 0.399	1.130 / 0.400	1.124 / 0.404
20				
21		1.268, / 0.447		
22	1.322 / 0.461	1.311, / 0.462	1.300 / 0.460	1.277 / 0.459
23			1.350 / 0.478	
24		1.384 / 0.487	1.370 / 0.485	1.348 / 0.485
25	1.414 / 0.493			
26				

O C T A N A L

Table 1 continued/

TABLE 1 (Continued)

Peak Number	Agathosma ovata	Agathosma clavisepela	Agathosma apiculata	Agathosma puberula
27			1.529 / 0.541	1.480 / 0.532
28				
29			1.566 / 0.554	1.521 / 0.547
30	1.631 / 0.569		1.590 / 0.563	
31	1.653 / 0.576	1.643 / 0.579	1.631 / 0.577	1.580 / 0.568
32	1.691 / 0.590		1.667 / 0.590	1.620 / 0.582
33		1.734 / 0.611	1.716 / 0.607	1.670 / 0.600
34	1.769 / 0.617	1.754 / 0.618	1.741 / 0.616	1.688 / 0.607
35				
36	1.814 / 0.632	1.790 / 0.630	1.774 / 0.628	1.733 / 0.623
37				
38				1.764 / 0.634
39			1.845 / 0.653	
40	1.892 / 0.660	1.866 / 0.657	1.860 / 0.658	

Table 1 continued/

TABLE 1 (Continued)

Peak Number	Agathosma ovata	Agathosma clavisepela	Agathosma apiculata	Agathosma puberula
41				
42	1.913 / 0.667	1.901 / 0.669	1.882 / 0.666	1.835 / 0.660
43	1.933 / 0.674			
44	1.947 / 0.679	1.922 / 0.677	1.907 / 0.675	1.853 / 0.666
44(a)				1.861 / 0.669
44(b)				
45		1.983 / 0.698	1.939 / 0.686	
46			1.966 / 0.696	
47	2.064 / 0.720			
48	2.036 / 0.713	2.029 / 0.715	2.013 / 0.712	1.947 / 0.700
49				1.993 / 0.717
50			2.027 / 0.718	
51			2.077 / 0.735	
52				

Table 1 continued/

TABLE 1 (Continued)

Peak Number	Agathosma ovata	Agathosma clavisepela	Agathosma apiculata	Agathosma puberula
53	2.127 / 0.742	2.116 / 0.745	2.097 / 0.742	2.035 / 0.732
54	2.150 / 0.753			
55		2.163 / 0.762		2.067 / 0.743
56			2.138 / 0.757	2.083 / 0.749
57				
58	2.220 / 0.774		2.181 / 0.772	2.127 / 0.765
59				
60	2.251 / 0.785	2.230 / 0.785	2.216 / 0.784	2.150 / 0.773
61	2.303 / 0.803	2.271 / 0.800	2.262 / 0.801	2.190 / 0.787
62		2.304 / 0.811		
63			2.296 / 0.813	2.227 / 0.801
64				
65				2.260 / 0.813
66	2.399 / 0.837			2.283 / 0.821

Table 1 continued/

TABLE 1 (Continued)

Peak Number	Agathosma ovata	Agathosma clavisepela	Agathosma apiculata	Agathosma puberula
67				
68	2.477 / 0.864		2.442 / 0.865	
69		2.478 / 0.873	2.460 / 0.871	2.407 / 0.865
70				
71	2.589 / 0.903	2.564 / 0.903	2.546 / 0.901	
72				
73			2.593 / 0.918	2.480 / 0.892
74				
75				
76	2.746 / 0.958			
77	2.770 / 0.966			
78			2.752 / 0.974	2.693 / 0.968
79	2.814 / 0.981		2.771 / 0.981	

Table 1 continued/

TABLE 1 (Continued)

Peak Number	Agathosma ovata	Agathosma clavisepela	Agathosma apiculata	Agathosma puberula
80				2.760 / 0.992
81	2.867 / 1.000	p - C R E S O L 2.839 / 1.000	2.825 / 1.000	2.781 / 1.000
82				
83	2.949 / 1.029			
84	2.963 / 1.033	2.935 / 1.034	2.922 / 1.034	
85				
86	3.006 / 1.048			

TABLE 2

ADJUSTED RELATIVE RETENTION INDICES ON APIEZON L.

Peak Number	Agathosma ovata	Agathosma clavisepela	Agathosma apiculata	Agathosma puberula
1				
2	-0.153 / -0.109	-0.145 / -0.103	-0.144 / -0.101	-0.148 / -0.112
3	-0.126 / -0.090	-0.113 / -0.080	-0.111 / -0.077	-0.114 / -0.087
4	-0.085 / -0.061	-0.079 / -0.056	-0.079 / -0.056	-0.080 / -0.060
5	-0.048 /-0.032 minute amount	-0.048 /-0.032 minute amount	-0.049 / -0.034	-0.049 /-0.035 minute amount
6	0.048 / 0.034	0.045 / 0.032	0.045 / 0.032	0.049 / 0.037
7	0.057 / 0.041	0.056 / 0.040	0.056 / 0.039	0.076 / 0.057
8	0.104 / 0.074	0.101 / 0.072	0.102 / 0.071	0.104 / 0.079
9	0.140 / 0.100			
10			0.170 / 0.122 minute amount	0.170 / 0.122 minute amount
11	0.185 / 0.132	0.180 / 0.128	0.185 / 0.131	0.187 / 0.140

Table 2 continued/

TABLE 2 (Continued)

Peak Number	Agathosma ovata	Agathosma clavisepela	Agathosma apiculata	Agathosma puberula
12	0.202 / 0.145	0.193 / 0.137	0.196 / 0.137	0.205 / 0.154
13	0.230 / 0.164	0.225 / 0.160	0.221 / 0.155	0.247 / 0.186
14	0.246 / 0.176	0.238 / 0.169	0.244 / 0.171	
15	0.266 / 0.190	0.260 / 0.185	0.259 / 0.181	0.283 / 0.213
16	0.282 / 0.201	0.276 / 0.196	0.275 / 0.192	0.302 / 0.227
17	0.304 / 0.217	0.293 / 0.208	0.293 / 0.205	
18				
19	0.345 / 0.246	0.338 / 0.240		0.357 / 0.269
20	0.363 / 0.259	0.356 / 0.253	0.356 / 0.249	0.375 / 0.282
21	0.399 / 0.285	0.386 / 0.274	0.390 / 0.273	0.416 / 0.313
22			minute amount	
23	0.443 / 0.316	0.437 / 0.310	0.437 / 0.306	0.452 / 0.340
24			minute amount	minute amount
25		minute amount		

Table 2 continued/

TABLE 2 (Continued)

Peak Number	Agathosma ovata	Agathosma clavisepela	Agathosma apiculata	Agathosma puberula
26	0.525 / 0.375	0.514 / 0.365	0.512 / 0.358	0.537 / 0.404
27				
28		0.548 / 0.389	minute amount	0.555 / 0.417
29	0.569 / 0.406	0.561 / 0.398	0.562 / 0.393	0.596 / 0.448
30	0.596 / 0.426	0.592 / 0.420	0.591 / 0.413	
31			minute amount	
32				
33	0.657 / 0.469	minute amount	minute amount	0.659 / 0.496
34		0.674 / 0.478	0.673 / 0.471	0.687 / 0.517
35	0.696 / 0.497			0.723 / 0.543
36	0.722 / 0.516	0.699 / 0.496	0.721 / 0.504	0.742 / 0.558
36(a)		0.721 / 0.512		
37	0.741 / 0.529	0.749 / 0.532	0.749 / 0.524	
38	0.772 / 0.551	0.777 / 0.552	0.773 / 0.540	0.775 / 0.583
39		0.800 / 0.568	0.803 / 0.562	

Table 2 continued/

TABLE 2 (Continued)

Peak Number	Agathosma ovata	Agathosma clavisepela	Agathosma apiculata	Agathosma puberula
40				
41				
42	0.854 / 0.609			
43	0.870 / 0.621	0.882 / 0.626		0.879 / 0.661
44	0.896 / 0.640	0.896 / 0.636	0.892 / 0.624	0.896 / 0.674
45		minute amount	0.913 / 0.639	
46	0.930 / 0.664			
47	0.947 / 0.676	0.944 / 0.670	0.945 / 0.661	0.918 / 0.690
48	0.963 / 0.688	minute amount	minute amount	0.951 / 0.715
49	1.000 / 0.714	A N E T H O L E 1.000 / 0.710	1.000 / 0.699	
50	1.034 / 0.738	1.031 / 0.732	1.042 / 0.729	
51	1.053 / 0.752	1.055 / 0.749		1.102 / 0.829
52	1.111 / 0.793	1.110 / 0.788	1.118 / 0.782	
53		1.134 / 0.805		1.143 / 0.860
54	1.170 / 0.835	1.171 / 0.831	1.176 / 0.822	1.190 / 0.895

Table 2 continued/

TABLE 2 (Continued)

Peak Number	Agathosma ovata	Agathosma clavisepela	Agathosma apiculata	Agathosma puberula
55				
56				
57	1.231 / 0.879	1.234 / 0.876	1.252 / 0.875	1.245 / 0.936
58			1.282 / 0.897	
59		1.290 / 0.916		minute amount
60				
61		1.348 / 0.957		1.294 / 0.973
62				
63	1.382 / 0.986	1.392 / 0.988	1.419 / 0.992	1.294 / 0.973
64	1.401 / 1.000	E U G E N O L 1.408 / 1.000	A C E T A T E 1.430 / 1.000	1.330 / 1.000
65				
66	1.486 / 1.061		minute amount	
67				
68	1.538 /1.098		1.583 / 1.107	

Table 2 continued/

TABLE 2 (Continued)

Peak Number	Agathosma ovata	Agathosma clavisepela	Agathosma apiculata	Agathosma puberula
69	1.581 / 1.129		1.640 / 1.147	
70	minute amount		1.709 / 1.195	
71				
72				
73	1.767 / 1.262			
74	1.811 / 1.293			
75				
76				
77				
78				

TABLE 3

PERCENTAGES AND IDENTIFICATION ON F.F.A.P.

Peak Number	Agathosma ovata	Agathosma clavisepela	Agathosma apiculata	Agathosma puberula	Identification
1	7.32%	4.76%	4.24%	5.20%	∝-Pinene
1(a)	0.25%	0.15%	0.24%	0.05%	
2	12.80%	19.05%	19.53%	6.61%	β-Pinene
3	9.51%	4.10%	5.82%	1.93%	
4	0.05%	0.61%	0.65%	0.05%	
5	7.47%	1.62%	2.18%	24.82%	Myrcene
6					
7	0.35%	0.76%	1.12%	0.10%	
8	8.72%	2.79%	2.18%	2.98%	Limonene
9	40.39%	1.81%	1.82%	2.91%	
10		0.35%	0.24%	0.21%	
11	0.15%	1.22%	0.88%	8.38%	

Table 3 continued/

TABLE 3 (Continued)

Peak Number	Agathosma ovata	Agathosma clavisepela	Agathosma apiculata	Agathosma puberula	Identification
12	0.45%	0.35%	0.59%	0.42%	
13	1.05%	7.40%	4.29%	6.56%	
14	0.55%	0.35%	0.35%	0.21%	
15					
16	0.35%	0.41%	0.29%	0.16%	
17					
18	0.40%	0.61%	0.71%	0.26%	
19	2.54%	1.67%	4.29%	0.10%	
20					
21		0.05%			
22	1.10%	10.89%	8.00%	14.11%	Ocimene
23			0.06%		
24		0.30%	1.12%	0.10%	

Table 3 continued/

TABLE 3 (Continued)

Peak Number	Agathosma ovata	Agathosma clavisepela	Agathosma apiculata	Agathosma puberula	Identification
25	0.05%				
26					
27			0.06%	0.05%	
28					
29			0.06%	0.05%	
30	0.10%		0.06%		
31	0.45%	0.30%	0.41%	0.31%	
32	0.05%		0.06%	0.99%	
33		15.05%	30.71%	5.79%	
34	1.15%	11.70%	3.18%	15.09%	Linalool
35					
36	0.05%	0.10%	0.24%	0.10%	
37					

Table 3 continued/

TABLE 3 (Continued)

Peak Number	Agathosma ovata	Agathosma clavisepela	Agathosma apiculata	Agathosma puberula	Identification
38				0.05%	
39			0.06%		
40	0.05%	0.05%	0.12%		
41					
42	0.75%	0.66%	0.47%	0.78%	
43					
44	0.55%	0.55%	1.50%	1.14%	Caryophyllene
44(a)				0.99	
44(b)					
45		2.89	0.06		
46			0.06		
47	0.05%				
48	0.10%	4.61%	0.24%	0.16%	Isopulegol

Table 3 continued/

TABLE 3 (Continued)

Peak Number	Agathosma ovata	Agathosma clavisepela	Agathosma apiculata	Agathosma puberula	Identification
49				0.26%	
50			0.06%		
51			0.06%	0.10%	
52					
53	1.15%	3.80%	0.12%	0.42%	Terpineol
54	0.10%				
55		0.05%		0.05%	
56			0.06%	0.05%	
57					
58	0.05%		0.06%	0.05%	
59					
60	0.15%	0.10%	0.18%	0.21%	
61	0.25%	0.10%	0.06%	0.52%	

Table 3 continued/

TABLE 3 (Continued)

Peak Number	Agathosma ovata	Agathosma clavisepela	Agathosma apiculata	Agathosma puberula	Identification
62		0.05%			
63			0.06%	0.16%	
64					
65				0.05%	
66	0.05%			0.05%	
67					
68	0.05%		0.06%		
69		0.10%	0.18%	0.05%	
70					
71	0.05%	0.10%	0.12%		
72					
73			0.06%	0.10%	
74					

Table 3 continued/

TABLE 3 (Continued)

Peak Number	Agathosma ovata	Agathosma clavisepela	Agathosma apiculata	Agathosma puberula	Identification
75					
76	0.05%				
77	0.20%				
78			1.35%	0.05%	
79	0.10%		0.06%		
80				0.10%	
81	0.30%	0.05%	0.06%	0.10%	p-Cresol
82					
83	0.05%				
84	0.05%	0.10%	0.12%		
85					
86	0.20%				

TABLE 4

PERCENTAGES AND IDENTIFICATION ON APIEZON L.

Peak Number	Agathosma ovata	Agathosma clavisepela	Agathosma apiculata	Agathosma puberula	Identification
1					
2	0.04%	1.32%	1.35%	2.38%	
3	0.08%	0.04%	0.08%	1.71%	
4	10.80%	4.72%	4.28%	5.98%	α-Pinene
5	0.04%	0.04%	0.04%	0.04%	
6	3.59%	1.78%	2.33%	3.46%	
7	6.38%	2.10%	3.85%	23.06%	Myrcene
8	18.51%	19.34%	21.68%	6.97%	β-Pinene
9	0.04%				
10			0.04%		
11	1.25%	0.04%	0.04%	1.03%	
12	3.40%	0.80%	0.80%	1.12%	

Table 4 continued/

TABLE 4 (Continued)

Peak Number	Agathosma ovata	Agathosma clavisepela	Agathosma apiculata	Agathosma puberula	Identification
13	2.15%	14.37%	11.36%	16.24%	Ocimene
14	5.78%	1.36%	0.42%		
15	2.49%	7.90%	4.53%	8.73%	
16	8.99%	1.96%	1.99%	4.05%	Limonene
17	23.65%	0.91%	1.82%		
18					
19	0.11%	0.04%		1.08%	
20	1.13%	0.70%	0.89%	1.12%	
21	0.94%	9.44%	2.20%	15.02%	Linalool
22			0.04%		
23	0.57%	0.38%	0.50%	0.67%	
24			0.04%	0.36%	
25		0.04%			

Table 4 continued/

TABLE 4 (Continued)

Peak Number	Agathosma ovata	Agathosma clavisepela	Agathosma apiculata	Agathosma puberula	Identification
26	0.45%	2.10%	0.50%	0.94%	
27					
28		0.04%	0.04%	0.27%	
29	0.19%	0.14%	0.67%	0.49%	
30	2.46%	2.10%	5.50%		
31			0.04%		
32					
33	0.04%	0.04%	0.04%	0.90%	
34		15.00%	29.40%	5.26%	
35	0.04%			0.81%	
36	1.13%	2.76%	0.50%	0.33%	Terpineol
36(a)		0.80%			
37	0.08%	0.10%	0.04%		

Table 4 continued/

TABLE 4 (Continued)

Peak Number	Agathosma ovata	Agathosma clavisepela	Agathosma apiculata	Agathosma puberula	Identification
38	1.32%	0.28%	0.21%	1.12%	
39		0.04%	0.04%		
40					
41					
42	0.08%				
43	0.08%	3.11%		0.31%	
44	0.08%	1.95%	1.86%	1.21%	
45		0.04%	0.04%		
46	0.11%				
47	0.15%	3.08%	0.42%	0.22%	Isopulegol
48	0.11%	0.07%	0.04%	0.27%	
49	0.45%	0.07%	0.08%	0.04%	Anethole
50	0.30%	0.28%	0.21%		

Table 4 continued/

TABLE 4 (Continued)

Peak Number	Agathosma ovata	Agathosma clavisepela	Agathosma apiculata	Agathosma puberula	Identification
51	0.04%	0.04%		0.13%	
52	0.04%	0.07%	0.04%		
53		0.04%		0.22%	
54	0.19%	0.07%	0.08%	0.18%	
55					
56					
57	0.15%	0.04%	0.08%	0.22%	
58			0.04%		
59		0.07%		0.09%	
60					
61		0.07%			
62					
63	1.17%	0.28%	1.44%	1.17%	Caryophyllene

Table 4 continued/

TABLE 4 (Continued)

Peak Number	Agathosma ovata	Agathosma clavisepela	Agathosma apiculata	Agathosma puberula	Identification
64				0.04%	Eugenol Acetate
65					
66	0.04%		0.08%		
67					
68	0.04%		0.08%		
69	0.04%		0.08%		
70	0.04%		0.04%		
71					
72					
73	0.04%				
74	0.04%				
75					
76					

TABLE 5

COMPARISON OF PEAKS ON APIEZON L AND F.F.A.P.

Agathosma ovata (Apiezon L)	Agathosma ovata (F.F.A.P.)	Agathosma clavisepela (Apiezon L)	Agathosma clavisepela (F.F.A.P.)	Agathosma apiculata (Apiezon L)	Agathosma apiculata (F.F.A.P.)	Agathosma puberula (Apiezon L)	Agathosma puberula (F.F.A.P.)
α-Pinene 10.8%	7.3%	4.7%	4.8%	4.3%	4.2%	6.0%	α-Pinene 5.2%
β-Pinene 18.5%	12.8%	19.3%	19.1%	21.7%	19.5%	7.0%	β-Pinene 6.6%
Myrcene 6.4%	7.5%	2.1%	1.6%	3.9%	2.2%	23.1%	Myrcene 24.8%
Limonene 9.0%	8.7%	2.0%	2.8%	2.0%	2.2%	4.1%	Limonene 3.0%
Ocimene 2.2%	1.1%	14.4%	10.9%	11.4%	8.0%	16.2%	Ocimene 14.1%
Linalool 1.0%	1.2%	9.4%	11.7%	2.2%	3.2%	15.0%	Linalool 15.1%
Caryophyllene 1.2%	0.5%	0.3%	0.6%	1.4%	1.5%	1.2%	Caryophyllene 1.1%

Table 5 continued/

TABLE 5 (Continued)

Agathosma ovata (Apiezon L)	Agathosma ovata (F.F.A.P.)	Agathosma clavisepela (Apiezon L)	Agathosma clavisepela (F.F.A.P.)	Agathosma apiculata (Apiezon L)	Agathosma apiculata (F.F.A.P.)	Agathosma puberula (Apiezon L)	Agathosma puberula (F.F.A.P.)
Sulphur cpd		15.0%	15.1%	29.4%	30.7%	5.3%	Sulphur pcd 5.8%
Isopulegol 0.2%	0.1%	3.1%	4.6%	0.4%	0.2%	0.2%	Isopulegol 0.2%
Terpineol 1.1%	1.2%	2.8%	3.8%	0.5%	0.1%	0.3%	Terpineol 0.4%
50.4%	40.4%	73.1%	75.0%	77.2%	71.8%	78.4%	76.3%

Corrected to one decimal place.

GAS CHROMATOGRAPH RECORDS

1) SAMPLE

(i) Source: *Agathosma apiculata* + 3 standards (camphene, octanal and p-cresol) as 5 : 1.

(ii) Quantity injected: 0.06ul. (Vent open for 1 minute.)

2) COLUMNS

(i) Type and Dimensions: 1 x 50' support-coated open tubular column.

(ii) Support or Coating: -- Particle size: --

(iii) Liquid Phase: F.F.A.P. Quantity: prepacked

3) CARRIER. NITROGEN

(i) Pressure: 2.5 p.s.i.

(ii) Flow A: 3.7ml/minute B: --

(iii) Auxiliary Gas: 25.5ml/minute

4) DETECTORS (F.I.D.)

(i) Hydrogen Pressure A: -- B: 17 p.s.i.

(ii) Air Pressure: 62 p.s.i.

5) TEMPERATURES

(i) Injector: 275° Manifold: 275°

(ii) Column: 60°

(iii) Program:

Initial Temperature: 60° Initial Time: 13 min.

Final Temperature: 210° Rate °C/min.: 4°

Final Time mins.: 20 min. Cool Rate: 10°C/min.

6) AMPLIFIER

(i) Range: 100 Attenuation: 32

(ii) Polarity Recorder: +

(iii) Polarity Zero: +

7) RECORDER

(i) Ground:

(ii) Polarity: -

(iii) Chart Speed: 10mm/minute

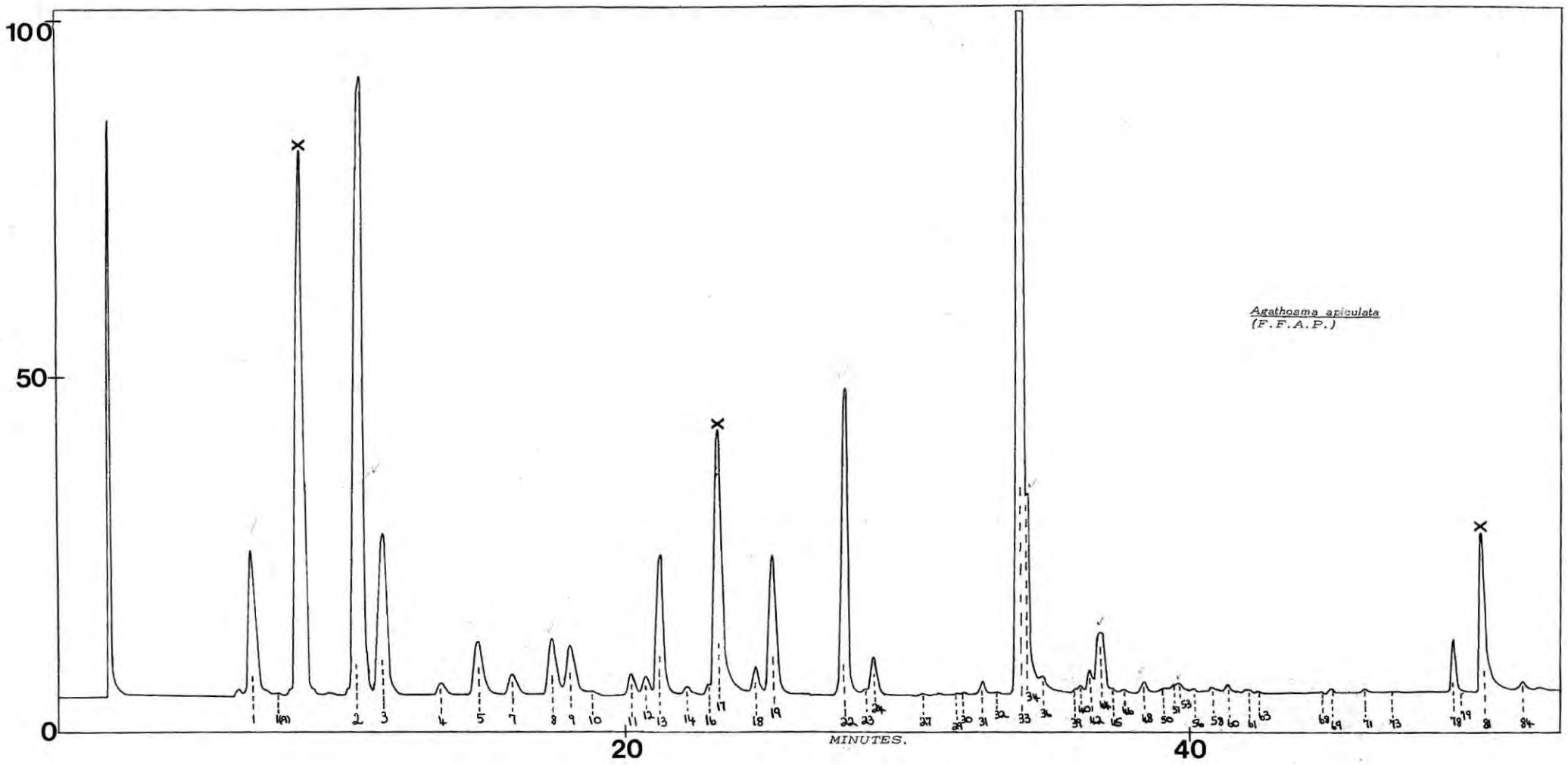


Figure 1

GAS CHROMATOGRAPH RECORDS

1) SAMPLE

(i) Source: Agathosma clavisepela + 3 standards (camphene, octanal and p-cresol) as 5 : 1.

(ii) Quantity injected: 0.06ul. (Vent open for 1 minute.)

2) COLUMNS

(i) Type and Dimensions: 1 x 50` support-coated open tubular column.

(ii) Support or Coating: -- Particle size: --

(iii) Liquid Phase: F.F.A.P. Quantity: prepacked

3) CARRIER. NITROGEN

(i) Pressure: 2.5 p.s.i.

(ii) Flow A: 3.7ml/minute B: --

(iii) Auxiliary Gas: 25.5ml/minute

4) DETECTORS (F.I.D.)

(i) Hydrogen Pressure A: -- B: 17 p.s.i.

(ii) Air Pressure: 62 p.s.i.

5) TEMPERATURES

(i) Injector: 270° Manifold: 265°

(ii) Column: 60°

(iii) Program:

Initial Temperature: 60° Initial Time: 13 min.

Final Temperature: 210° Rate °C/min.: 4°

Final Time mins.: 30 min. Cool Rate: 10°C/min.

6) AMPLIFIER

(i) Range: 100 Attenuation: 32

(ii) Polarity Recorder: +

(iii) Polarity Zero: +

7) RECORDER

(i) Ground

(ii) Polarity: —

(iii) Chart Speed: 10mm/minute

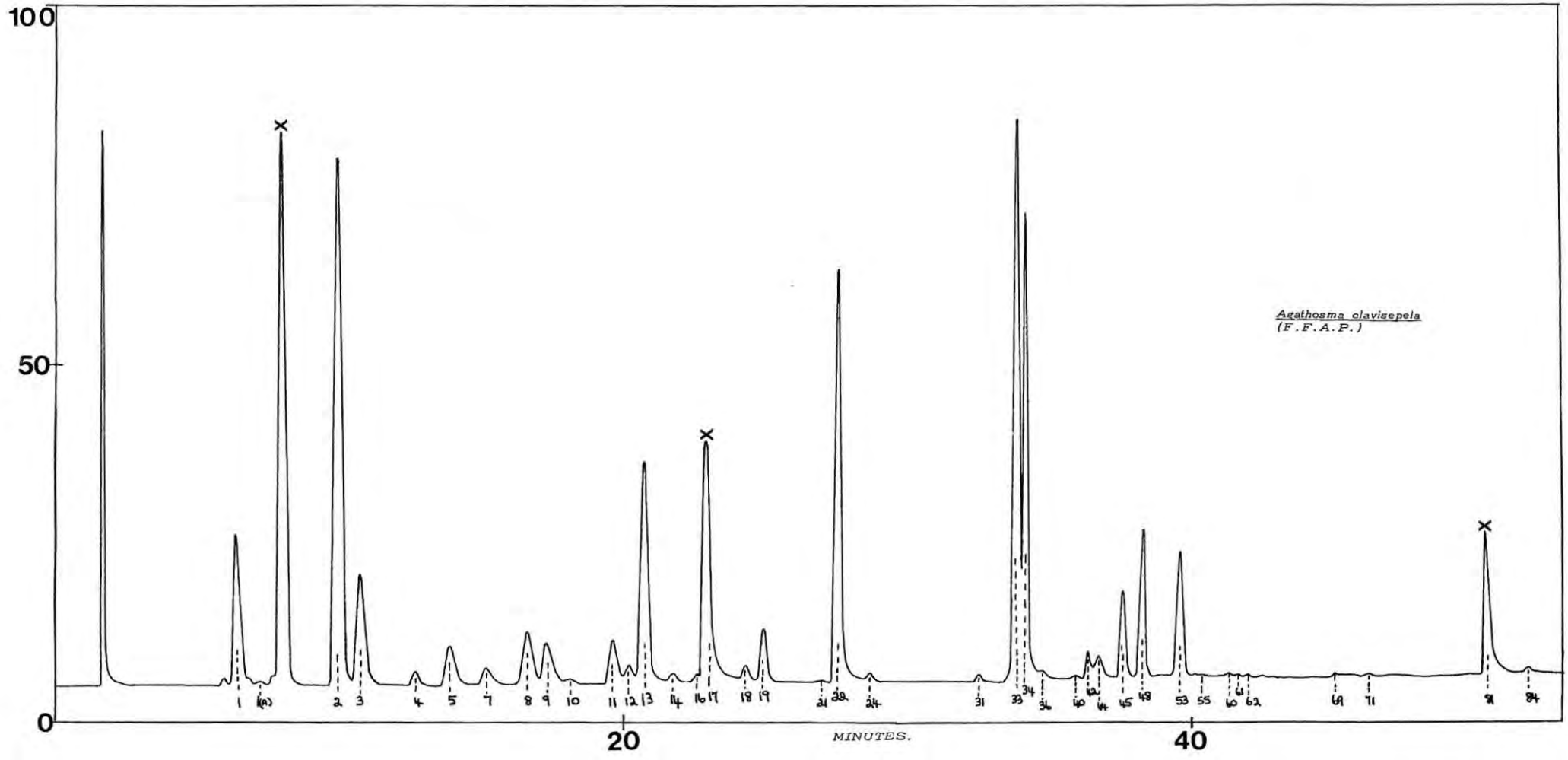


Figure 2

GAS CHROMATOGRAPH RECORDS

1) SAMPLE

(i) Source: Agathosma ovata + 3 standards (camphene, octanal and p-cresol) as 5 : 1.

(ii) Quantity injected: 0.06ul. (Vent open for 1 minute.)

2) COLUMNS

(i) Type and Dimensions: 1 x 50` support-coated open tubular column.

(ii) Support or Coating: -- Particle size: --

(iii) Liquid Phase: F.F.A.P. Quantity: prepacked

3) CARRIER. NITROGEN

(i) Pressure: 2.5 p.s.i.

(ii) Flow A: 3.7ml/minute B: --

(iii) Auxiliary Gas: 25.5ml/minute

4) DETECTORS (F.I.D.)

(i) Hydrogen Pressure A: -- B: 17 p.s.i.

(ii) Air Pressure: 62 p.s.i.

5) TEMPERATURES

(i) Injector: 250° Manifold: 270°

(ii) Column: 60°

(iii) Program:

Initial Temperature: 60° Initial Time: 13 min.

Final Temperature: 210° Rate °C/min.: 4°

Final Time mins.: 15 min. Cool Rate: 10°C/min.

6) AMPLIFIER

(i) Range: 100 Attenuation: 32

(ii) Polarity Recorder: +

(iii) Polarity Zero: +

7) RECORDER

(i) Ground

(ii) Polarity: -

(iii) Chart Speed: 10mm/minute

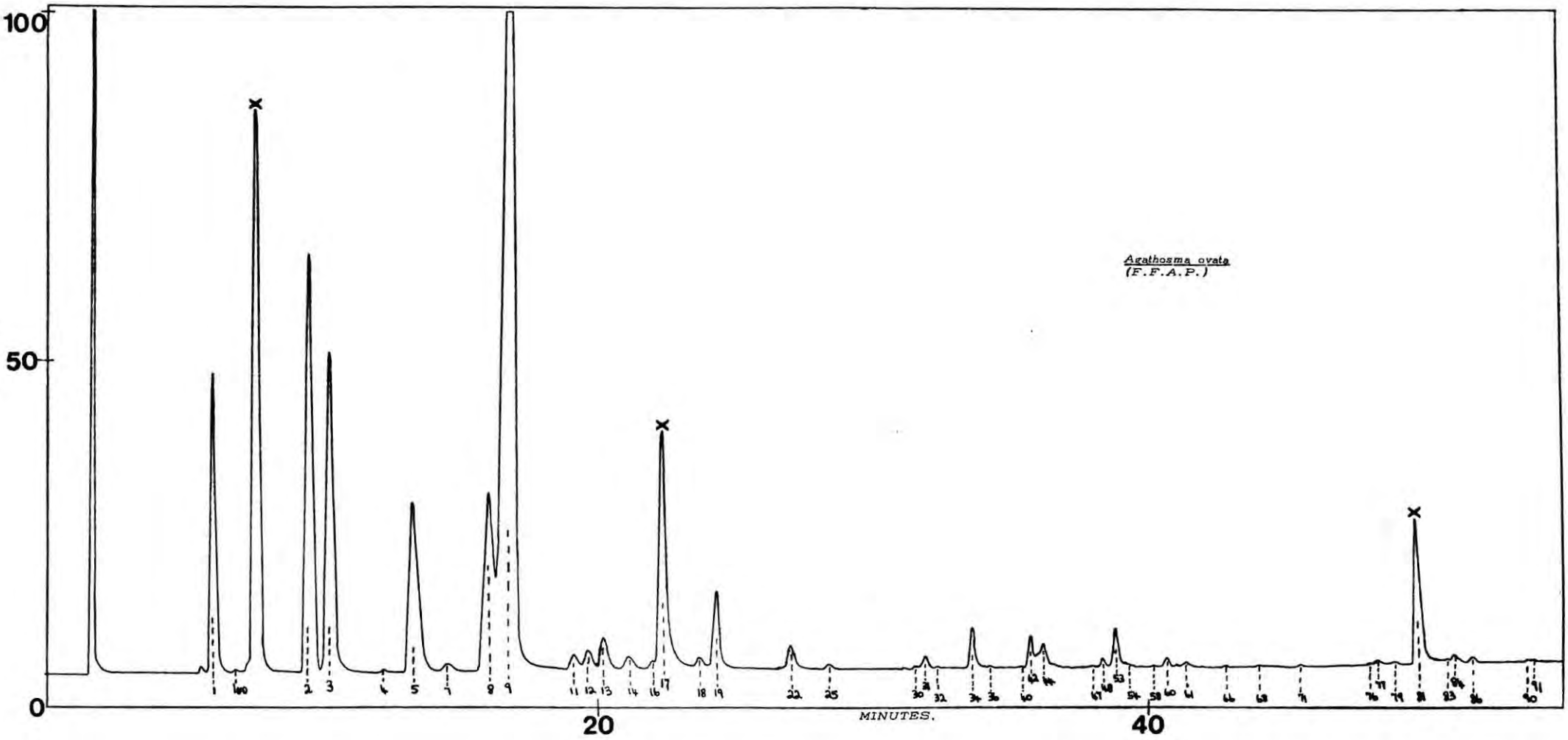


Figure 3

GAS CHROMATOGRAPH RECORDS

1) SAMPLE

(i) Source: *Agathosma puberula* + 3 standards (camphene, octanal and p-cresol) as 5 : 1.

(ii) Quantity injected: 0.1ul. (Vent open for 1 minute.)

2) COLUMNS

(i) Type and Dimensions: 1 x 50' support-coated open tubular column.

(ii) Support or Coating: -- Particle size: --

(iii) Liquid Phase: F.F.A.P. Quantity: prepacked

3) CARRIER. NITROGEN

(i) Pressure: 2.5 p.s.i.

(ii) Flow A: 3.2ml/minute B: --

(iii) Auxiliary Gas: 35.0ml/minute

4) DETECTORS (F.I.D.)

(i) Hydrogen Pressure A: -- B: 20 p.s.i.

(ii) Air Pressure: 60 p.s.i.

5) TEMPERATURES

(i) Injector: 250° Manifold: 220°

(ii) Column: 60°

(iii) Program:

Initial Temperature: 60° Initial Time: 13 min..

Final Temperature: 210° Rate °C/min.: 4°

Final Time mins.: 30 min. Cool Rate: 10°C/min.

6) AMPLIFIER

(i) Range: 100 Attenuation: 32

(ii) Polarity Recorder: +

(iii) Polarity Zero: -

7) RECORDER

(i) Ground

(ii) Polarity: +

(iii) Chart Speed: 10mm/minute

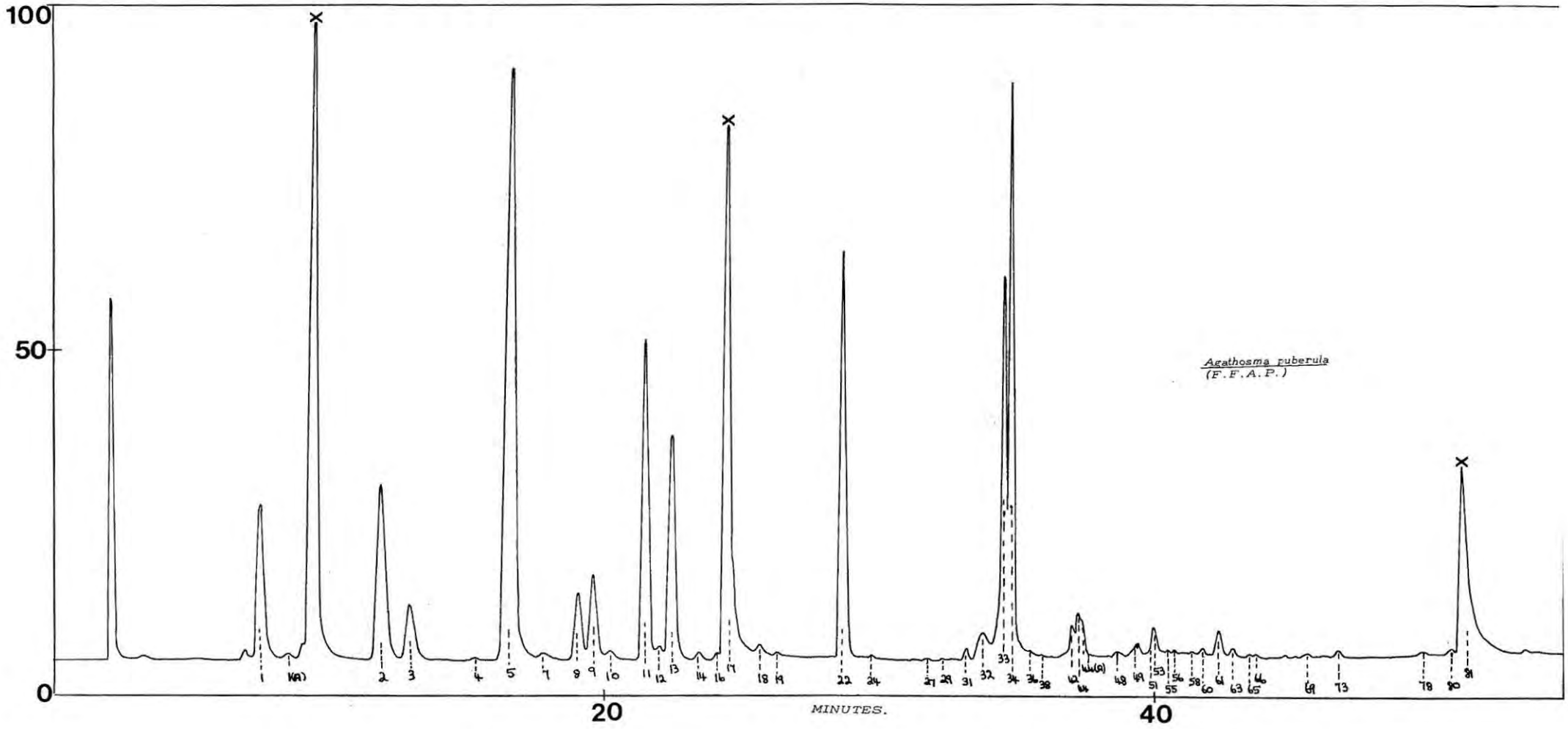


Figure 4

GAS CHROMATOGRAPH RECORDS

1) SAMPLE

(i) Source: *Agathosma apiculata* + {camphene: 2 anethole: eugenol acetate} 3 5 } as 6 : 1.

(ii) Quantity injected: 0.15ul.

2) COLUMNS

(i) Type and Dimensions: 2 x 12′ stainless steel columns connected in series.

(ii) Support or Coating: Chromosorb W
Particle size: 80 - 100 mesh

(iii) Liquid Phase: Apiezon L Quantity: prepacked

3) CARRIER. NITROGEN

(i) Pressure: 60 p.s.i.

(ii) Flow A: 13ml/minute B: --

4) DETECTORS (F.I.D.)

(i) Hydrogen Pressure A: -- B: 23 p.s.i.

(ii) Air Pressure: 62 p.s.i.

5) TEMPERATURES

(i) Injector: 210°C Manifold: 230°C

(ii) Column: 60°C

(iii) Program:

Initial Temperature: 60°C Initial Time: 20 min.
Final Temperature: 200°C Rate °C/min.: 2°
Final Time mins.: hold Cool Rate: --

6) AMPLIFIER

(i) Range: 100 Attenuation: 32

(ii) Polarity Recorder: +

(iii) Polarity Zero: +

7) RECORDER

(i) Ground

(ii) Polarity: -

(iii) Chart Speed: 5mm/minute

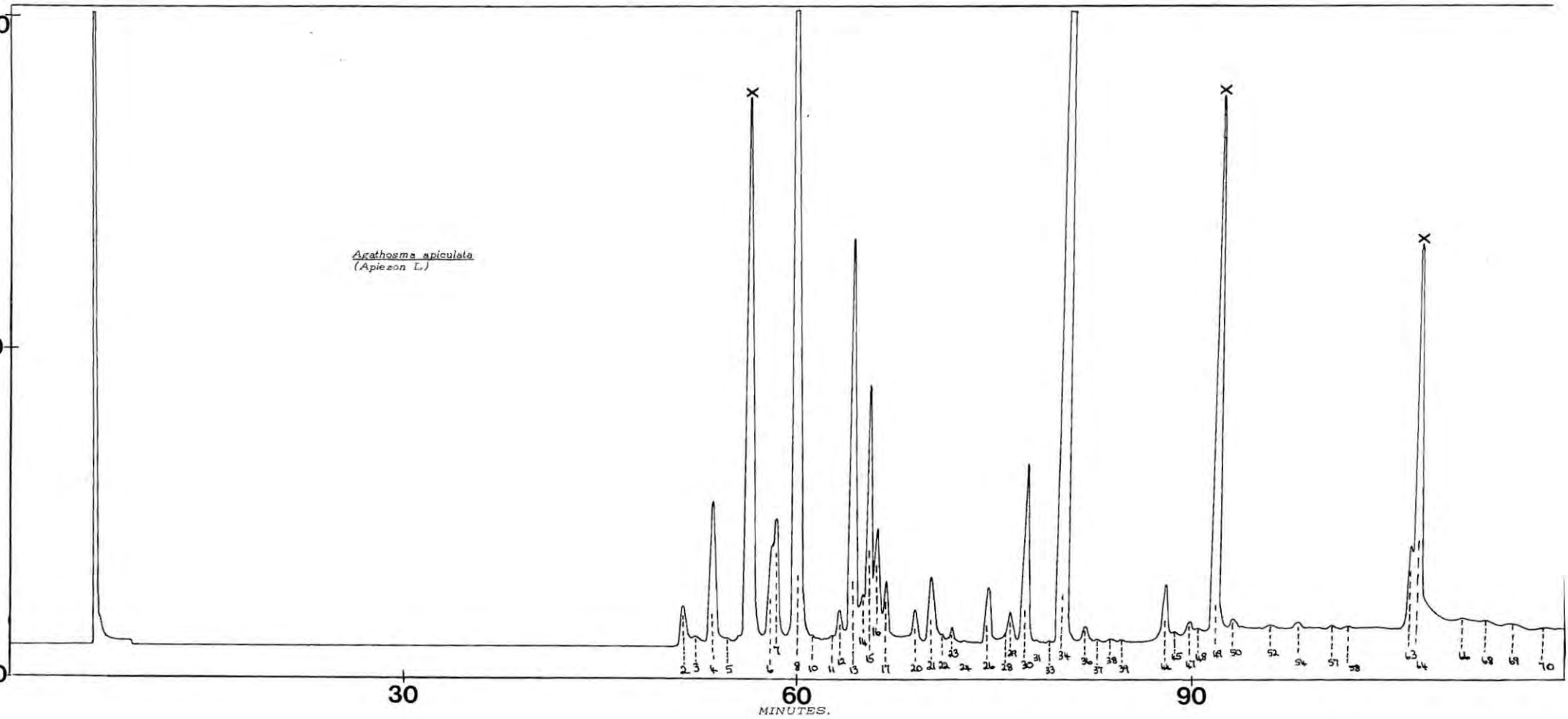


Figure 5

GAS CHROMATOGRAPH RECORDS

1) SAMPLE

(i) Source: Agathosma clavisepela + {camphene: 2 anethole: 3 eugenol acetate: 5} as 6 : 1

(ii) Quantity injected: 0.15ul

2) COLUMNS

(i) Type and Dimensions: 2 x 12′ stainless steel columns connected in series.

(ii) Support or Coating: Chromosorb W
Particle size: 80 - 100 mesh

(iii) Liquid Phase: Apiezon L Quantity: prepacked

3) CARRIER. NITROGEN

(i) Pressure: 60 p.s.i.

(ii) Flow A: 14ml/minute B: --

4) DETECTORS (F.I.D.)

(i) Hydrogen Pressure A: -- B: 23 p.s.i.

(ii) Air Pressure: 62 p.s.i.

5) TEMPERATURES

(i) Injector: 265°C Manifold: 230°C

(ii) Column: 60°C

(iii) Program:

Initial Temperature: 60°C Initial Time: 20 min.
Final Temperature: 200°C Rate °C/min.: 2°
Final Time mins.: hold Cool Rate: --

6) AMPLIFIER

(i) Range: 100 Attenuation: 32

(ii) Polarity Recorder: +

(iii) Polarity Zero: +

7) RECORDER

(i) Ground

(ii) Polarity: -

(iii) Chart Speed: 5mm/minute

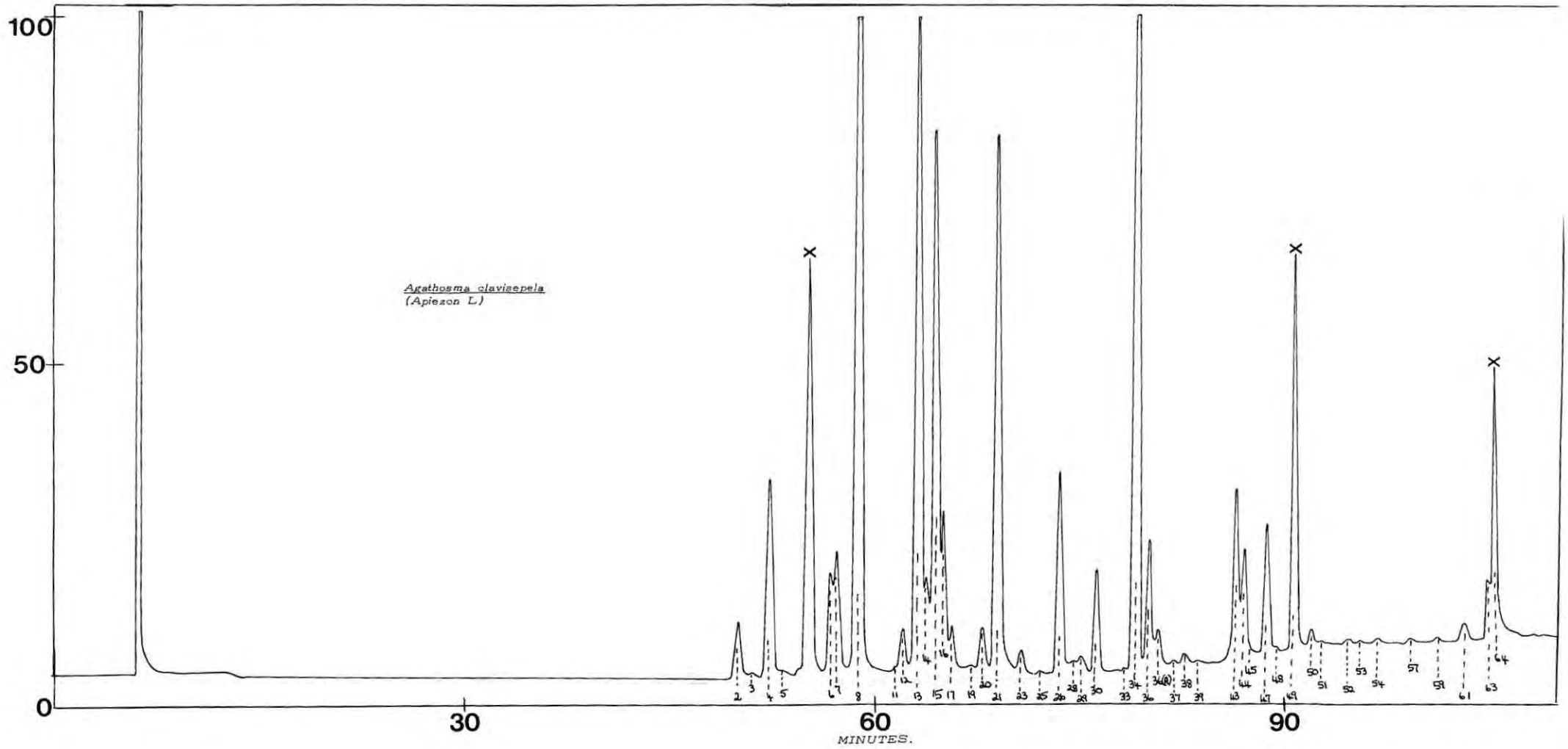

100
50
0
30
60
90
MINUTES.
Agathosma clavisepela
(Apiezon L)
X
X
X

Figure 6

GAS CHROMATOGRAPH RECORDS

1) SAMPLE

(i) Source: Agathosma ovata + {camphene:anethole: 2 3 eugenol acetate} 5 } as 6 : 1.

(ii) Quantity injected: 0.15ul

2) COLUMNS

(i) Type and Dimensions: 2 x 12′ Stainless steel columns connected in series.

(ii) Support or Coating: Chromosorb W
Particle size: 80 - 100 mesh

(iii) Liquid Phase: Apiezon L Quantity: prepacked

3) CARRIER. NITROGEN

(i) Pressure: 60 p.s.i.

(ii) Flow A: 13ml/minute B: --

4) DETECTORS (F.I.D.)

(i) Hydrogen Pressure A: -- B: 23 p.s.i.

(ii) Air Pressure: 62 p.s.i.

5) TEMPERATURES

(i) Injector: 260°C Manifold: 230°C

(ii) Column: 60°C

(iii) Program:

Initial Temperature: 60°C Initial Time: 20 min.
Final Temperature: 200°C Rate °C/min.: 2°
Final Time mins.: hold Cool Rate: --

6) AMPLIFIER

(i) Range: 100 Attenuation: 32

(ii) Polarity Recorder: +

(iii) Polarity Zero: +

7) RECORDER

(i) Ground

(ii) Polarity: -

(iii) Chart Speed: 5mm/minute

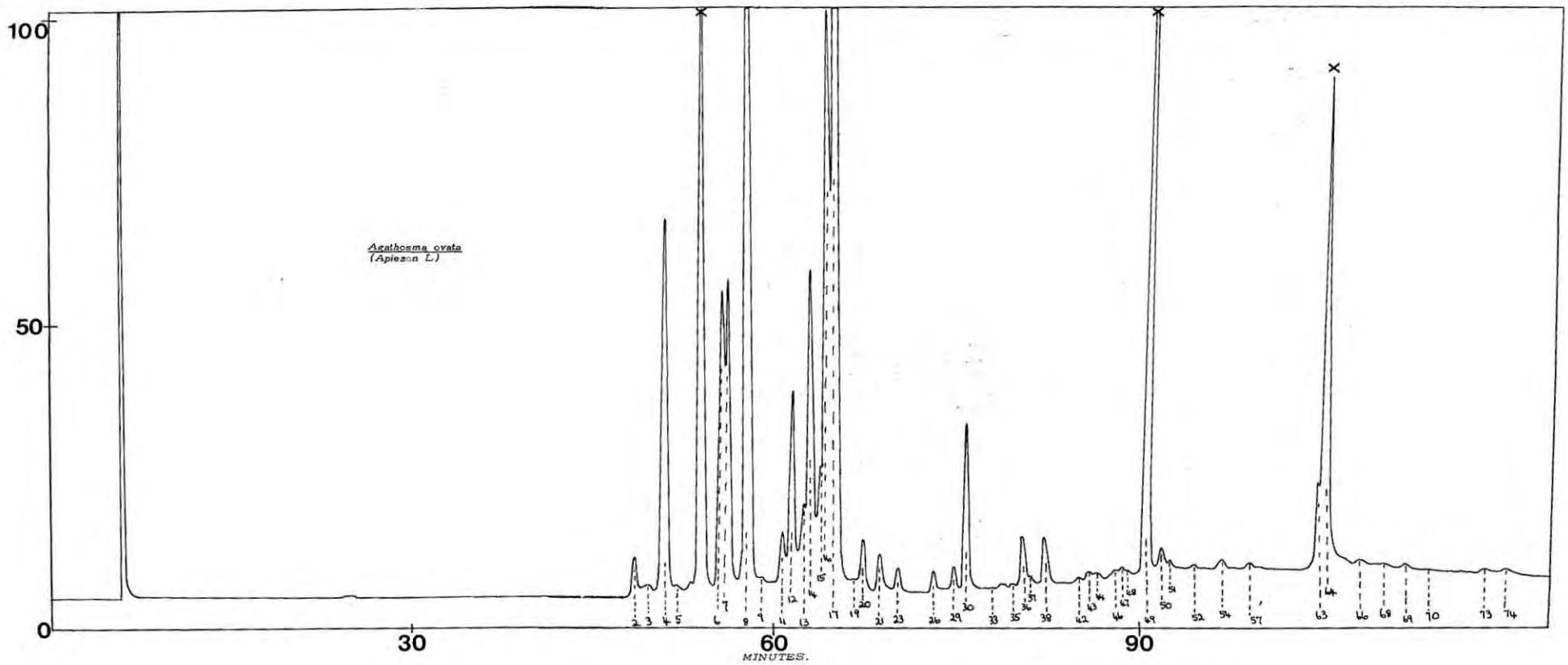


Figure 7

GAS CHROMATOGRAPH RECORDS

1) SAMPLE

(i) Source: Agathosma puberula + {camphene: 2 anethole: 3 eugenol acetate: 5} as 6 : 1.

(ii) Quantity injected: 0.15ul

2) COLUMNS

(i) Type and Dimensions: 1 x 20′ stainless steel column

(ii) Support or Coating: Chromosorb W
Particle size: 80 - 100 mesh

(iii) Liquid Phase: Apiezon L
Quantity: Prepacked (4%)

3) CARRIER. NITROGEN

(i) Pressure: 50 p.s.i.

(ii) Flow A: -- B: 17.0ml/minute

4) DETECTORS (F.I.D.)

(i) Hydrogen Pressure A: -- B: 20 p.s.i.

(ii) Air Pressure: 60 p.s.i.

5) TEMPERATURES

(i) Injector: 260°C Manifold: 260°C

(ii) Column: 80°C

(iii) Program:

Initial Temperature: 80°C Initial Time: 20 min.
Final Temperature: 200°C Rate °C/min.: 2°
Final Time mins.: hold Cool Rate: --

6) AMPLIFIER

(i) Range: 100 Attenuation: 8

(ii) Polarity Recorder: +

(iii) Polarity Zero: -

7) RECORDER

(i) Ground

(ii) Polarity: +

(iii) Chart Speed: 5mm/minute

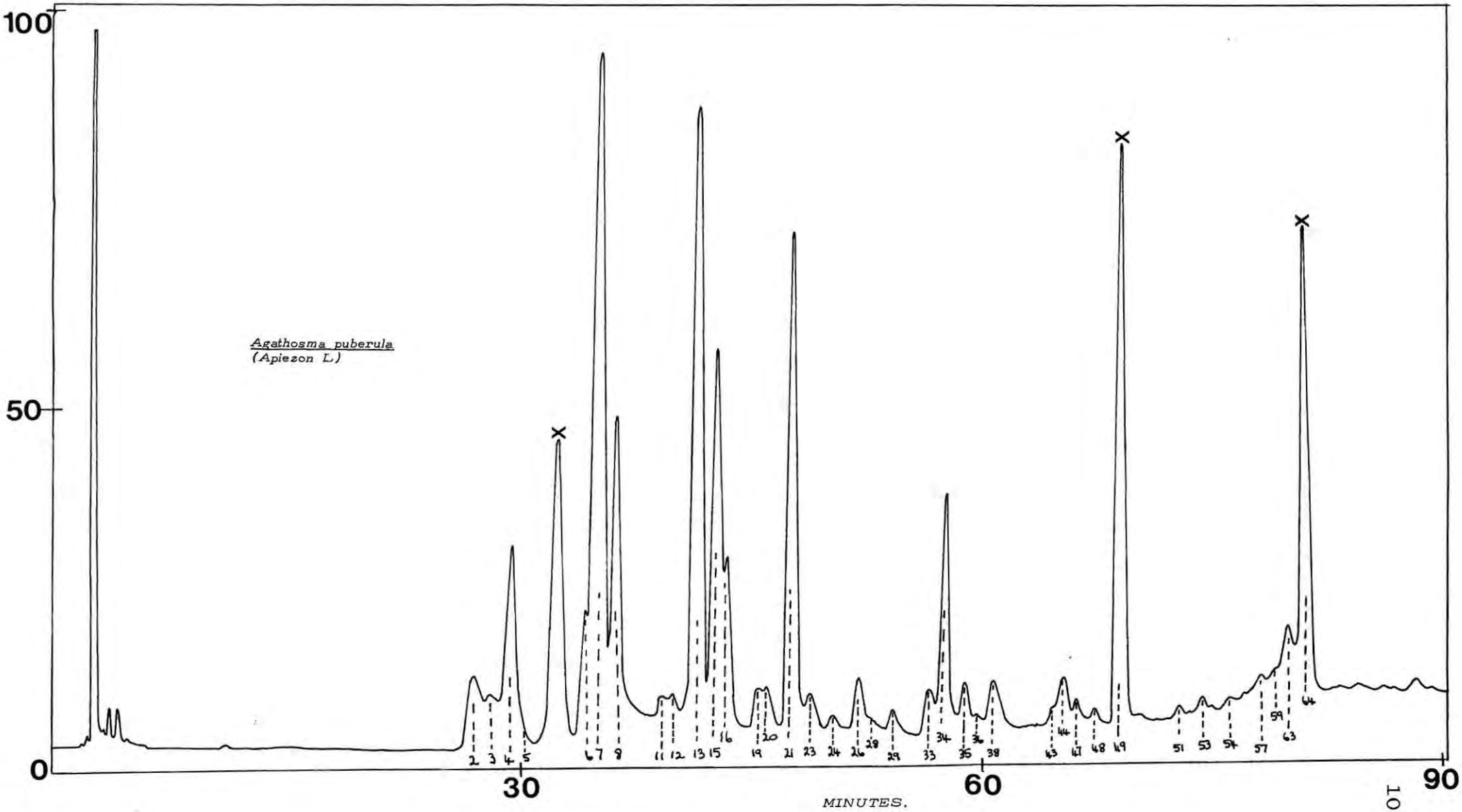

100
50
0
30
60
90
MINUTES.
Agathosma puberula
(Apiezon L)
2 3 4 5 6 7 8 11 12 13 15 16 19 20 21 23 24 26 28 29 33 34 35 36 38 43 44 47 48 49 51 53 54 57 59 63 64

Figure 8