STUDY OF OPOPANAX OIL FROM GUM - OPOPANAX OF PLANT COMMIPHORA ERYTHREA VAR. GLABRESCENS (FAMILY BURSERACEAE) FOUND IN NORTH EASTERN PARTS OF KENYA AND WESTERN SOMALILAND

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1984

"Dedicated to my beloved parents, brothers and sisters"

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LITERATURE SURVEY:

Gum-opopanax is an oleo-gum-resin from Burseraceae family, Plant species called commiphora erythrea Var glabrescens Engler. It is a sun dried exudation from the bark of the plant. Commiphora erythrea is a tall tree growing in North-Eastern Kenya and Western parts of Somaliland. Much of the secretion is obtained by spontaneous exudation from cracks and fissures which commonly form in the bark, and some are obtained by the natives (4). The gum is associated with oil and resins. The oil can be isolated by steam distillation method. Resins are insoluble in water but dissolve in alcohol, ether and chloroform. Gums are, on the other hand, insoluble in alcohol, chloroform and ether. Accordingly the resins can easily be osolated by extracting exhaustively from the gums using either chloroform as described below (7).

PHYSICAL CHARACTERISTICS OF THE VOLATILE OIL (8):

Appearance: Yellowish-green when freshly isolated but turns to intense green colour on standing. On exposure to air the oil resinifies.

Odour and Taste: Has aromatic odour with a bitter taste.

Solubility: Soluble in 1 to 10 vol. of 90% alcohol, sometimes with opalescence or turbidity.

Specific gravity at 15^0 ..... 0.870 to 1.006

Optical rotation, d_{20} ..... -19^0 28' to -8^0 0'

Refractive Index at 20^0 ..... 1.489 to 1.5165

Acid Number ..... 0.6 to 3.7

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Ester Number ..... 5.6 to 20

Ester Number after acetylation ..... 29.9 to 57.9

The yield varies from 5 to 10%.

The values obtained experimentally are:

Specific gravity at 26°C 0.95002

Optical rotation d_{21}° ..... $^{+}7.07$

Acid Number 3.70

Ester value 7.390

Refractive Index ($n_{d}^{21^{\circ}}$) 1.512

Solubility in 90% Alcohol ..... Soluble in 1 to 20 volumes of 90% Alcohol.

The yield of the oil on moisture free basis = 13.60% $^{v}/_{w}$.

The oil was separated from the gum - resins by steam distillation. The thin layer chromatographic studies of the oil done, Column Chromatography and Gas Liquid Chromatographic studies carried out. Ultra violet and infra-red spectroscopy were also carried out. Physico - Chemical Properties determined.

Microbiological assey of both the oil and resins were carried out. The resins were isolated from the gum by means of continous soxhlet extractor using Chloroform as the extracting solvent.

INTRODUCTION

INTRODUCTION

Commiphora erythrea from plant family Burseraceae is the source of gum-opopanax. The family is composed of 16 genera and 500 species. Commiphora makes up 185 species in the family and is found in less humid parts of tropical and sub-tropical Africa, Madagascar, tropical Arabia, Pakistan, India, Southern Iran and Ceylon. Difference between species groups within the genus seem less important than those between other groups. Commiphora is a name from Greek meaning gum-bearing, they are small trees attaining the height of lometres.

Since they are having minor differences it is of value to cite examples of commiphora spp. which are thought to be of medicinal use. These include:

Commiphora Africana - Bark yields gum-resin which gives an agreeable odour when burning and is used to fumigate clothing. The gum-resin is obtained by incision in the trunk to the depth of the bark. The exudate is white and clear, but solidifies soon and takes a yellowish tint. Boiled for treatment of inflammation of the eyes by holding the face over the steaming pot. For Scorpion bite the bark is applied after chewing it along with natron. The gum-resin alone has been used in West Africa as an insecticide especially as a repellant of termite. Also as a stomachic and eye remedy (10).

Commiphora molmol - Oleo-gum-resin obtained by spontaneous exudation from the cracks and fissures which commonly form in the bark and some from incisions made by the natives; is used as an incense and in perfumery. It has local stimulant and antiseptic properties.

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And is chiefly employed in medicine in the form of mouth wash as an astringent (10).

Commiphora habessinica - used as stomachic in children. The gum-resin is well known in tropical countries as a mosquito repellant, the usual mode of use being to burn it as incense sticks. The gum-resin contains volatile oil consisting of cuminol and euginol (10).

In general resin of Commiphora species is valued as a dressing for wounds. Decoction of barks useful as Purgative and Antihelmintic. Has been used as a disinfectant for wounds in form of resin (11). The history of myrrh dates back to ancient times. Has been used as incense, perfume and medicine from remote antiquity. It is mentioned in the Old testament and New Testament and some form of it was used by the ancient Hebrews and Arabs as a gift (probably the gum-resin from Commiphora erythrea var. glabrescens or Bisabol). It is cited in Herodotus as one of the substances used in embalming the dead. The part used is oleo-gum-resin. African myrrh is considered the best variety because it is the most Aromatic. Arabian myrrh is less aromatic (9).

The gum-opopanax used in the project is from Commiphora erythrea, and is the current commercial gum opopanax. Gum-opopanax is also called Bisabol-myrrh or sweet-myrrh. It is the sun dried exudate from the bark of the plant, Commiphora erythrea var. glabrescens. The plant is a tall tree growing in the Western parts of Somaliland and widely distributed in Northern Province. The gum is collected by the natives, sold on the market of Berbera and shipped to Aden, from where it is exported to

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Europe, India and China. In the Far East the gum is employed widely as a constituent of incense (8). Associated with the gum is volatile oil and resins so the whole material (exudate) is an oleo-gum-resin. The oil can be isolated by steam distillation and the yield varies from 5 to 10. Resins are insoluble in water but dissolve in high proof alcohol, chloroform and ether. The alcohol -, chloroform - or ether - insoluble portion of the oleo-gum-resin is the gum which is composed of proteins and carbohydrate derivatives (4). Accordingly the resins can be isolated by dissolving the gum-opopanax in either of the above given solvents, and concentrating filtrate preferably in vacuo to yield the so-called resinoid opopanax, a very viscous mass. When resins are separated and purified, they are usually brittle amorphous solids which fuse readily upon heating after passing a preliminary stage of softening. Resins contain acids and some of their metallic salts (resinates) are used extensively in the manufacture of cheap soaps and varnishes for instance commiphoric acid in myrrh.

THE MEDICINAL APPLICATION OF GUM-OPOPANAX

Oil of opopanax is a most valuable perfume ingredient. It has got warm, balsamic and exotic odour which is of value in perfumery. The oil was formerly employed as antispasmodic and deobstruent, but is now rarely used in Pharmacy.

Resinoid of opopanax is a very useful fixative. Has got an odour similar to that of oil, but less pronounced, imparting strength and lasting tonalities to perfume compounds (8).

The gum is claimed to be of value in treatment of snake bite. The gum-opopanax is known by the natives to possess some insect repellant

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rties. And has been used as disinfectant for wounds (10). It is
nown to the natives that gum-opopanax decrease sexual urge.
lingly the natives boil and drink in a view to decreasing the libido
youth seminaries. The insect-repellant properties attributable to
opanax can be of use by burning it as an incense stick, this has
eported by maradufu et al.

PHYSICOCHEMICAL PROPERTIES OF OPOPANAX OIL:

hese vary within quite wide limits as described in literature, and
asons are not known:

Specific gravity at 15°C	0.870 to 1.006
Optical rotation (d_{20}^{o})	-19^{o} 28'
Refractive index at 20°C	1.512
Acid value	0.6 to 3.08
Ester value	5.6 to 20
Ester number after acerylation	29.9 to 57.9
The oil content, yield	5.0 to 10.0%.

ve figures or values are quoted in "The Textbook of Essential
OILS" VOLUME IV".

CHEMICAL COMPOSITION OF OPOPANAX OIL:

ttle is known about the chemical composition, however, the oil is
to contain sesquiterpene hydrocarbons. Sesquiterpene hydrocarbons
anax oil were studied, using a combination of separation techniques
nclude column chromatography on alumina, silver nitrate adduction,
chromatography. The compounds isolated were identified by Infra
troscopy (IR) and nuclear Magnetic Resonance (NMR) Spectroscopy.

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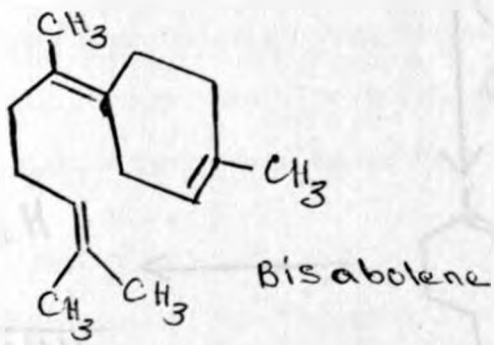
Eighteen sesquiteperne hydrocarbons were identified as follows: Δ- elemene, α- cubene, α- copaene, cis -α- bergamotene, β- elemene, α- santalene, trans - α- bergamotene, caryphyllene, γ- elemene, epi-β- santalene, β- santalene, humulene, γ- muurolene, trans - α- bisabolene, β- bisabolene, ar - curcumene, Δ- cadinene, and γ- cadinene. The major products obtained when hydrocarbons are regenerated from bisabolene trihydrochloride using sodium ethoxide (NaOAC), and ethanol (nOAC) as reagents, were also characterised and identified as cis -α- and β -bisabolene (12).

Bisabolene is a monocyclic sesquiterpenoid hydrocarbon with empirical formula ($C_{15}H_{24}$) . It occurs most widely distributed in nature and has the following structure:



Bisabolene

It is a monocyclic sesquiterpene and the position of double bonds were established by oxidation with ozone in glacial acetic acid, which gave mainly acetone and levulinic acid. It was first isolated by Tucholka from Bisabol myrrh and later by Burgess and page from oil of bergamot. As mentioned above, bisabolene can be characterised by the preparation of a trihydrochloride, melting point (79 - 80)^{o}C, from which it can be regenerated by the action of sodium acetate in acetic acid solution.

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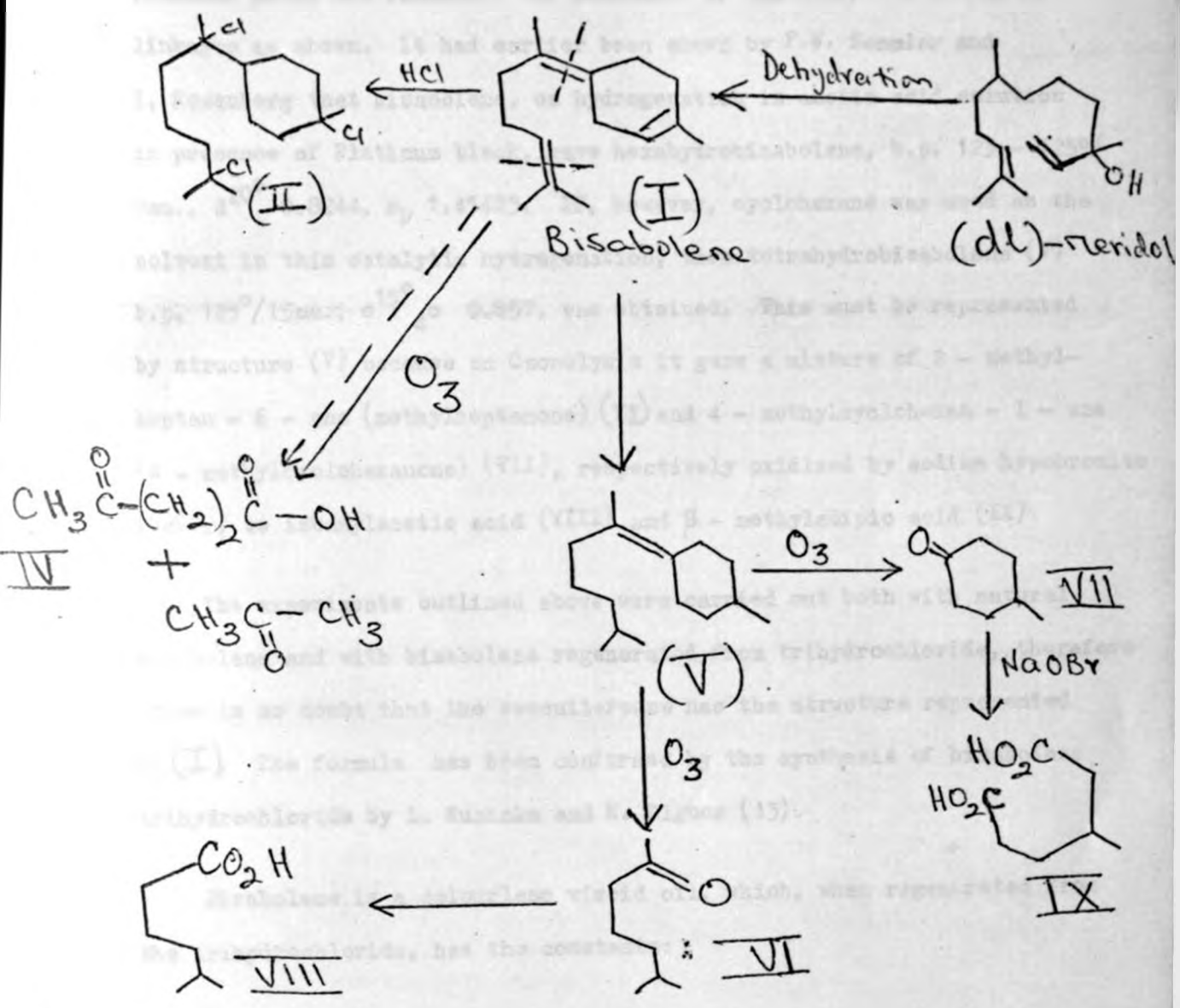
The reactions mentioned above are as follows:



The compound gives a characteristic saturated trihydrochloride (II) which regenerates bisabolene on treatment with sodium acetate in acetic acid solution. Bisabolene must be therefore a monocyclic and contain three ethylenic linkages. The carbon skeleton was established by observation of L. Ruzicka and E. Capato that the compound was obtained on dehydration of racemic (dl -) neridol (III).

Ozonolysis of bisabolene gives acetone, levulinic acid and small amount of succinic acid. This establishes the presence of the isopro-

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pylidene group and indicates the positions of the other two ethylenic linkages as shown. It had earlier been shown by P.W. Semmler and I. Rosenberg that bisabolene, on hydrogenation in acetic acid solution in presence of Platinum black, gave hexahydrobisabolene, b.p. 123 - 125°/8mm., $d^{20°}$ 0.8244, n_D 1.45423. If, however, cyclohexane was used as the solvent in this catalytic hydrogenation, then tetrahydrobisabolene (V) b.p. 125°/15mm., $d^{15°}_{4°}$ 0.857, was obtained. This must be represented by structure (V) because on Ozonolysis it gave a mixture of 2 - methylheptan - 6 - one (methylheptanone) (VI) and 4 - methylcyclohexan - 1 - one (4 - methylcyclohexanone) (VII), respectively oxidised by sodium hypobromite ($NaOBr$) to Isoamylacetic acid (VIII) and β - methyladipic acid (IX).

The experiments outlined above were carried out both with natural bisabolene and with bisabolene regenerated from trihydrochloride, therefore there is no doubt that the sesquiterpene has the structure represented by (I). The formula has been confirmed by the synthesis of bisabolene trihydrochloride by L. Ruzicka and M. Liguor (13).

Bisabolene is a colourless viscid oil, which, when regenerated from the trihydrochloride, has the constants:

b.p. = (133 - 134)° C/12 mm.

$d^{21°}_{4°}$ = 0.8717 and,

$n^{21°}_{D}$ = 1.4923

The proposed structures of some of the sesquiterpene hydrocarbons are as follows:

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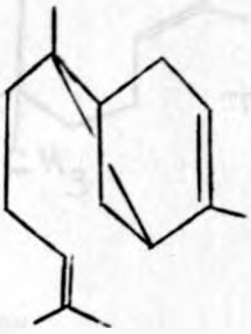
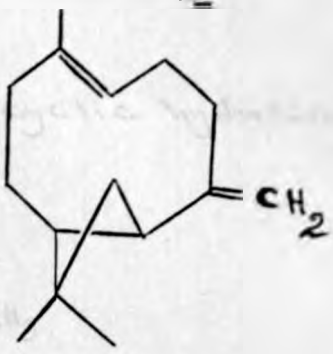
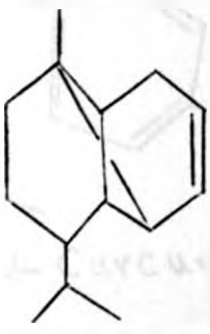
Trans -α- bergamotene



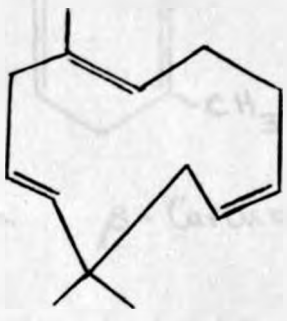
Caryophyllene



Copaene

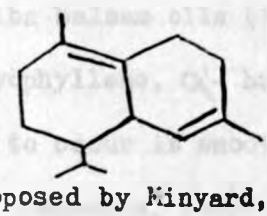


α- Humulene



Humulene is readily oxidizable on exposure to air and is claimed to be identical with α- Caryophyllene (13)

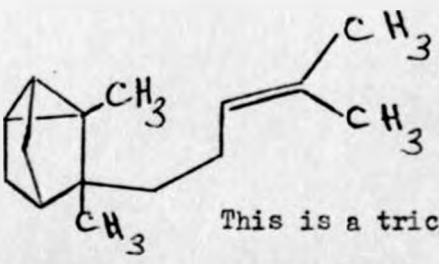
δ- Cadinene



Above structures were proposed by Kinyard, J.P. et al (15).

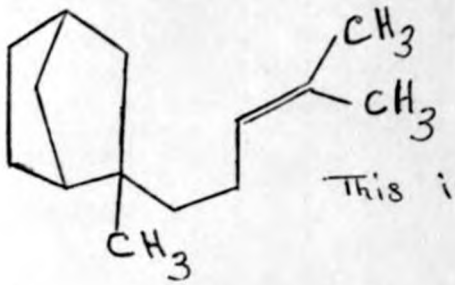
Also the structures for α- Santalene, β- Santalene, Curcumenes have been proposed by Simonsen, J.L. and Barton, D.H.R. (13). And are as follows:

α- Sentalene



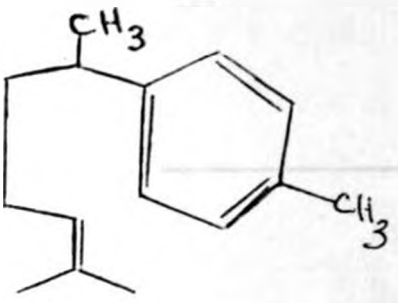
This is a tricyclic hydrocarbon

β- Santalene

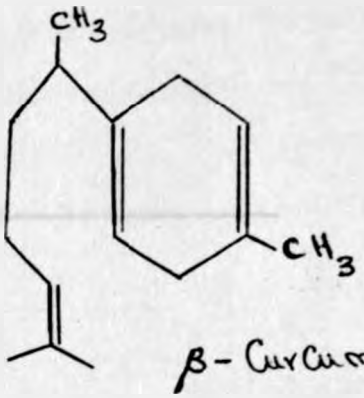


This is a dicyclic hydrocarbon.

Curcumene:



α- Curcumene.



β- Curcumene.

Some of the sesquiterpene hydrocarbons have been isolated from various other sources. For instance β - bisabolene has been isolated from bergamot, ginger, and copaiba balsam oils (14). Copaene, L- trans - α- bergamotene, L- caryophyllene, α- humulene, and L:δ - cadinene among others have been reported to occur in smooth leaf variety cotton (Gossypium hirsutum) plant buds (squares) (15).

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EXPERIMENTAL WORK

REAGENTS AND APPARATUS

Reagents:

Reagents used in the work were as follows:

0.1N Potassium hydroxide.

0.5N Alcoholic potassium hydroxide.

0.1N Alcoholic potassium hydroxide.

0.5N Hydrochloric Acid.

Absolute Alcohol (95 - 100% Ethanol)

Chloroform

Diethyl Ether

n - hexane

Benzene: Acetone mixture (97.5:2.5)

Chloroform: Methanol mixture (1:9)

Vanillin - Conc.Sulphuric Acid (1% $W/_V$).

Apparatus:

Apparatus used were as follows:

Atago Polarimeter.

10ml pycnometer.

Abbe Refractometer

Perkin - Elmer Infra - Red Spectrometer Model 727 B.

Ultraviolet spectrometer - unicam sp800 A.

Chromatograph - Pye unicam series 104.

Continuous Soxhlet Extractor.

Silica gel $60GF_{254}$.

Alumina (Aluminium oxide 90) - MERCK Kieselgel G/u 254.

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Media:

Nutrient - Agar.

MacConkey's Agar.

Micro -organisms:

Escherichia Coli.

Staphylococcus Aureus.

DETERMINATION OF THE OIL CONTENT

The oil content of oleo-gum-resin of gum-opopanax was determined by steam distillation method, method 1 as described by the British Pharmacopoea 1973 - Appendix A 37, for oil lighter than water. The apparatus used were shown in figure 1.

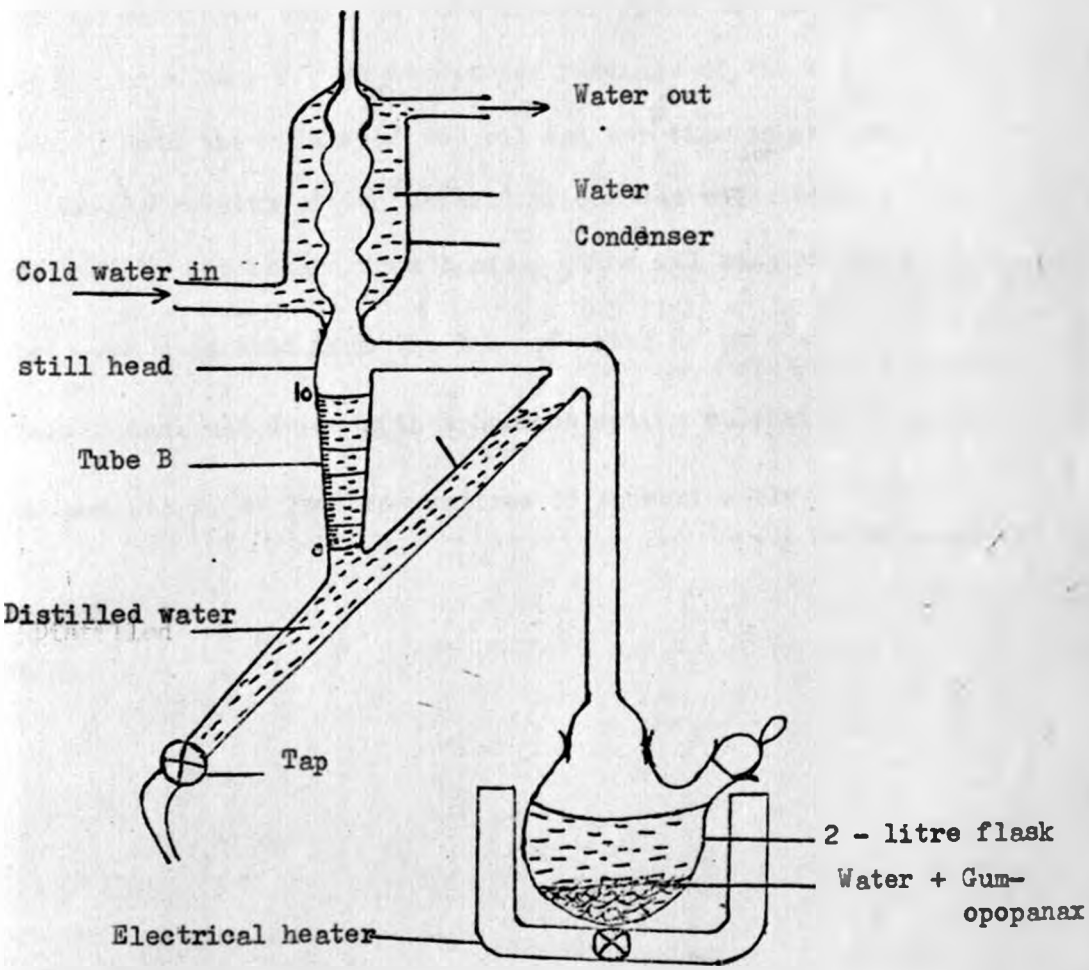

Fig. 1

Approximately 25 gm of powdered gum-opopanax was weighed accurately into the 2 - litre flask and about 300 mls of water added. Distilled water was poured into still head until it overflowed into the 2 - litre flask through tube A. Then the 2 - litre flask electrically heated, noting the time as the first drop of oil enters the graduated tube (tube B). Distillation continued until no more oil was given out as indicated by the constancy of two successive readings of the oil collected. Both the volume of the oil and the time taken were noted. Average results of two determinations was calculated and expressed on a moisture free basis. The oil samples collected were separated from the bulk of water using a separatory funnel and dried with anhydrous sodium sulphate. Filtered and stored at low temperatures of approximately 4 ^{o}C.

DETERMINATION OF MOISTURE CONTENT:

The determination of moisture content of gum-opopanax was done gravimetrically according to European pharmocopoea Vol. 1 (1969).

The gum-opopanax was thoroughly commimuted and about 2g of the powdered sample weighed accurately into a previously dried petridish. The Petridish and its contents were dried in an oven at 105°C to constant weight. Three determinations were carried out and the average value calculated.

ISOLATION OF OPOPANAX OIL FOR FURTHER INVESTIGATION

In order to obtain larger quantities of the oil, a 10 litre flask and 100 g of the comminuted gum-opopanax were used. Sufficient water to cover the material was added and the apparatus arranged as shown in Figure 1. Distilled water poured into still head until it overflowed into the 10 litre flask through tube K. Then the flask was electrically heated. The oil collected was separated from the bulk of water using a separatory funnel. Dried with anhydrous sodium sulphate, filtered and stored at low temperatures of approximately 4°C.

DETERMINATION OF PHYSICO-CHEMICAL PROPERTIES OF THE OIL

Physical Properties:

(a) Appearance: (i.e. colour), odour and taste of the isolated were determined.

(b) Solubility: Solubility of the oil in 90% Alcohol was determined according to the method described by GUENTHER VOLUME 1.

In general factors such as adulteration and aging of the oil are known to affect solubility. Accordingly, freshly isolated oil sample was used.

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(c) Specific gravity: This is the ratio of a given volume of liquid at a specified temperature to that of an equal volume of water at the same temperature. It is an important criteria as far as quality and purity of an essential oil are concerned. The specific gravity was determined at 26°C in a 10 ml pycnometer.

A clean pycnometer together with a cork was weighed empty. Then filled with distilled water before inserting the cork having a capillary hole at the centre.

The cork was inserted so that excess water could go out through the hole. After removing excess water with a dry cloth the weight was determined. After emptying and wiping the pycnometer dry with the cloth, the procedure was repeated using the oil instead of distilled water. Specific gravity calculated according to the following formula:

$$\text{Specific gravity} = \frac{W_o - x}{W_w - x}$$

Where,

W_o = Weight of the oil with the Pycnometer.

W_w = Weight of the water with the Pycnometer.

x = Weight of the empty Pycnometer.

(d) Optical Rotation ($\alpha_D^{21°C}$ - Determination of this physical constant is based on the fact that when a beam of polarised light passes through a layer of liquid, it possesses a property of rotating the plane of polarisation clockwise (dextrorotatory) or anti-clockwise (laevorotatory). It is

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an important constant for both identification and quality characterisation of an oil. The determination was performed at 21°C using Atago Polarimeter (Japan) according to the method described by E. GUENTHER (Vol.1).

Opopanax oil dissolved in n-hexane (2% $^{v}/_{v}$) was placed into Atago Polarimeter making sure that it is completely filled. The direction and degrees of rotation were then measured. Six readings taken and the average value calculated. The same procedure was repeated with pure n-hexane, and finally with commercial opopanax oil.

The difference between the average value of the oil dissolved in n-hexane (solvent) and that of pure solvent was taken as the optical rotation in each case.

(e) Refractive Index (n) - This is the ratio of the sine of the angle of incidence to the angle of refraction of a beam of light when it passes from a less dense to a more dense medium. The constant was determined using Abbe Refractometer with Sodium D line at 21°C according to the method recommended by E. GUENTHER (vol. 1).

The prisms of the instrument were carefully cleaned with dilute alcohol and allowed to dry. A few drops of the oil applied onto the lower prism and the instrument closed. The instrument adjusted such that two portions, a brightly illuminated and darkly illuminated were brought to the field of vision as viewed through the eye-piece. Further adjustment done so that the two portions were equally illuminated and the readings

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taken. Four readings were taken and the average value calculated. The above procedure was repeated but instead of the isolated oil, the commercial opopanax oil was used.

CHEMICAL PROPERTIES

(a) Acid value: - This was determined according to the method described by E. GUENTHER (Vol.1).

0.1 N potassium hydroxide solution was prepared and standardised. About 2.5 g of oil accurately weighed was dissolved in 15 ml of neutral 95% alcohol in 100 ml suponification flask. 3 drops of 1% phenolphthalein added and the mixture titrated with 0.1 N potassium hydroxide solution adding the alkali drop ise at the rate of 30 drops per minute, with continual agitation. The end point was taken as the appearance of a reddish-pink colour which did not fade within 10 secs. Two determinations carried out and the average value calculated. Blank was carried out following the same procedure but without the oil. The difference between the blank and the value of the sample taken as the number of 0.1 N potassium hydroxide required. Acid value is given by the following formula:

$$\text{Acid value} = \frac{(\text{No. of mls of 0.1 N Potassium hydroxide required} \times 5.61)}{\text{Weight of the oil used in gms.}}$$

(b) Ester value: - This is the number of milligrams of potassium hydroxide required to neutralise the acids resulting from the hydrolysis of 1 gm of the oil. The determination was carried out according to the method described by E. GUNTHER (Vol. 1).

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About 1.5 g of the oil weighed accurately was dissolved in 5 ml of 95% alcohol, which had been boiled and neutralised to phenolphthalein solution, in a suponification flask. The free acids neutralised by titrating the mixtures with 0.1 N alcoholic potassium hydroxide using 0.2 ml of a 1% phenolphthalein solution. 10 ml of 0.5 N alcoholic potassium hydroxide added and the flask attached to a reflex condenser. Boiled on a water-bath for 1 hour and permitted to cool for 15 minutes at room temperature. Then 20 ml of water and 0.2 ml of 1% phenolphthalein added. The excess alkali in the mixture titrated with standardised 0.5 N hydrochloric acid. Two determinations were carried out and the average value calculated. The procedure was repeated but without the sample. The difference between the volume of the acid used in the blank and the acid used in the presence of the oil was taken as equal to the alkali required to saponify the esters. Ester value is given by the following equation:

$$\text{Ester value} = \frac{28.05\ (B - V)}{W}$$

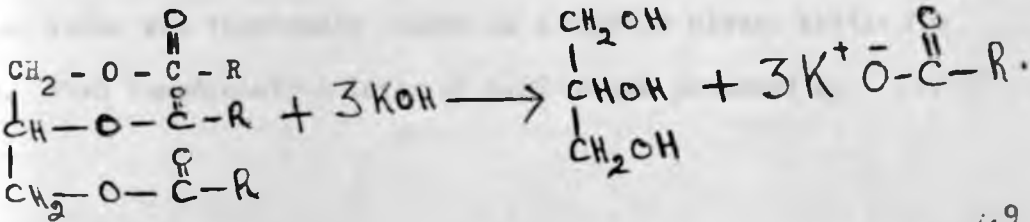
Where,

B = Volume in mls of 0.5 N Hydrochloric acid required to neutralise the blank.

V = Volume in mls of 0.5 N Hydrochloric acid required to neutralise the excess alkali.

W = Weight of the oil sample in grams.

The reaction taking place in the saponification flask during hydrolysis is:



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THIN LAYER CHROMATOGRAPHY OF THE OIL

Thin layer chromatography was carried out according to the method described by Stahl (1969). Ascending Technique was employed.

Preliminary work was done using microscope slides in order to obtain a suitable mobile-phase. The slurry was prepared by shaking a mixture of 35g of silica gel 60^{GF}254 and a 100 ml of Chloroform - methanol (2:1) vigorously for 5 minutes in a stoppered conical flask. A pair of clean, dry microscope slides held back - to - back was dipped into the slurry and slowly withdrawn. The two slides separated and the solvent allowed to evaporate to dryness. Meanwhile, mobile solvent was poured into a small development tank, closed and allowed to saturate the tank. Using capillary tubes spotting was done and the oil used was dissolved in n - hexane 2. $^{v}/_{v}$. After development visualisation was first carried out using ultraviolet light then vanillin - sulphuric acid reagent. The plates were sprayed with the vanillin - sulphuric acid reagent (1% $^{w}/_{v}$) and heated at 105^{o} for 10 minutes to enable maximum colour intensity to occur. This was repeated with several mobile - solvent systems and Benzene - Acetone 97.5 : 2.5 respectively was found to be the suitable solvent system.

Spotting and development was then carried out on larger plates 20 x 20 cm using ascending technique and the same mobile - phase. The 20 x 20 cm plates were washed with tap water using a small amount of detergent. The detergent removed by washing the plates further with copious quantities of water. Dried and rinsed with acetone. Placed on the table and tightly clumped. 30 gm of silica gel 60 GF_{254} and 60 ml of distilled water was vigorously shaken in a tightly closed bottle for 10 minutes. Then immediately a layer of 0.25 mm was prepared by

.../20

spreading the slurry on the plates using a desaga spreader. Plates allowed to dry at room temperature and then activated at 105°C for 30 minutes.

Mobile - solvent, Benzene - Acetone (97.5 : 2.5) was poured into development tanks and allowed to saturate the tanks for 1 hour.

The base line was marked at least 1 cm from the edge and the oil dissolved in n - hexane 2% $^{v}/_{v}$ was applied. Spotting was done using capillary tubes with the aid of a template. Then the plates put on development tanks and the solvent front (length of run) of at least 15 cm allowed. Visualisation was carried out as in the case of microscope slide. Then the chromatogram was traced.

COLUMN CHROMATOGRAPHY OF THE OIL

Differential movement of individual solute through a porous medium is the basis of this technique. Those solutes which are least adsorbed move more rapidly than those which are strongly adsorbed. This difference causes separation. The column chromatography was carried out according to the method recommended by Trease and Evans (1972) using wet packing method.

A glass column with sintered glass at the lower end was prepared by alumina. Alumina was made into a slurry with Benzene: Acetone (97.5 : 2.5) and poured directly into the column with persistent tapping of the column to ensure even packing. Excess of the solvent allowed to drain until it was only about 1 cm above the top level of the alumina. 0.2 ml of pure oil was introduced on the adsorbent top using a 1 ml pipette. Then the elution was carried out using benzene: acetone (97.5 : 2.5), collecting the eluate in fractions of 20 ml aliquots. Finally, elution

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was carried out using chloroform: methanol (1:9 respectively) Thin layer chromatographic studies of the eluate was carried out. Then the fractions allowed to evaporate under toom temperature and atmospheric pressure in a dark cupboard.

GAS LIQUID CHROMATOGRAPHY (G. L. C.)

Qualitative Assay of Opopanax oil was done according to the method described by H.P. Burchfield and Eleanor E. Storrs (1962). Pye unicam apparatus series 104 chromatograph was used. The column used was a coiled shaped glass, 1.5 m long and 4 mm internal diameter (i.d.) with carbowax 20 m as stationary phase on chromosob WHp. The column was operated by temperature programming at 2°C per minute using Nitrogen as carrier gas with flow rate of 20 ml per minute. 1 ml of 2% $^{v}/_{v}$ opopanax oil dissolved in n - hexane was injected.

The column was conditioned by putting on the chromatograph for 1 hr., the flow rate of carrier gas adjusted and the paper chart movement set at 2 minutes per centimeter. Then 1 ml of the sample injected rapidly using Agla Syringe.

CONDITIONS FOR THE CHROMATOGRAPH

Chromatograph - pye unicam series 104.

Column Dimensions - 1.5 m coiled glass tube x 4 mm i.d.

Solid support - chromosob WHp

Stationary phase - 12% carbowax 20 M.

Temperature Prgramming - 75°C to 225°C at 2° per minute.

Chart speed - 2 minutes per centimeter.

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Attenuation - 50×10^4

Carrier gas - Nitrogen

Flow rate - 20 ml per minute

Detector - Flame ionization Detector (F.I.D.)

The qualitative assay of the oil was repeated using the same method. The conditions for the chromatograph were the same as above except for the following:

Instead of coiled glass tube, coiled metal tube of the same dimensions was used. 20% carbowax 20 M as stationary phase was replaced by 2.5% OVL. And the solid support chromosob wap was replaced by Diatomite CQ (80 to 120 mesh).

INFRA RED SPECTROSCOPY

In order to obtain pure samples of the constituents of the oil, Preparative Thin Layer Chromatography was first carried out.

Using 20 x 20 cm plates, washed and rinsed with acetone, a layer of 0.75 mm was prepared with a slurry made of Kieselgel G Fu 54 and distilled water. The layers allowed to dry at room temperature and activated at 105°C for 10 minutes. The same mobile phase - Benzene: Acetone (97.5 : 2.5) was used. Using a micropipette many spots were applied very close to one another so as to form a band of oil. Developed in a previously saturated tanks. After development plates were removed and allowed to dry. Some layer removed along the edge on either side so as to leave a layer of about 17 cm in the middle and a layer of 2 cm on either edge. The middle layer covered and the layers on the edges sprayed with 1% W/V vanillin-sulphuric acid reagent. Bands located and marked on either side of the plate. Each band was collected separately from the middle layer, dissolved in 20 ml chloroform and filtered.

Using Perkin - Elmer infra red spectrometer 727 B and a cell of path length 10 mm, infra red spectrum was developed. Solvent absorption was removed by use of another cell of the same path length containing only pure chloroform in the reference beam.

ULTRA VIOLET SPECTROSCOPY OF THE OIL

Isolation of individual components of the oil was done in same way as for infra red spectroscopy.

Ultra violet spectrum was developed using Recording ultra violet Spectrometer - Unicam Sp 800A.

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PART II

ISOLATION OF RESINS & DETERMINATION AND MICROBIOLOGICAL ASSAY OF BOTH THE RESINS AND THE OIL

PART 11

ISOLATION AND DETERMINATION OF RESINS OF GUM- OPOPANAX

Resins are amorphous solid or semi-solid fusible substances insoluble in water but soluble in 90% alcohol, ether and chloroform. The ether - , chloroform - and alcohol - insoluble portion of the gum contains proteins and carbohydrates. This difference provides a means of isolating the resins (Trease & Evans 11Th ED - 1978).

The isolation of the resins was done using a continuous soxhlet apparatus (Figure 2). About 25 gm of powdered gum-opopanax was weighed accurately into a thimble put into position and chloroform (extracting solvent) was poured, just enough to be syphoned, into the thimble compartment. A further 20 ml of chloroform added and the resins extracted exhaustively for 2 hrs, by heating under reflux over a water-bath. Timing was started as soon as the first syphoning had occurred. Meanwhile the weight of a clean, dry beaker was taken and 20 ml of distilled water poured into it.

The extract was concentrated under vacuum, and the concentrate poured into the beaker. Excess water poured off and the resins left to dry. The weight of the resin together with the beaker was taken. Two determinations were carried out and the average value calculated.

This general method of extracting resins is described in Remington's pharmaceutical Sciences 15Th Edition (1975).

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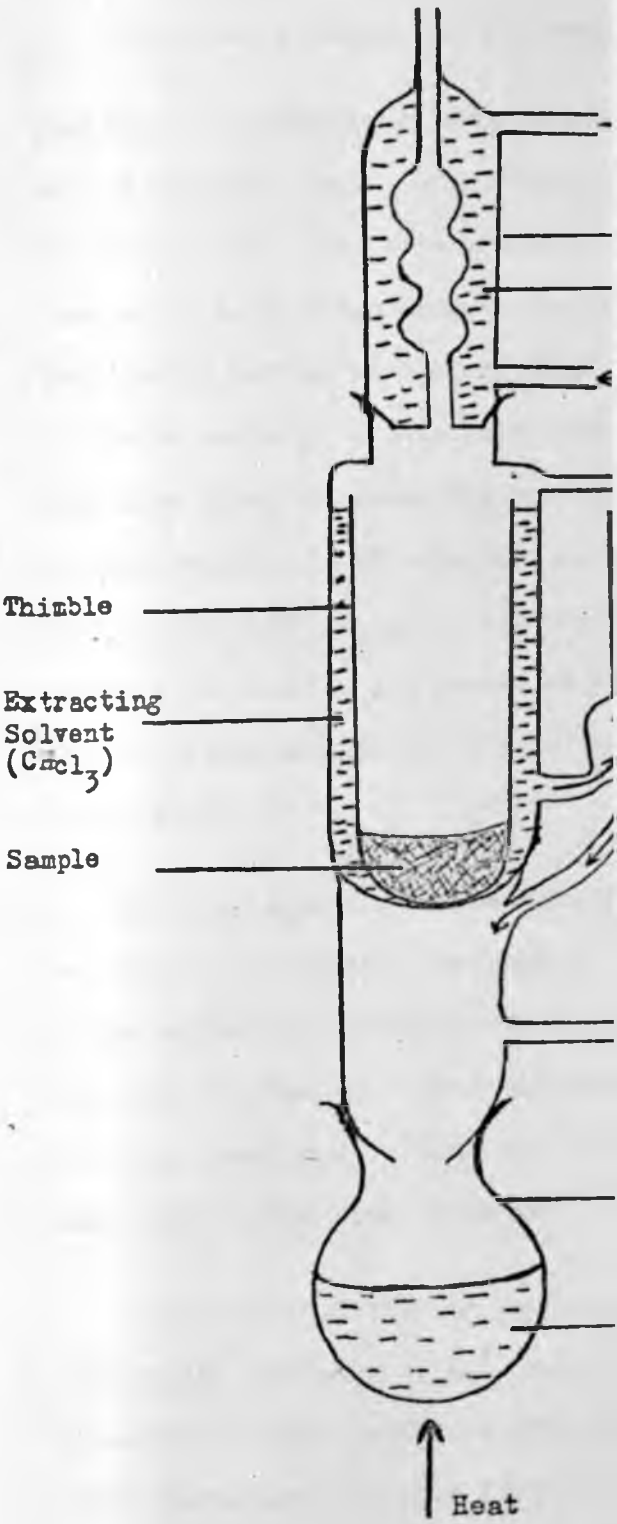
.../25

Figure 2



MICROBIOLOGICAL WORK OF THE OIL AND RESINS USING STAPHYLOCOCCUS AUREUS AND ESCHERICHIA COLI

The method followed was in accordance with Jawetz, E. (16).

Procedure: Petridishes were washed with tap water and rinsed with distilled water. Double-wrapped with paper bags and put in an autoclave. The media were prepared by suspending 4.2 g of Nutrient agar and 7.8. g of MacConkey's agar in 50 ml of distilled water separately in two bottles with tight fitting caps. Then put in the autoclave. The whole contents of the autoclave sterilised at 121°C for 15 minutes. Meanwhile asceptic screen was prepared by swabbing with cetrimide-Alcohol solution (0.5% cetrimide in 70% ethanol). The petridishes were transferred into the asceptic screen after removing the outer wrapping as close to the screen as possible. The bottles containing the media swabbed with the alcohol-cetrimide solution and placed in the screen.

Nutrient agar was casted onto three petridishes in the screen and to other 3 petridishes MacConkey's agar was casted. Left to dry in the screen then staphylococcus Aureus inoculated on the 3 petridishes containg Nutrient agar by swabbing. Using another swab, Escherichia Coli was inoculated on MacConkey's agar in the petridishes. Then each plate divided into 3 areas.

Paper discs previously impregnated in pure opopanax oil and dried were placed onto area 1, and paper discs impragnated in 5% $^{w}/_{v}$ resins dissolved in ether and dried were placed on area 2. Then to area 3 pape discs impregnated in pure diethyl ether and dried were placed. Then the media incubated at (37 - 38)°C for a period of 7 days. Observations

.../27

were made each day during this period. Zones of inhibition were measured.

Escherichia coli is an enteric gram negative organism.

Staphylococcus Aureus is a gram positive organism.

COMPOSITION OF NUTRIENT AGAR

Lab - Lemco powder	1 g
Yeast Extract	2 g
Peptone	5 g
Sodium Chloride	5 g
Agar No. 3	15 g

COMPOSITION OF MACCONKEY'S AGAR

Peptone	20 g
Bile salt	5 g
Lactose	10 g
Sodium Chloride	5 g
Neutral Red	0.075 g
Agar No. 3	12 g

RESULTS

Parameter / Test	Isolated oil	Commercial coconut
Moisture content	15.0 %	-
Solubility in diethyl	Insoluble	-
Specific gravity at 25°C	0.9500	-
Optical density ($D^{1\%}_{1cm}$)	4.20	3.80
Refractive index at 25°C	1.4500	1.4500
Acid value	2.02	-
Saponification value	1.12	-

R E S U L T S

THE YIELD AND PHYSICO - CHEMICAL PROPERTIES

Appearance: The isolated oil was yellowish-green but turned to intense green colour on standing.

Odour and Taste: The oil had aromatic odour and a bitter taste.

Moisture content: The moisture content was found to be 17.10% $^{v}/_{w}$.

TABLE 1

Parameter under Test	Isolated opopanax oil	Commercial opopanax
Yield % $^{v}/_{w}$ (on moisture free basis)	13.60% $^{v}/_{w}$	-
Solubility in 90% Alcohol	1:20	-
Specific gravity at 26°C	0.95002	-
Optical Rotation ($\alpha_D^{21^\circ}$)	$^{+}7.07$	$^{+}8.95$
Refractive Index at 21°C ($n_d^{21^\circ}$)	1.51200	1.50840
Acid value	3.70	-
Ester value	7.390	-

Fig. 3:- THIN-LAYER CHROMATOGRAPHIC SEPARATION OF OPOPANAX OIL

Technique: One way ascending
Adsorbent: Silica gel 60 GF254
Mobile phase: Benzene: Acetone (Ratio 97.5 : 2.5)
Length of run: 17 cm
Temperature: Room temperature ≃ 25°C
Sample: 2% $^{v}/_{v}$ opopanax oil in n-hexane
Visualisation reagent: 1% $^{w}/_{v}$ Vanillin in Conc. H_2SO_4

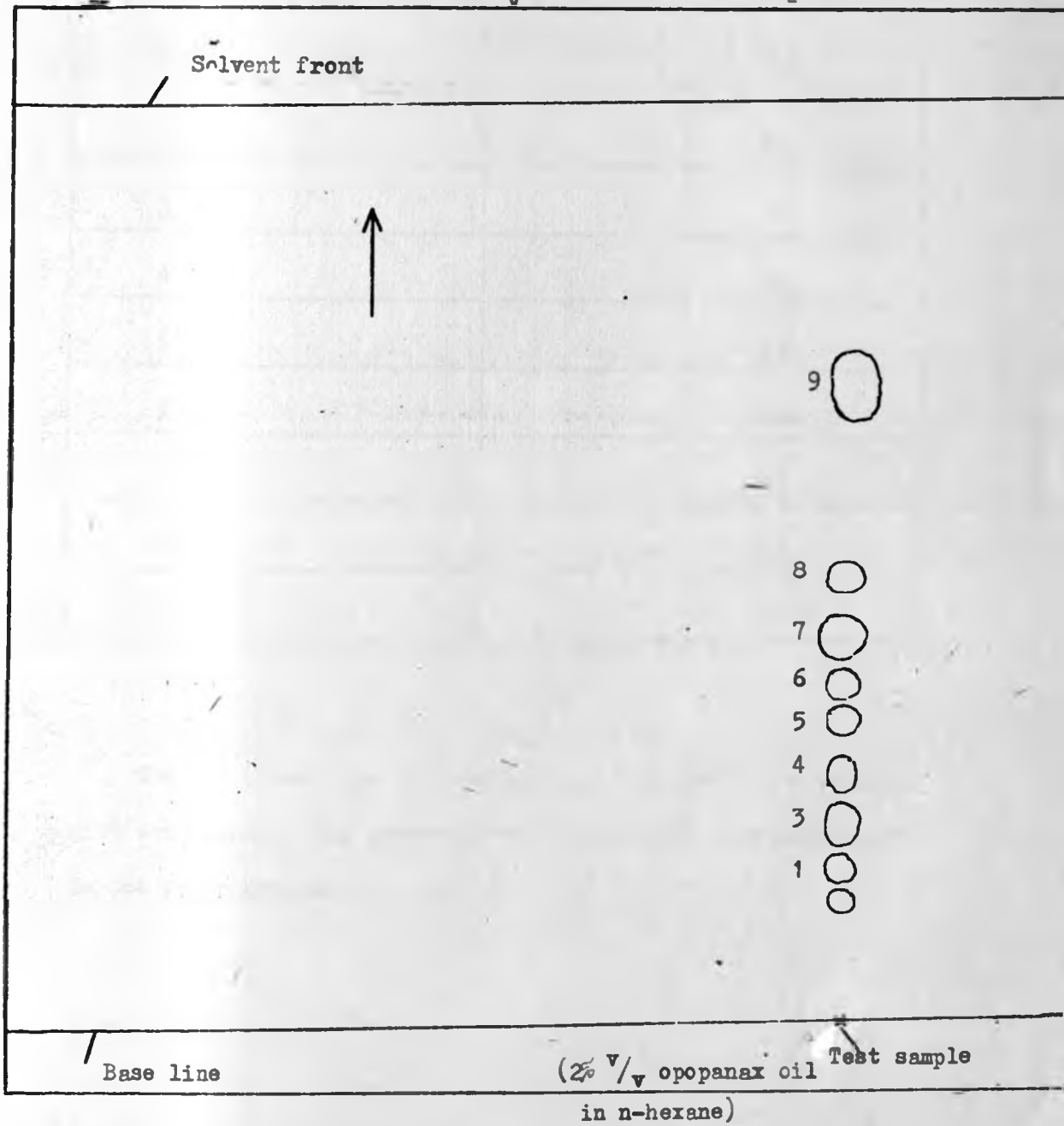


TABLE 2. TLC of opopanax oil Figure 3

Spot Number	$h^{R}f$ values
1	12
2	16
3	21
4	25
5	33
6	38
7	43
8	49
9	68

The TLC (thin layer chromatography) indicated the presence of 9 components. The component with higher $h^{R}f$ value appeared to be the major component.

COLUMN CHROMATOGRAPHY

Twenty five fractions each 20 ml were collected separately. Only twenty of these fractions indicated the presence of the sample when subjected to thin-layer chromatography (TLC.) Out of these fractions only four indicated the presence of an individual component on TLC. These were fractions 2 (Fig. 4), 11 (Fig. 6), 17 (Fig. 8) and 18(Fig.8) The other fractions indicated the presence of more than one component ranging from two to six.

The thin-layer chromatograms (TLC Chromatograms) were shown in Figures 4 to 8. The Rf value of each fraction spot-component was calculated. By multiplying by hundred the Rf values were converted to hRf values, and were as shown in Table.

In most cases the hRf values obtained were compareable with the hRf values obtained in TLC prior to column chromatographic separation (Table 2). However, better separation was achieved by first separating the sample by column chromatography followed by TLC as seen in figures 4 to 8.

After allowing the fractions to evaporate in a dark cupboard fraction 13 was seen to show resinification.

Fig. 4:-

T.L.C. AFTER COLUMN CHROMATOGRAPHY

Technique: One way ascending

Adsorbent: Kieselgel G/u 254.

Mophile phase: Benzene: Acetone (Ratio 97.5 : 2.5)

Temperature: Room temperature 25°C.

Sample: Opopanax oil in Benzene: Acetone mixture (Ratio 97.5 : 2.5).

Visualisation Reagent: 1% $^{w}/_{v}$ Vanillin in Conc. H_2SO_4.

Length of run: 16.5 cm.

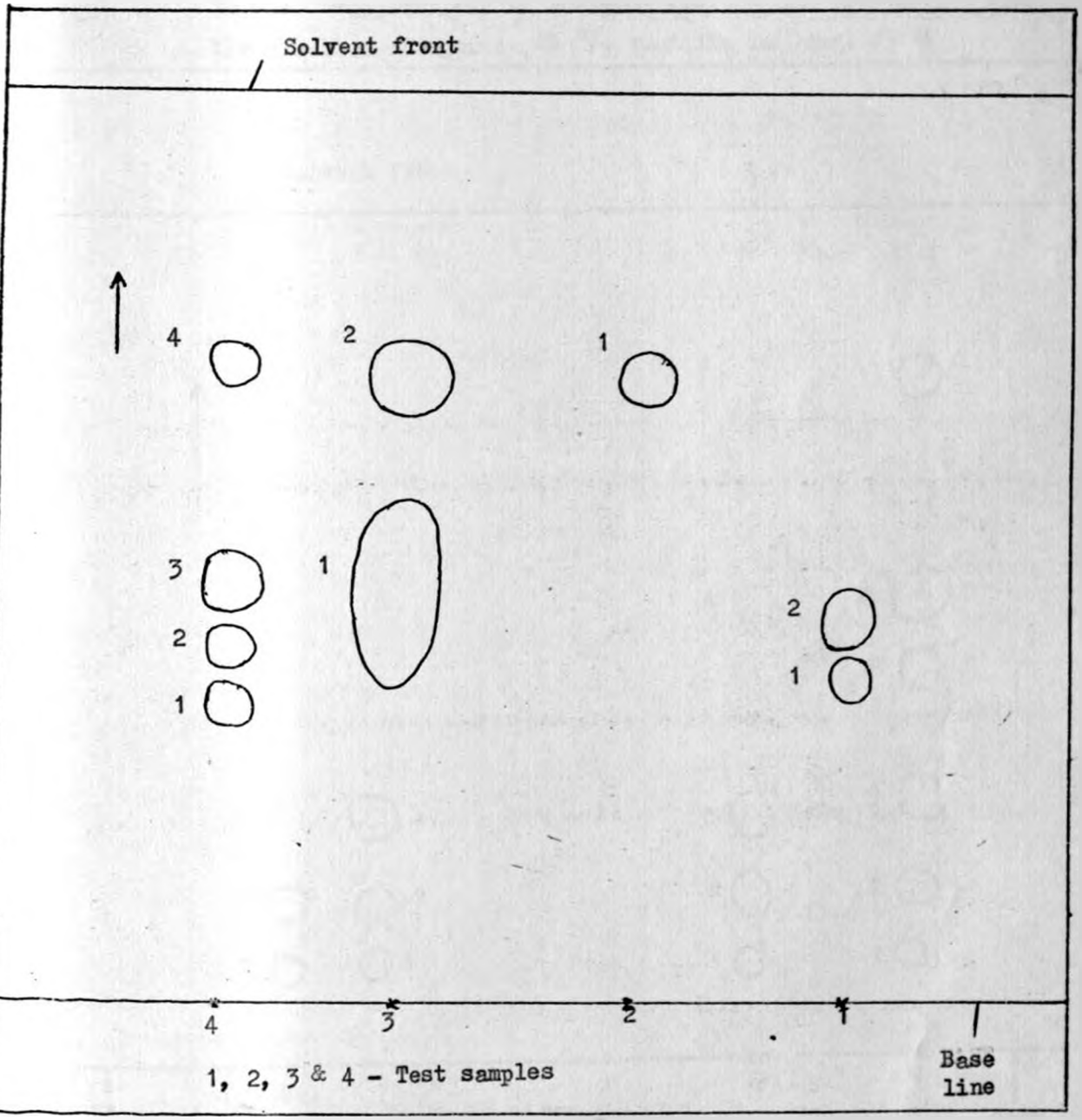


Fig 5 :- **T.L.C. AFTER COLUMN CHROMATOGRAPHY**

Technique: One way ascending.
Adsorbent: Kieselgel G/u 254
Mobile phase: Benzene: Acetone (Ratio 97.5 : 2.5)
Length of run: 15.4 cm.
Temperature: Room temperature $\simeq$ 25°C.
Sample: Opopanax oil in Benzene: Acetone mixture (97.5 : 2.5)
Visualisation:Reagent: 1% $^{W}/_{V}$ vanillin in Conc. H_2SO_4

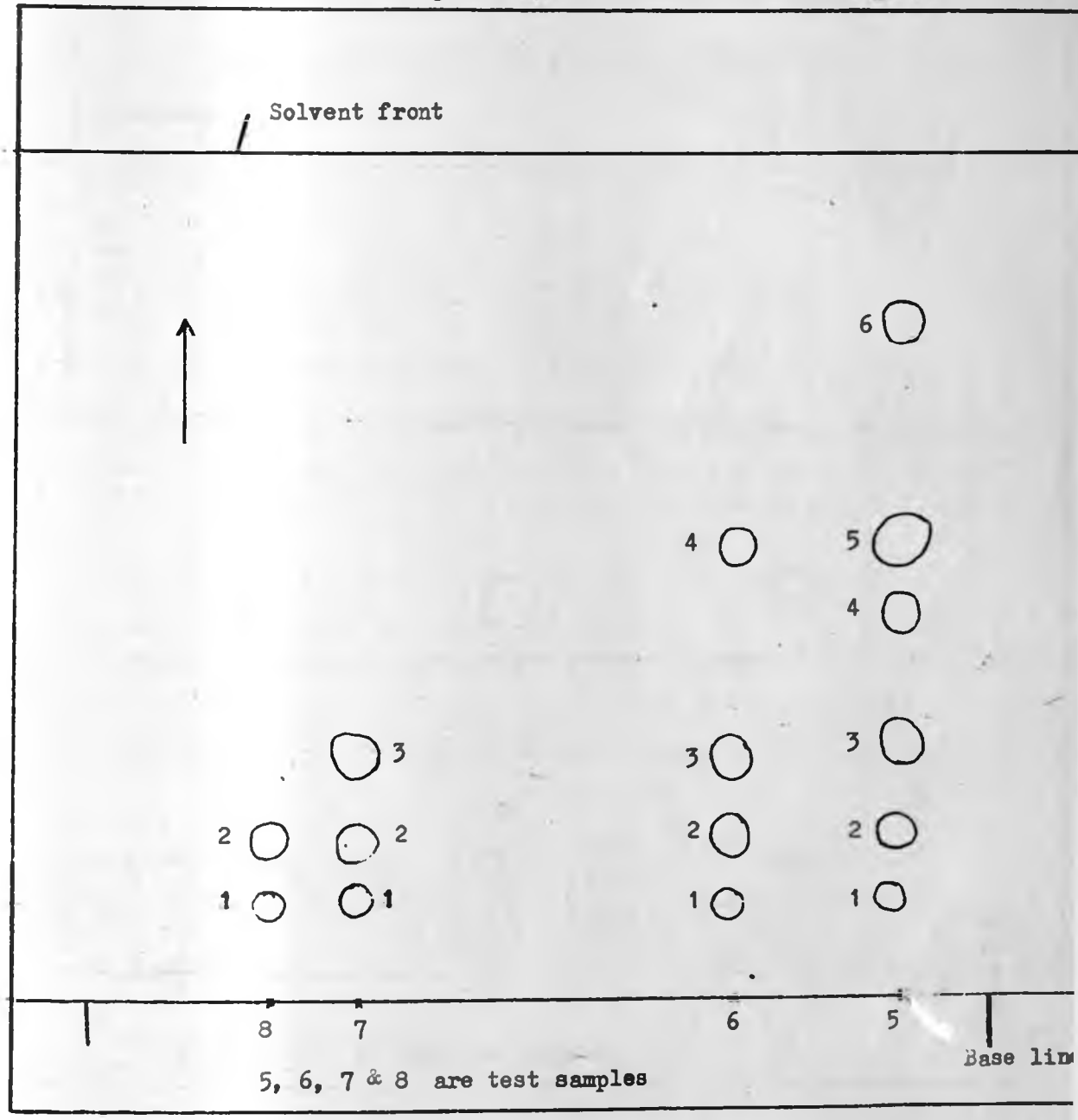


Fig. 6.- T.L.C. AFTER COLUMN CHROMATOGRAPHY

Technique: One way ascending
Adsorbent: Kieselgel G/u 254
Mophile phase: Benzene: Acetone (Ratio 97.5 : 2.5)
Length of run: 14.7 cm.
Temperature: Room temperature ≃ 25°C.
Sample: Opopanax oil in Benzene: Acetone mixture, (Ratio 97.5:2.5)
Visualisation: Reagent: 1% $^{w}/_{v}$ Vanillin in Conc. H_2SO_4

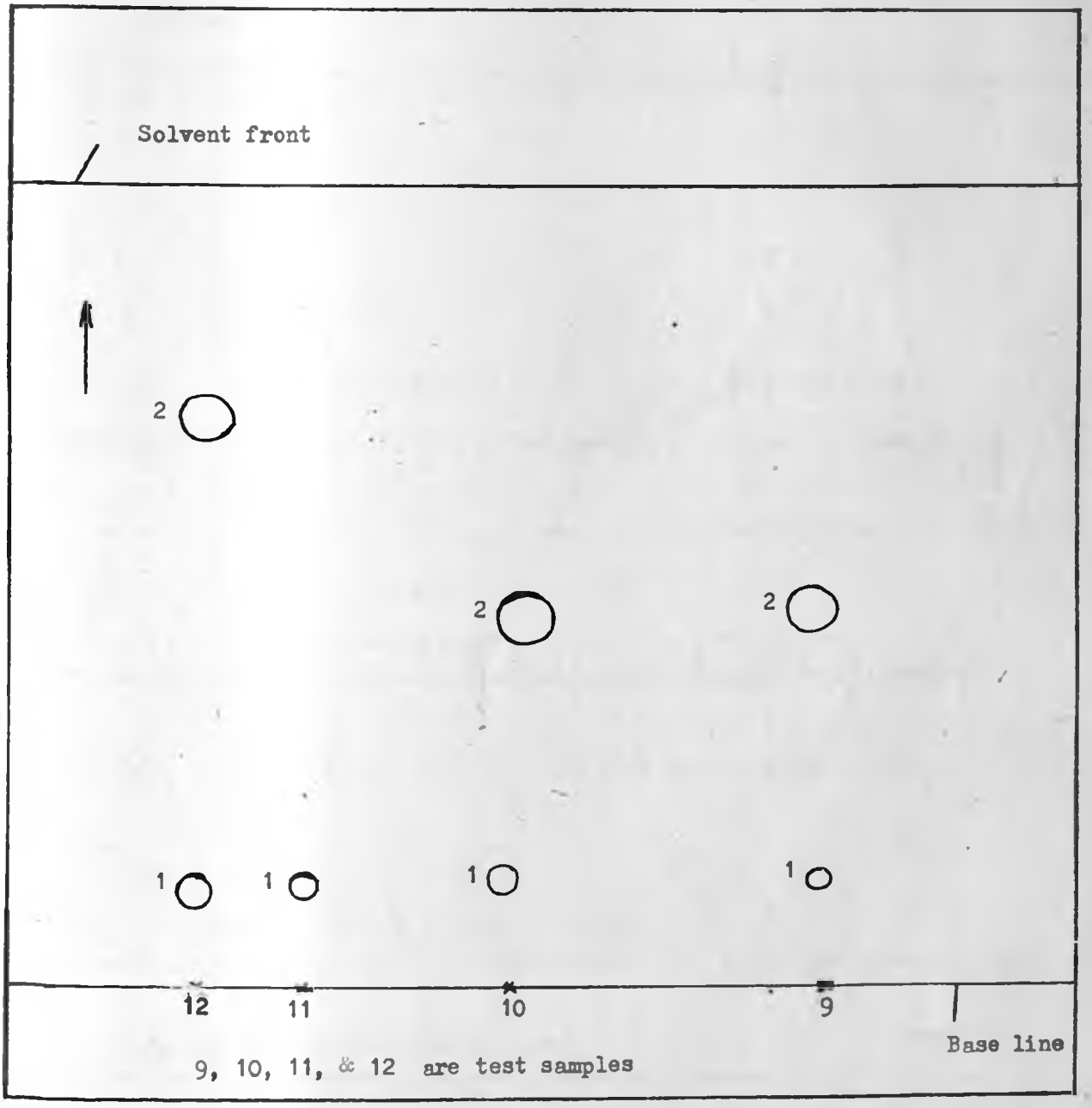
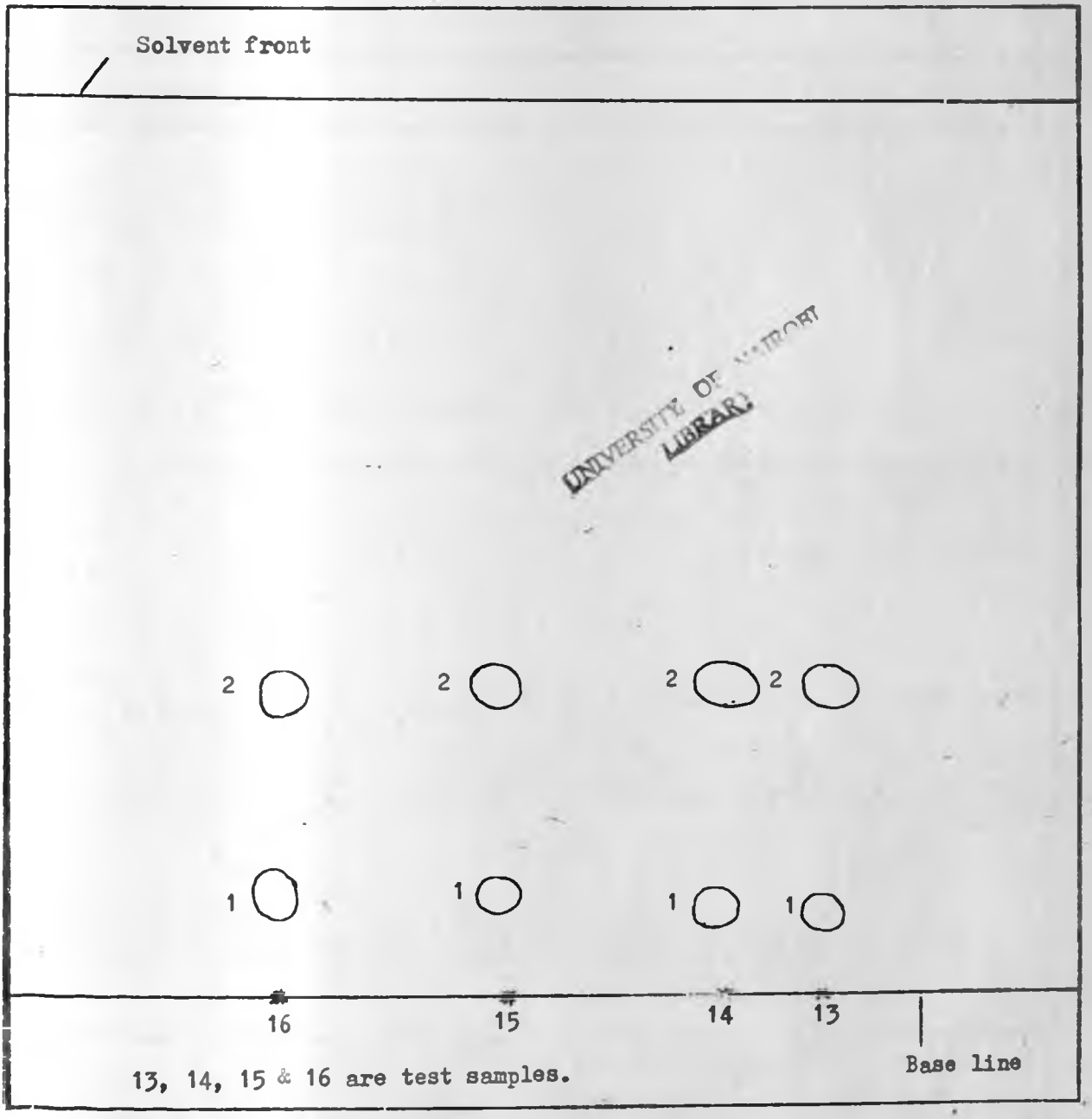


Fig. 7 :-

T.L.C. AFTER COLUMN CHROMATOGRAPHY

Technique: One way ascending.
Adsorbent: Kieselgel G/u 254.
Mobile phase: Benzene: Acetone (Ratio 97.5 : 2.5)
Length of run: 16.3 cm.
Temperature: Room temperature 25°C.
Sample: Opopanax oil
Visualising Reagent: 1% $^{w}/_{v}$ Vanillin in Conc. H_2SO_4.



NB: Chloroform - methanol mixture had been introduced when these fractions were trapped.

Fig 9:- T.L.C. AFTER COLUMN CHROMATOGRAPHY

Technique: One way ascending

Adsorbent: Kieselgel G/u 254

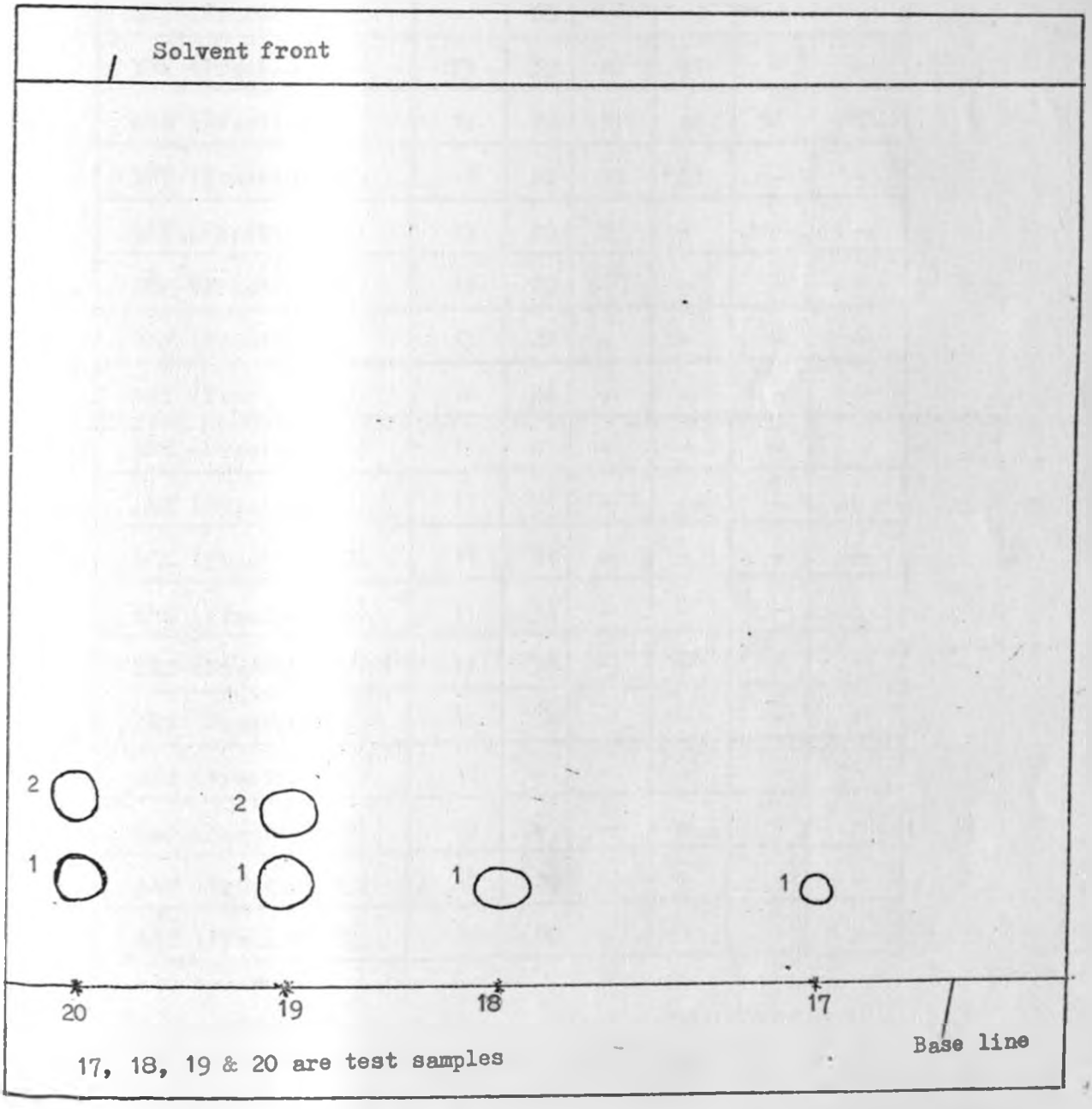
Mobile phase: Benzene: Acetone (Ratio 97.5 : 2.5)

Length of run: 16.5 cm.

Temperature: Room temperature $\simeq$ 25°C

Sample: Opopanax oil

Visualisation: 1% $^{w}/_{v}$ Vanillin in Conc. H_2SO_4



NB. Chloroform - methanol had been introduced when these fractions were being trapped.

hRf VALUES OF COMPONENTS OBTAINED BY SUBJECTING INDIVIDUAL FRACTIONS TO TLC

TABLE 3:

Spot number	1	2	3	4	5	6
hRf (Fraction 1)	37	44	-	-	-	-
hRf (Fraction 2)	69	-	-	-	-	-
hRf (Fraction 3)	-	68	-	-	-	-
hRf (Fraction 4)	33	39	46	71	-	-
hRf (Fraction 5)	12	20	31	46	54	81
hRf (Fraction 6)	12	20	29	53	-	-
hRf (Fraction 7)	12	20	29	-	-	-
hRf (Fraction 8)	12	20	-	-	-	-
hRf (Fraction 9)	13	47	-	-	-	-
hRf (Fraction 10)	14	46	-	-	-	-
hRf (Fraction 11)	12	-	-	-	-	-
hRf (Fraction 12)	13	71	-	-	-	-
hRf (Fraction 13)	11	34	-	-	-	-
hRf (Fraction 14)	11	34	-	-	-	-
hRf (Fraction 15)	11	34	-	-	-	-
hRf (Fraction 16)	11	34	-	-	-	-
hRf (Fraction 17)	12	-	-	-	-	-
hRf (Fraction 18)	12	-	-	-	-	-
hRf (Fraction 19)	12	20	-	-	-	-
hRf (Fraction 20)	12	20	-	-	-	-

GAS - LIQUID CHROMATOGRAPH (G.L.C.)

Figure 9: G.L.C. of opopanax oil dissolved in n - hexane (2% $^{v}/_{v}$).

Nine peaks were obtained implying the presence of 9 components.

CONDITIONS FOR THE CHROMATOGRAPH

Chromatograph - pye union series 104

Column dimension - 1.5 m coiled glass tube x 4 mm i.d.

Solid support - chromosob W.P.

Stationary phase - 12% carbowax 20 m

Temperature programming - 75°C to 225°C at 2° per minute.

Chart speed - 2 minutes per centimeter.

Attenuation - 5- x 10^{4}

Carrier gas - Nitrogen

Flow rate - 20 ml per minute

Detector - F.I.D.

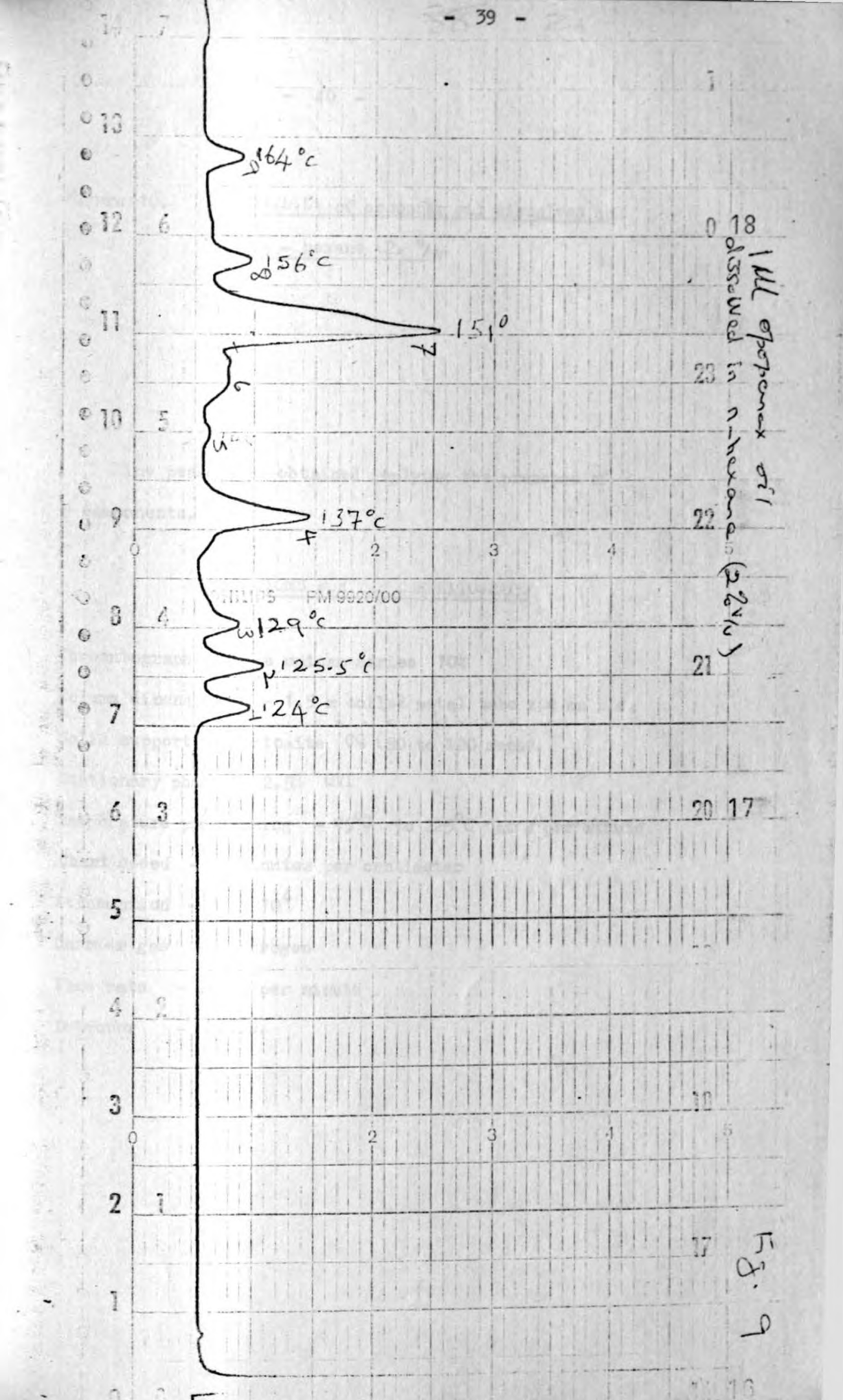

164°C
156°C
151°
137°C
129°C
125.5°C
124°C
PM 9920/00
1ML of penex oil dissolved in n-hexane (2%v/v)

Fig. 9

Figure 10. G.L.C. of opopanax oil dissolved in n - hexane (2 $^{v}/_{v}$

Nine peaks were obtained implying the presence of 9 components.

CONDITIONS FOR THE CHROMATOGRAPH

Chromatograph - bpye unicam series 104

Column dimensions - 1.5 m coiled metal tube x 4 mm i.d.

Solid support - Diatomite CQ (80 to 120 mesh).

Stationary phase - 2.5% OVI

Temperature programming - 75°C to 225°C at 2° per minute

Chart speed - 2 minutes per centimeter

Attenuation - 50×10^4

Carrier gas - Nitrogen

Flow rate - 20 ml per minute

Detector - F.I.D.

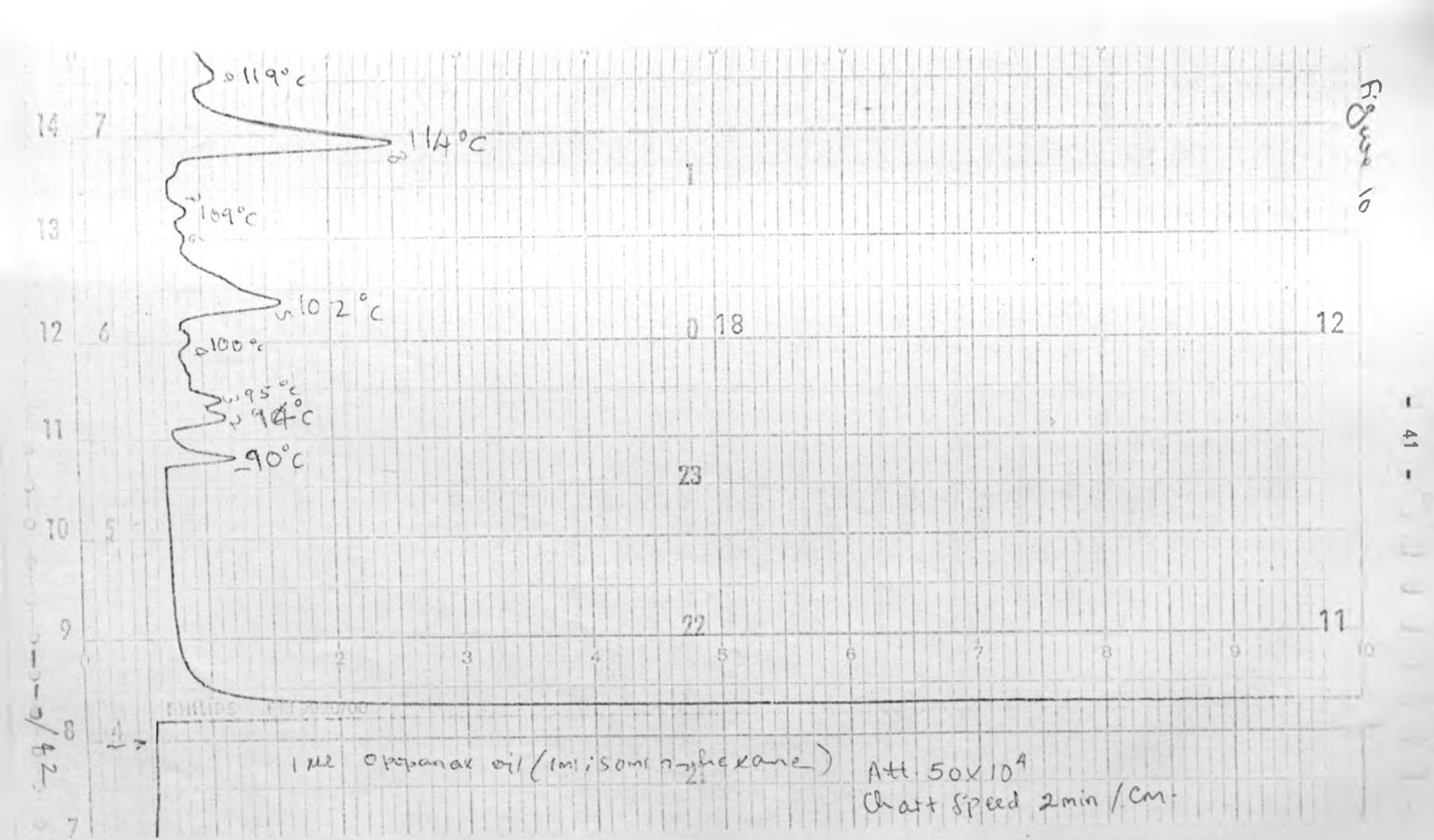

119°C
114°C
109°C
102°C
100°C
95°C
94°C
90°C
1 ml Oleoresin oil (1ml; isomn-hexane)
Att 50X10⁴
Chart speed 2min/cm.

Figure 10

RETENTION OF TIMES (Rt) Of THE OIL

TABLE 4: Rt of Isolated opopanax oil

Component	Rt (Chromatogram Fig. 9) (min)	Rt (Chromatogram Fig. 10) (min)
1	28.7	11.2
2	30.4	12.9
3	32.1	13.6
4	36.5	16.2
5	40.0	17.5
6	-	20.1
7	44.1	21.1
8	47.0	23.7
9	51.2	26.2

Figure 11. G.L.C. of Opopanax oil obtained from literature (14)

GAS CHROMATOGRAPHIC CONDITIONS FOR ANALYSES OF THE SESQUITERPENE HYDROCARBONS (14)

Chromatograph - Perkin - Elmer Model 881 gas chromatograph.

Column dimensions - 150 feet long x 0.01 i.d. capillary tube

Solid support - V930 (1%) = Versamid 930, Polyamide resin.

Stationary phase - OS138 (85%) and CO880 (14%)

OS138 = Polyphenylether (6 ring)

CO880 = Igepal CO880, nonyl phenoxy-Poly- (ethyleneoxy - ethanol.

Carrier gas flow N_2 - 0.7 ml per minute.

Temperature ^{o}C

Detector - 190^{o}C

Injector - 250^{o}C

Column - 170^{o}C

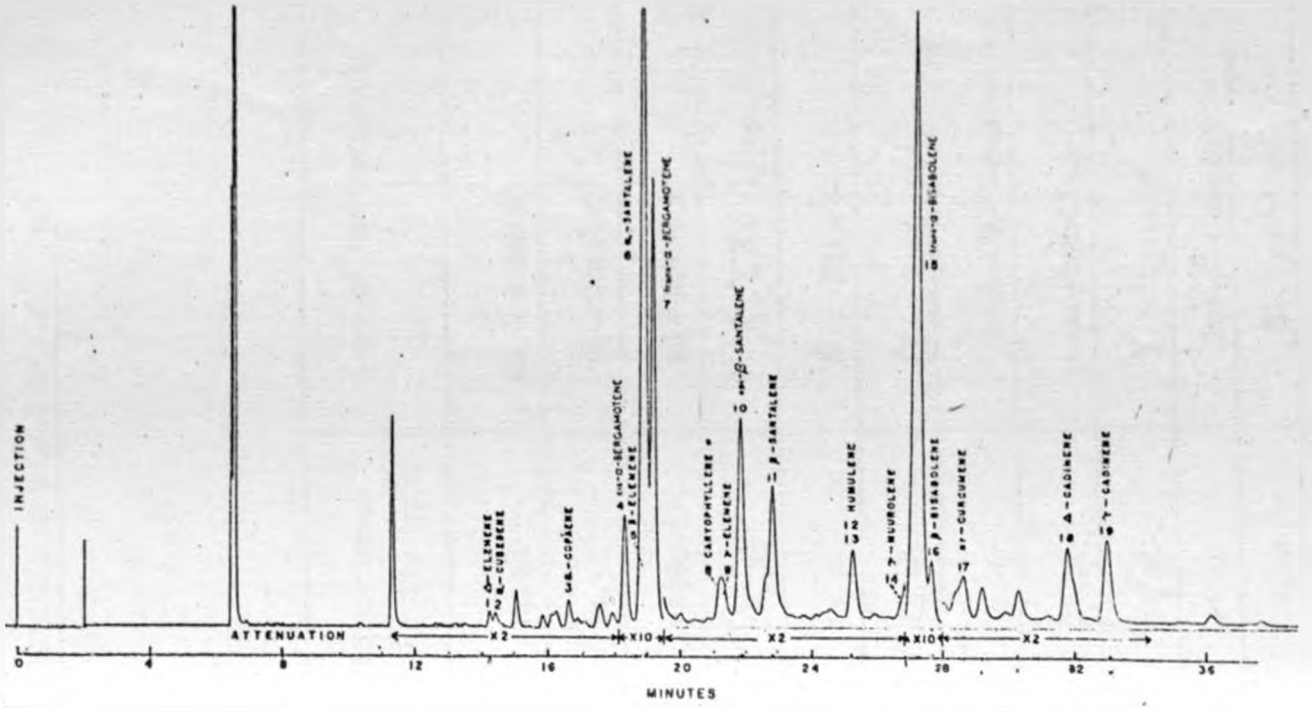


Figure 11 – Capillary column gas chromatogram of sesquiterpene hydrocarbon fraction of opoponax oil:

RETENTION TIME OF THE OIL (CHROMATOGRAM FIG. 11) OBTAINED BY WEINIGER J.A. (14)

TABLE 5:

Component	Rt (Chromatogram Fig. 11) (min)	Name of the compound
1	14.30	Δ - Elemene
2	14.50	α - Cubebene
3	16.60	α- Copaene
4	18.30	Cis -β- Bergamotene
5	18.70	β - Elemene
6	18.90	α- Santalene
7	19.20	trans- α- bergamotene
8	21.20	caryophyllene
9	21.20	γ - Elemene
10	21.60	Epi-β - santalene
11	22.80	β- santalene
12	25.20	Humulene
13	26.90	Muurolene
14	27.30	Trans- α- bisabolene
15	27.60	β - bisabolene
16	28.60	ar-curcumene
17	31.80	Δ - cadinene
18	33.00	γ- cadinene

RETENTION TIMES COMPARABLE WITH THOSE IN LITERATURE (14)

TABLE 6:

Component	Rt (Fig. 9) minutes	Rt (Fig. 10) minutes	Rt (Fig.11 minutes	Name of the compound
1	28.7	-	28.60	ar-curcumene
3	32.1	-	31.80	Δ-Cadinene
3	-	13.60	14.30	Δ-Elemene
4	-	16.20	16.60	α-Copaene
5	-	17.5	18.30	-Cis-β-bergamotene
6	-	20.0	19.20	Trans-α-bergamotene
7	-	21.1	21.20	Caryophyllene
8	-	23.7	22.80	β - Santalene
9		26.20	26.90	Muurolene

Rt = Retention Time

INFRA - RED SPECTROSCOPY (IR)

The IR Spectra were as shown in figures 12, 13, 14, 15, 16, 17, 18 and 19. Samples used correspond to the spots given in TLC. Spot 6 sample was not isolated.

ULTRAVIOLET SPECTROSCOPY (UV)

The UV Spectra were as shown in figures 20 and 21

NO. 007-1493

PERKIN ELMER

CONCENTRATION

THICKNESS

PHASE CHCl3

REMARKS

SCAN MODE ACCY. ☐ SURVEY ☑ HI ENERGY ☐ CAL. ☐ RESOLUTION ☐

OPERATOR KOMALAN DATE 1/3/84

SPECTRUM NO.

SAMPLE 1

ORIGIN OPOPANAX OIL.

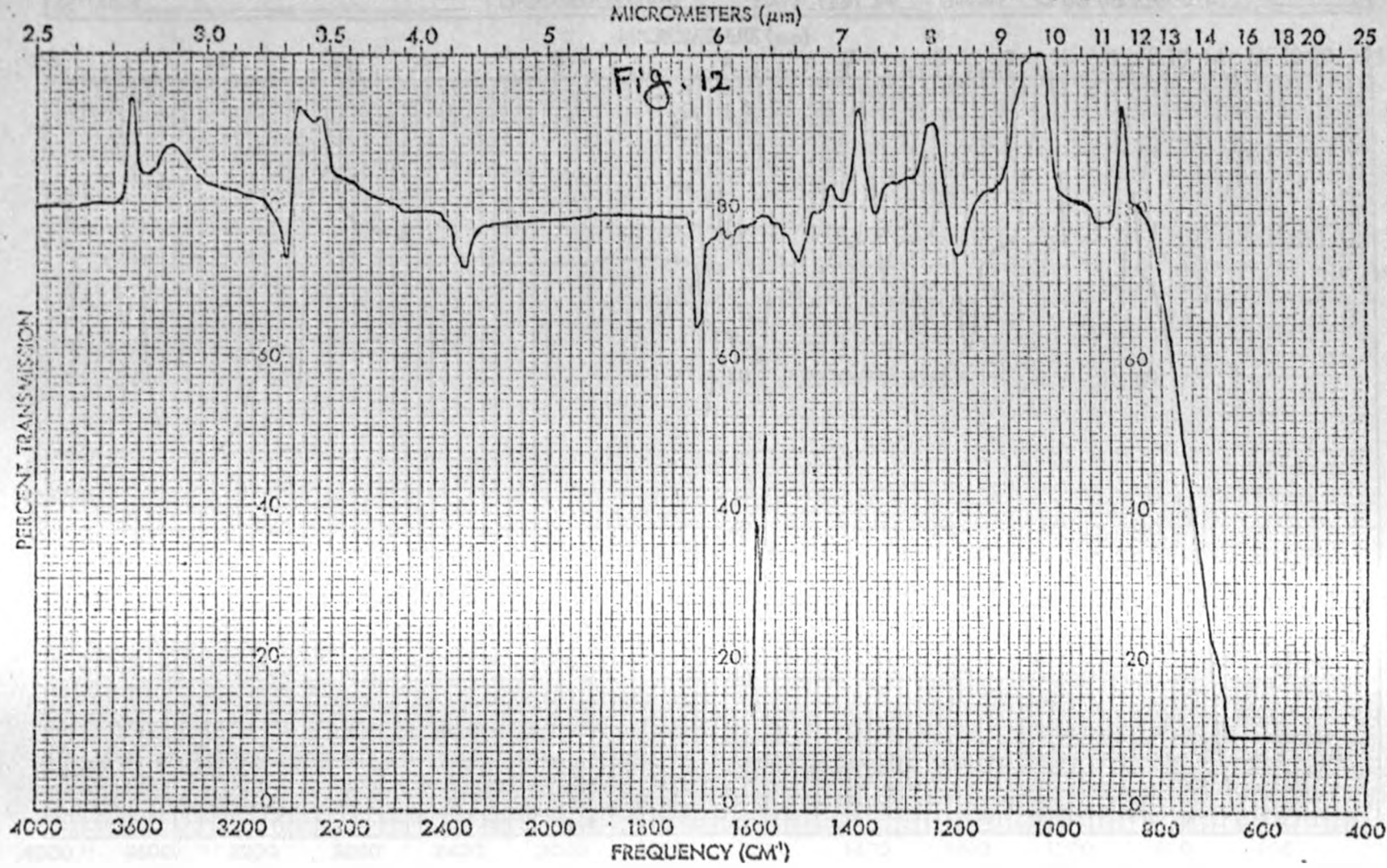


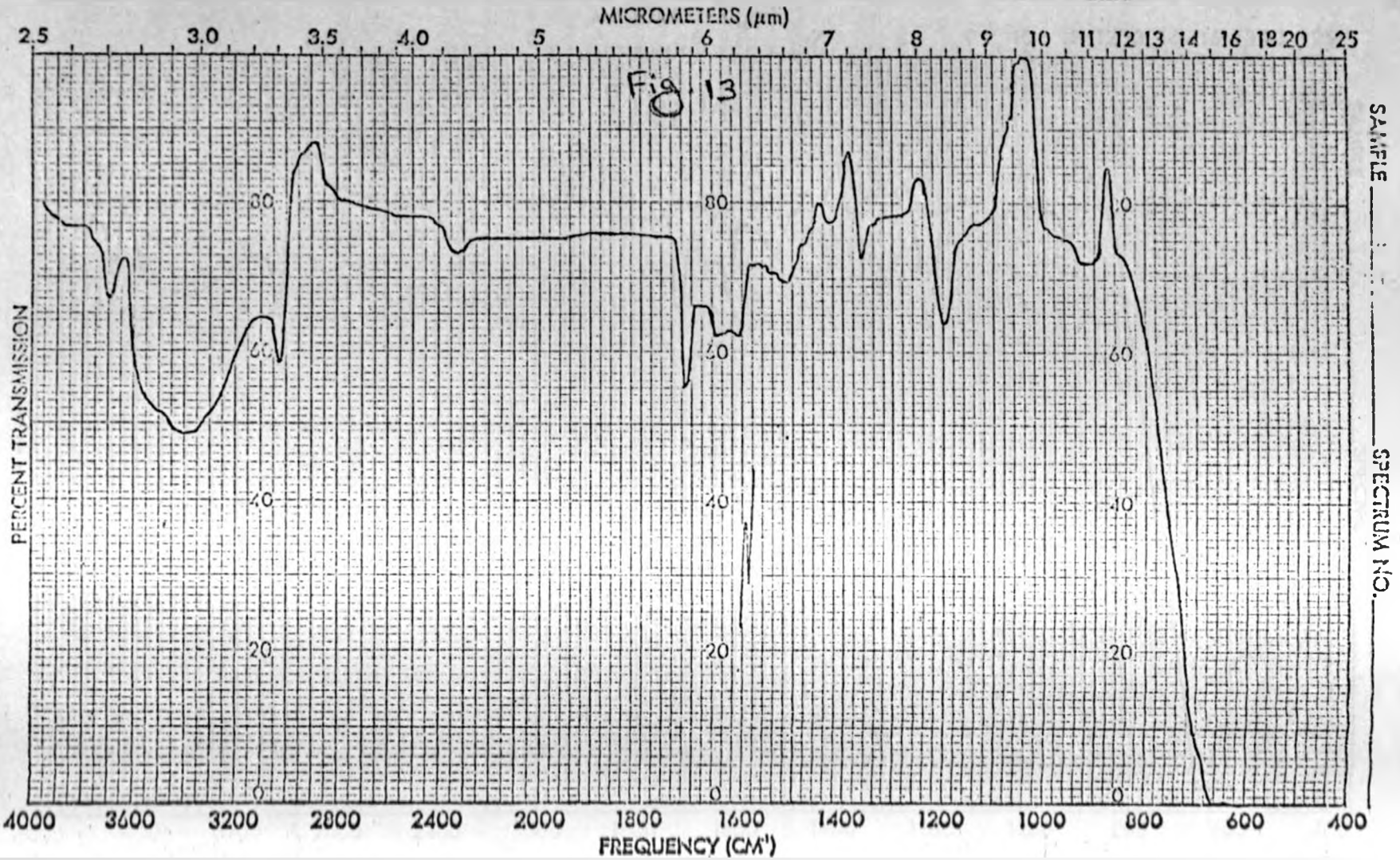
Fig. 12

NO. 057-1493

CONCENTRATION
THICKNESS
PHASE $CHCl_3$
REMARKS

SCAN MODE ACCY. ☐ SURVEY ☑
HI ENERGY ☐ CAL. ☐
RESOLUTION ☐
OPERATOR [illegible] DATE 1/3/84

SPECTRUM NO.
SAMPLE 2
ORIGIN Opopanax oil



— Fig. 13

NO. 007-1493

PERKIN-ELMER

CONCENTRATION

THICKNESS

PHASE $CHCl_3$

REMARKS

SCAN MODE ACCY. ☐ SURVEY ☑

HI ENERGY ☐ CAL. ☐

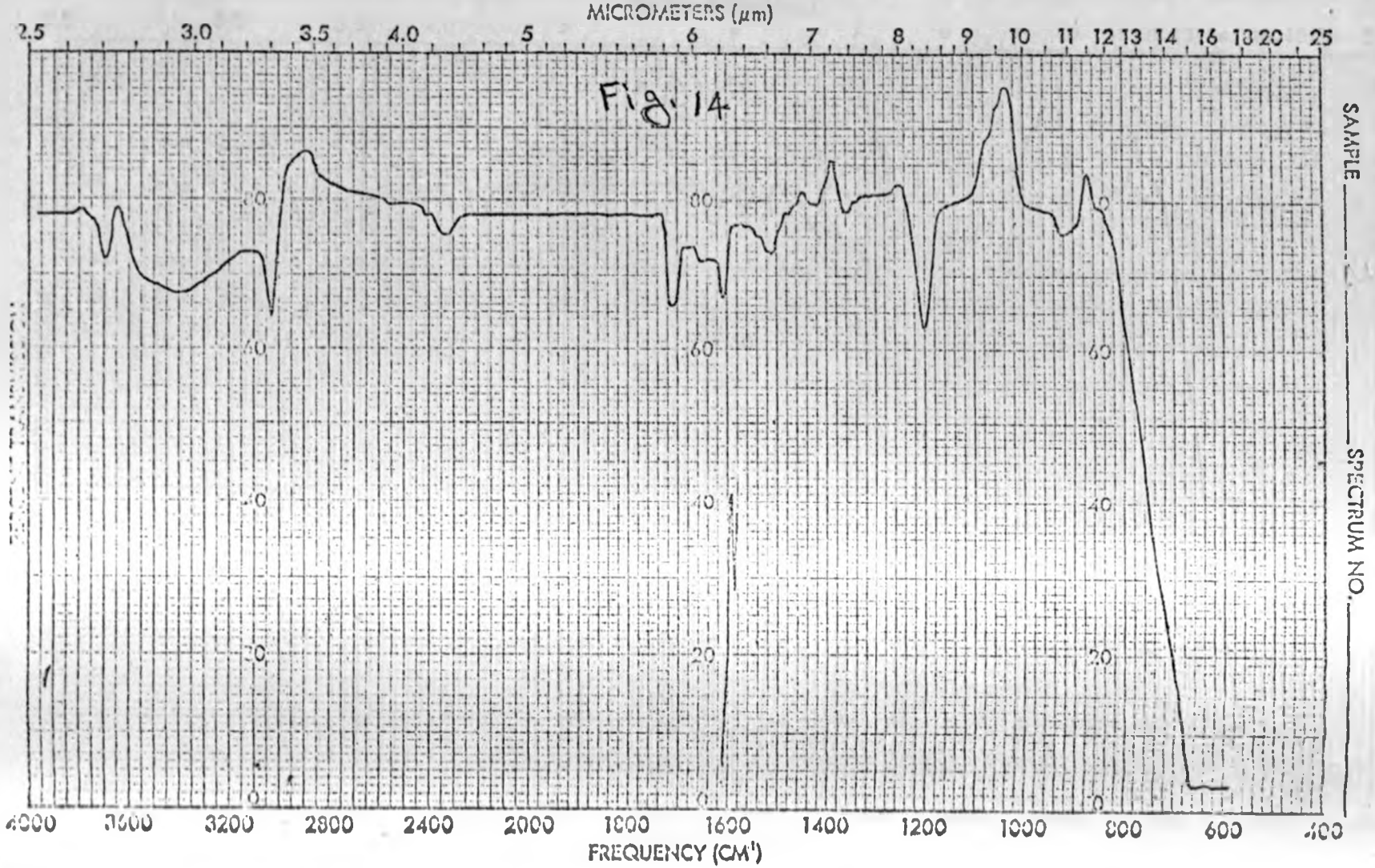
RESOLUTION ☐

OPERATOR [illegible] DATE 1/3/84

SPECTRUM NO.

SAMPLE 3

ORIGIN Opopanax oil



- 50 -

Fig. 14

NO. 007-1493

PERKIN-ELMER

CONCENTRATION

THICKNESS

PHASE $CHCl_3$

REMARKS

SCAN MODE ACCY. ☐ SURVEY ☑ HI ENERGY ☐ CAL. ☐ RESOLUTION ☐

OPERATOR [illegible] DATE 1/3/84

SPECTRUM NO.

SAMPLE 4

ORIGIN OPOPANAX OIL

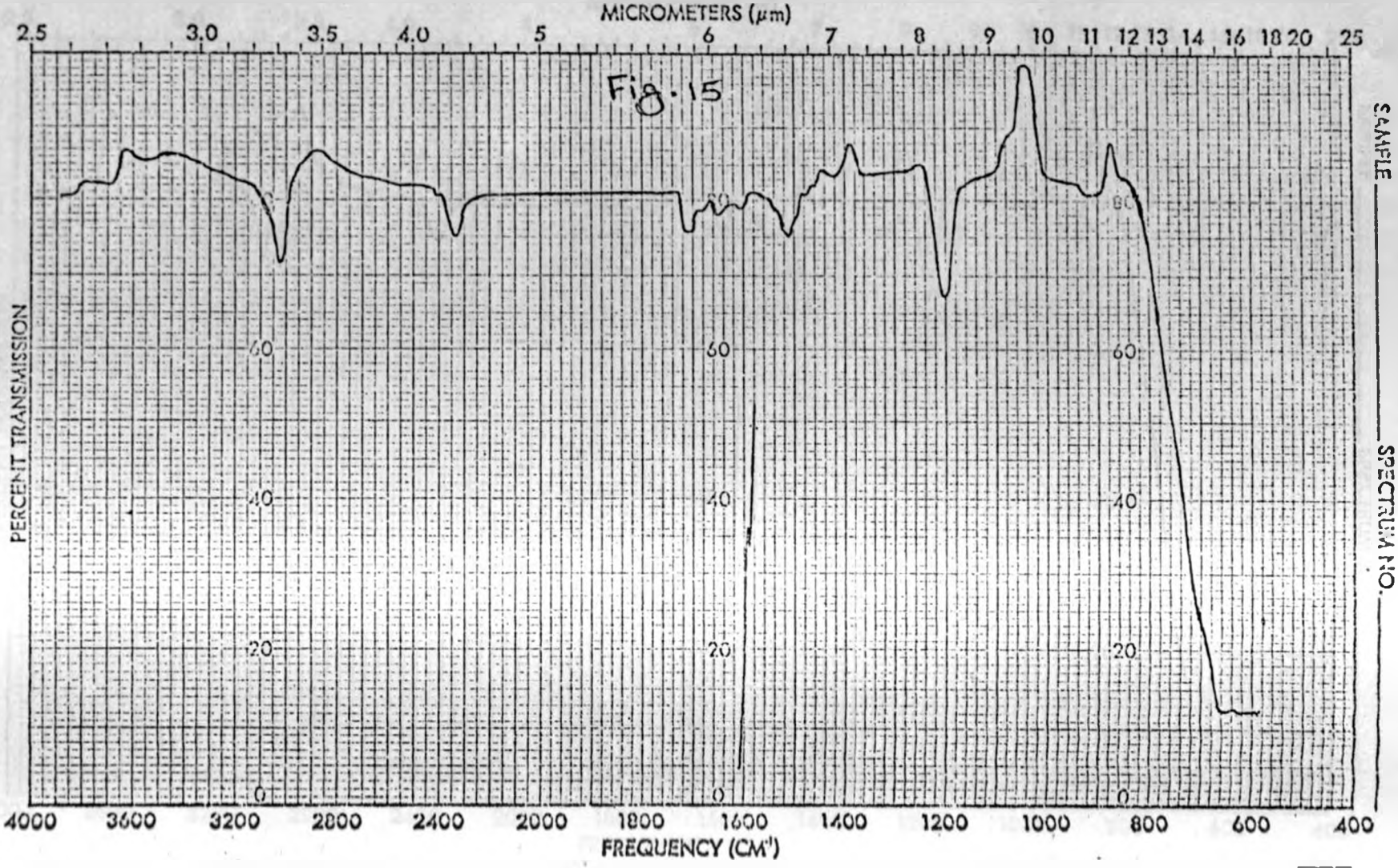


Fig. 15

NO. 007-1493

PERKIN-ELMER

CONCENTRATION

THICKNESS

PHASE $CHCl_3$

REMARKS

SCAN MODE ACCY. ☐ SURVEY ☑

HI ENERGY ☐ CAL. ☐

RESOLUTION ☐

OPERATOR [illegible] DATE 1/3/84

SPECTRUM NO.

SAMPLE 5

ORIGIN Apiezon oil

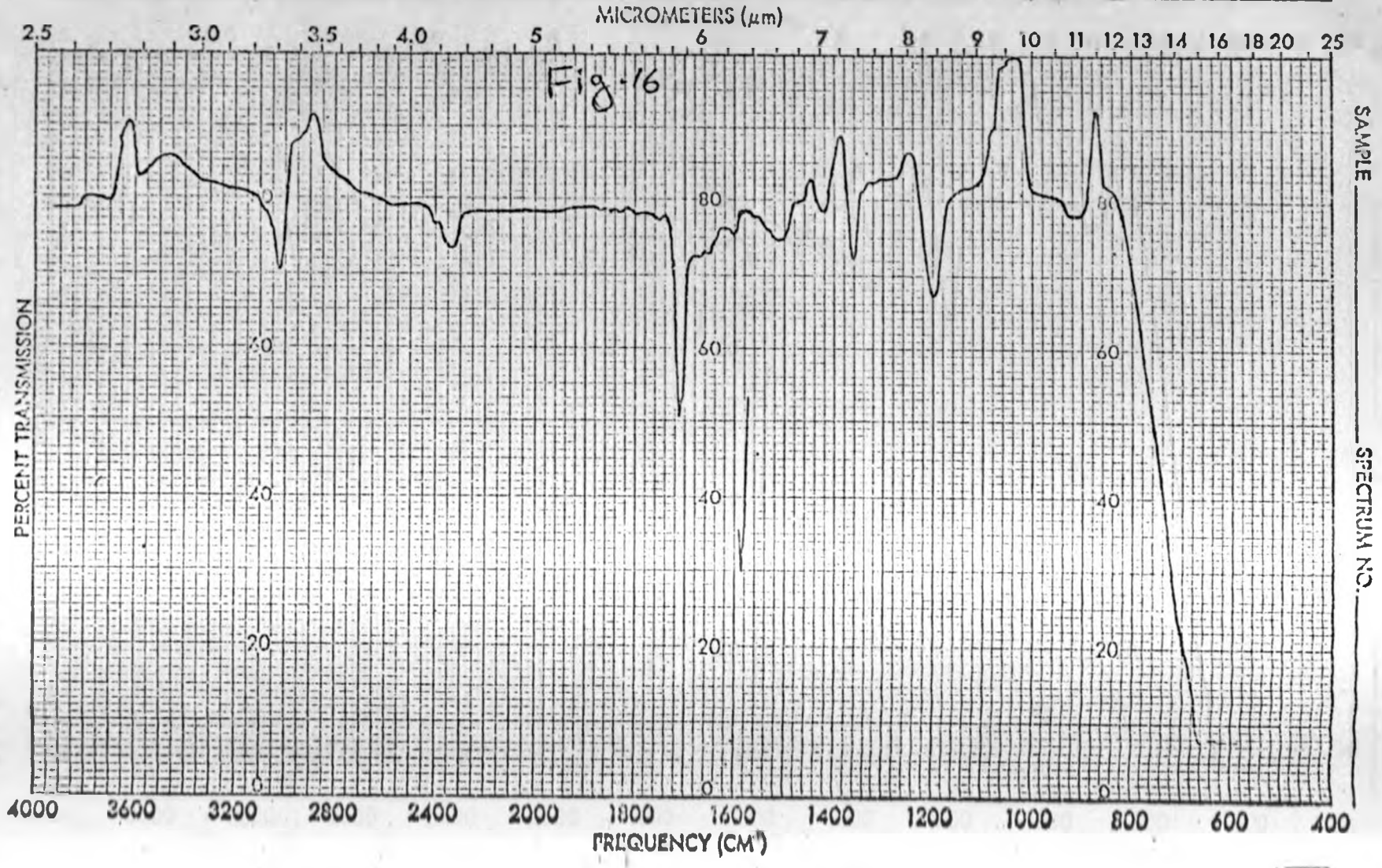


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Fig. 16

NO. 007-1493

PERKIN-ELMER

CONCENTRATION

THICKNESS

PHASE $CHCl_3$

REMARKS

SCAN MODE ACCY. ☐ SURVEY ☒

HI ENERGY ☐ CAL. ☐

RESOLUTION ☐

OPERATOR [illegible] DATE 1/3/84

SPECTRUM NO.

SAMPLE 7

ORIGIN OPOPANAX OIL

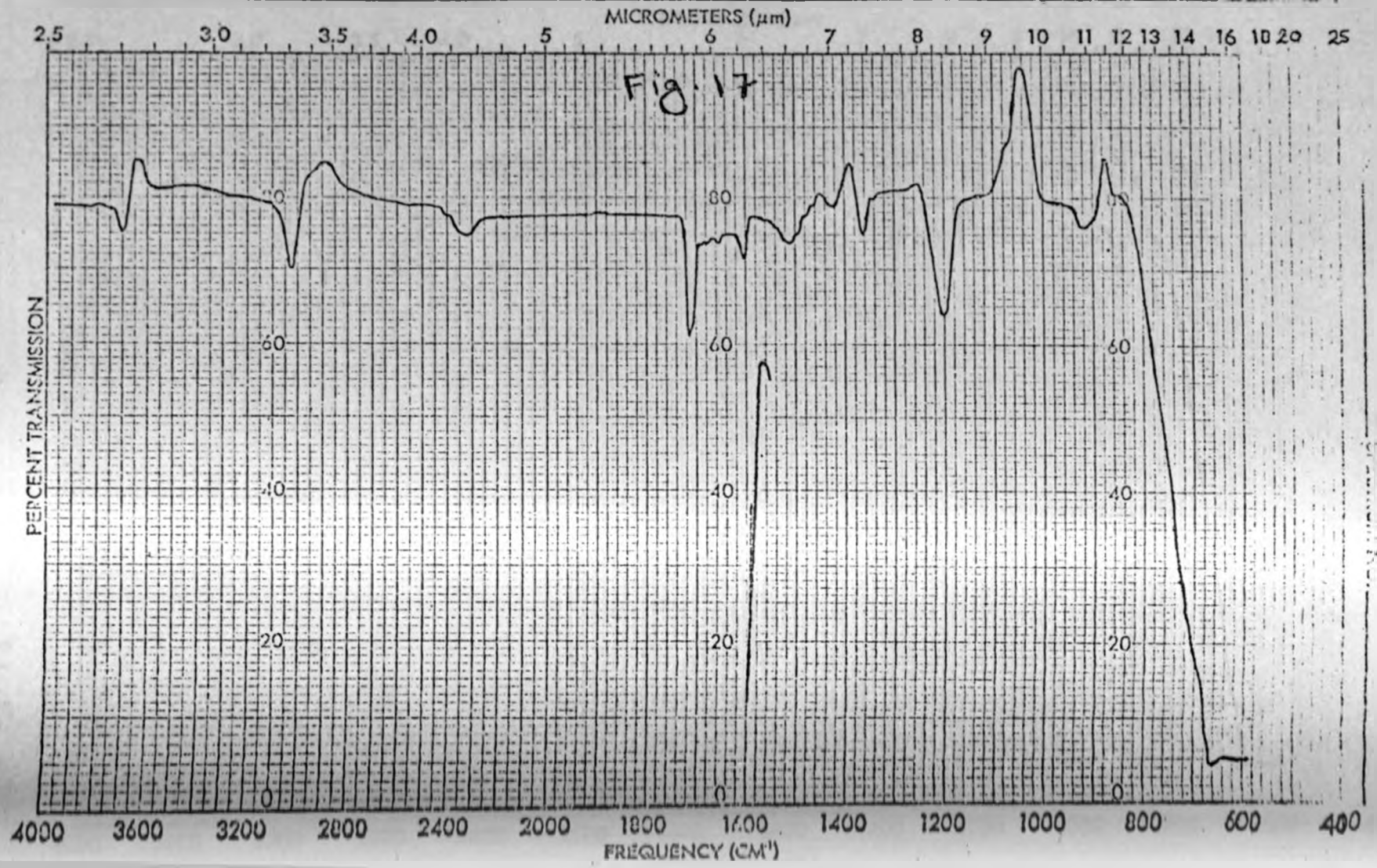


Fig. 17

—Fig. 17

NO. 007-1473

CONCENTRATION
THICKNESS
PHASE CH_2Cl_2
REMARKS

SCAN MODE, ACCY. ☐ SURVEY ☑
HI ENERGY ☐ CAL. ☐
RESOLUTION ☐
OPERATOR [illegible] DATE 1/3/84

SPECTRUM NO.
SAMPLE 8
ORIGIN OPOPANAX OIL.

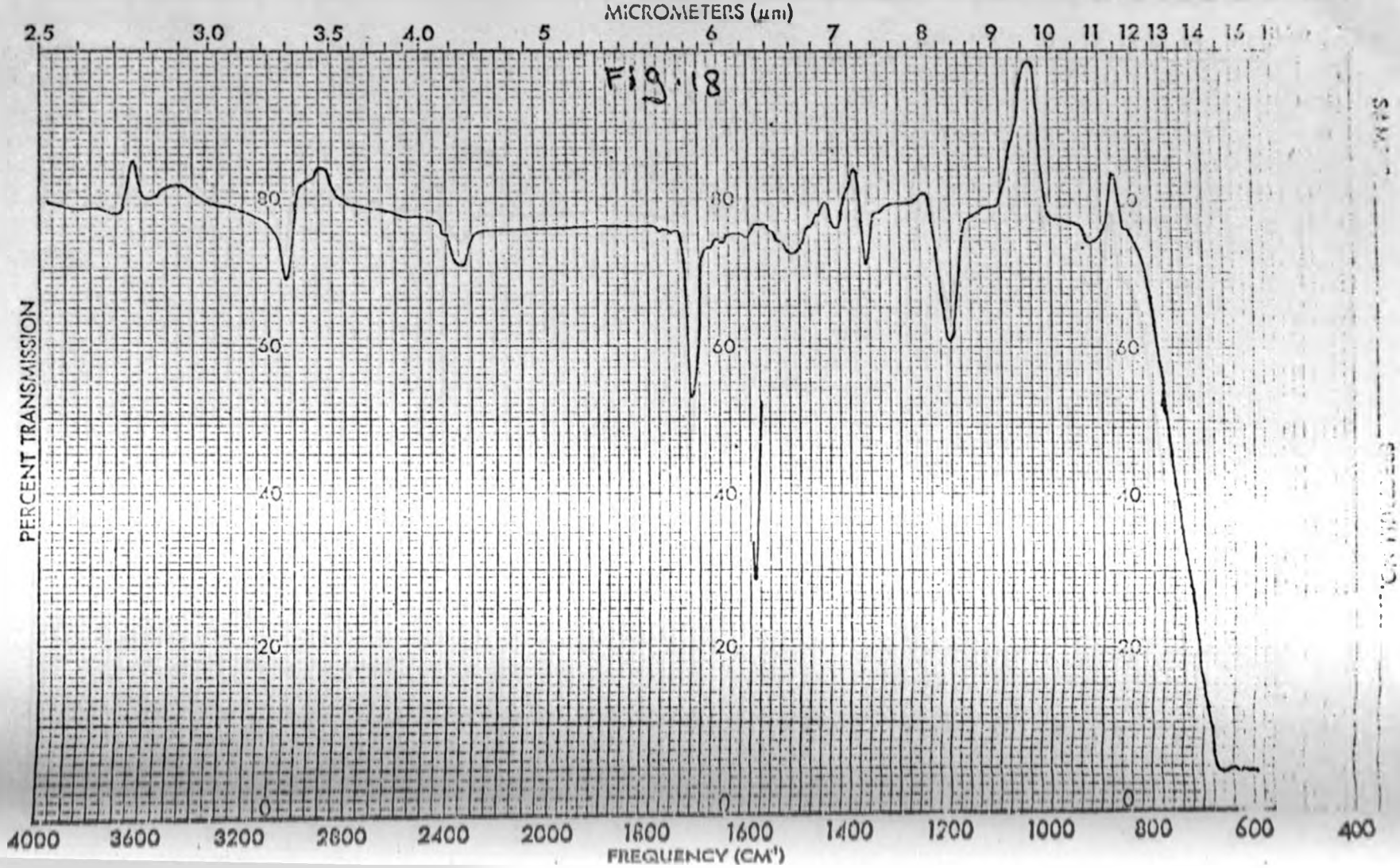


Fig. 18

Fig. 18

NO. 007-1493

PERKIN-ELMER

CONCENTRATION

THICKNESS

PHASE CH_2Cl_2

REMARKS

SCAN MODE ACCY. ☐ SURVEY ☑

HI ENERGY ☐ CAL. ☐

RESOLUTION ☐

OPERATOR [illegible] DATE 1/3/84

SPECTRUM NO.

SAMPLE 9

ORIGIN CARBOWAX OIL

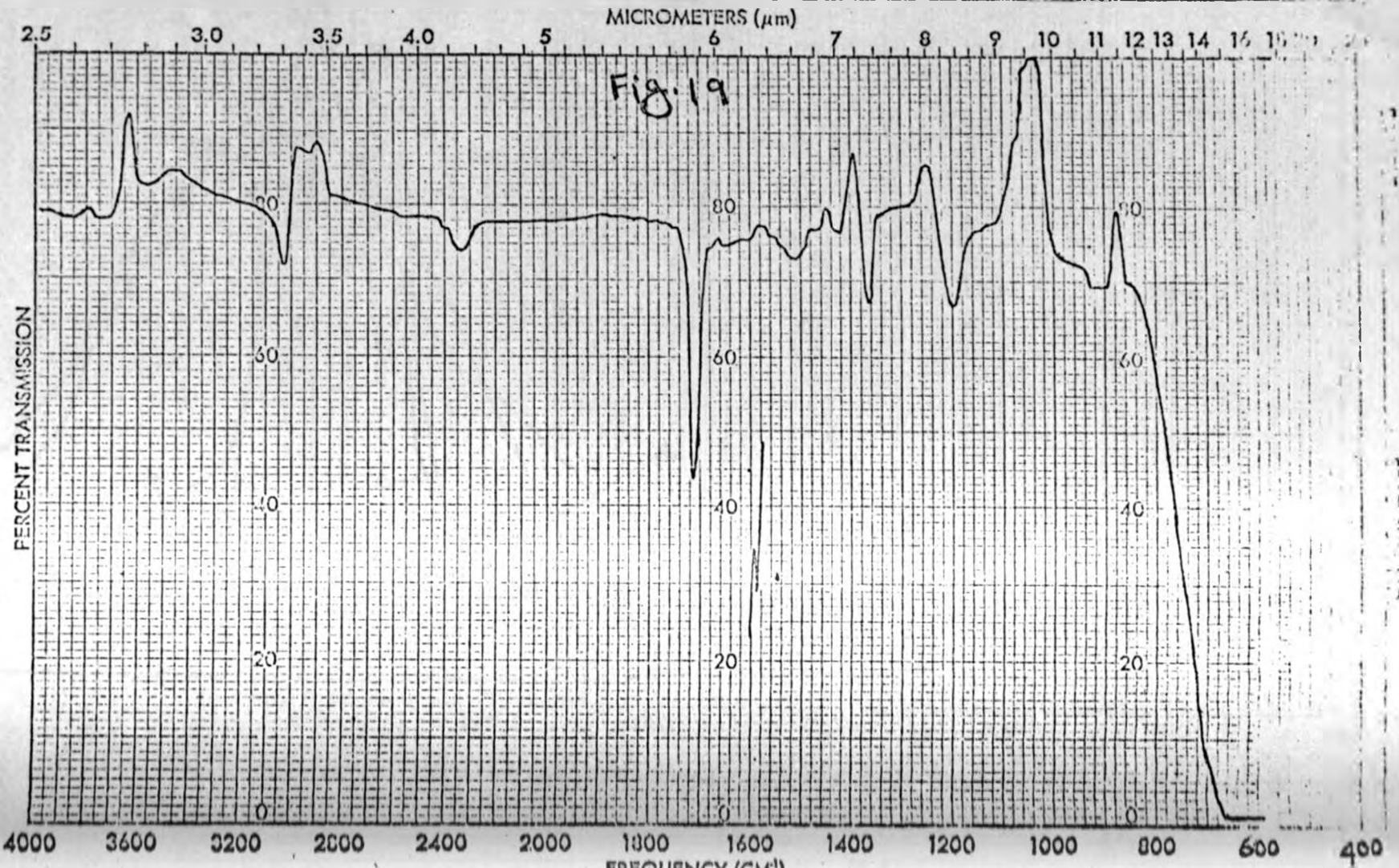
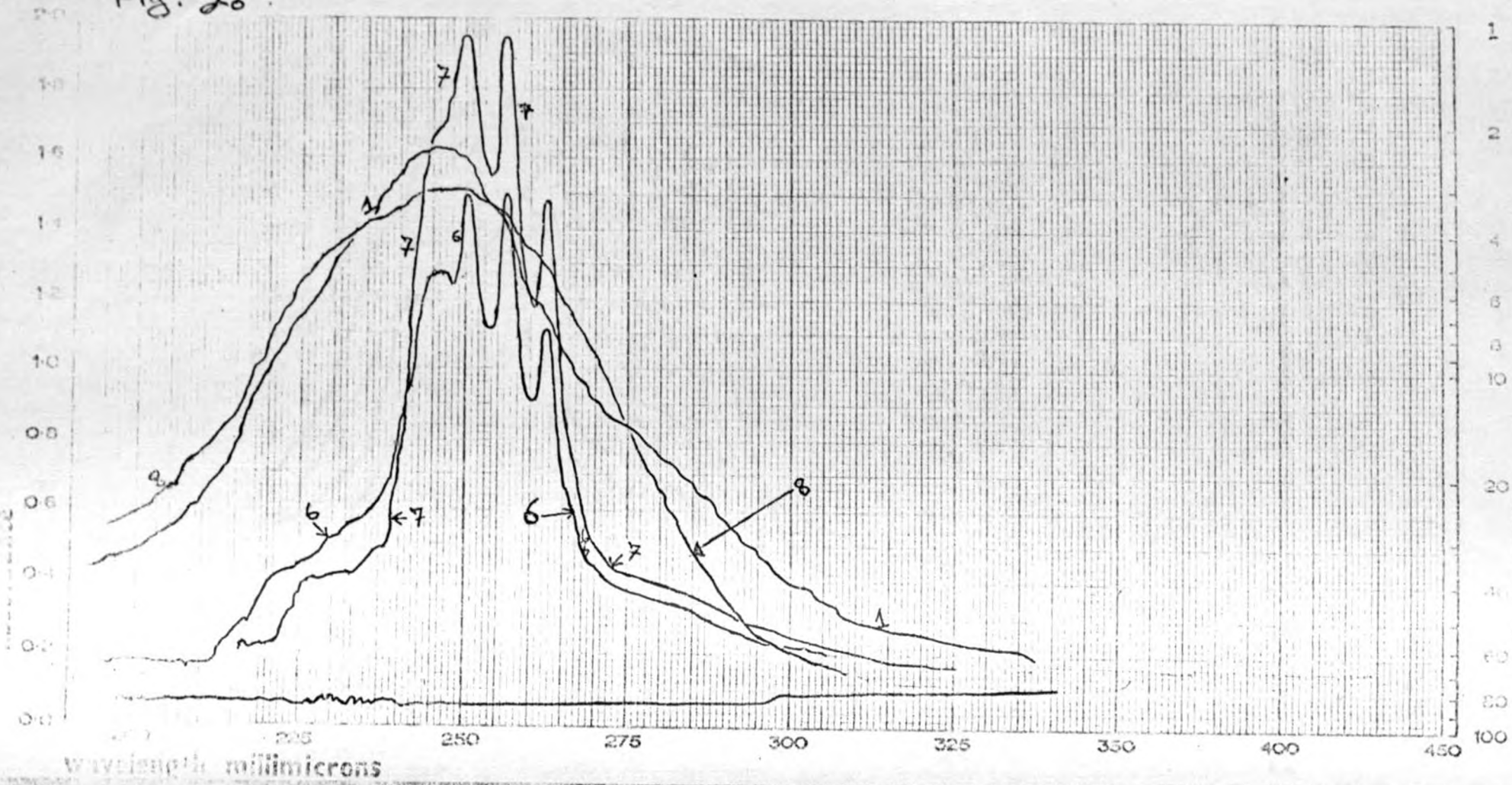


Fig. 19

Fig. 19

Fig. 20



ALIGN WITH [illegible] ON THE RECORDER

SAMPLE AND FORMULA

CONCENTRATION

REFERENCE

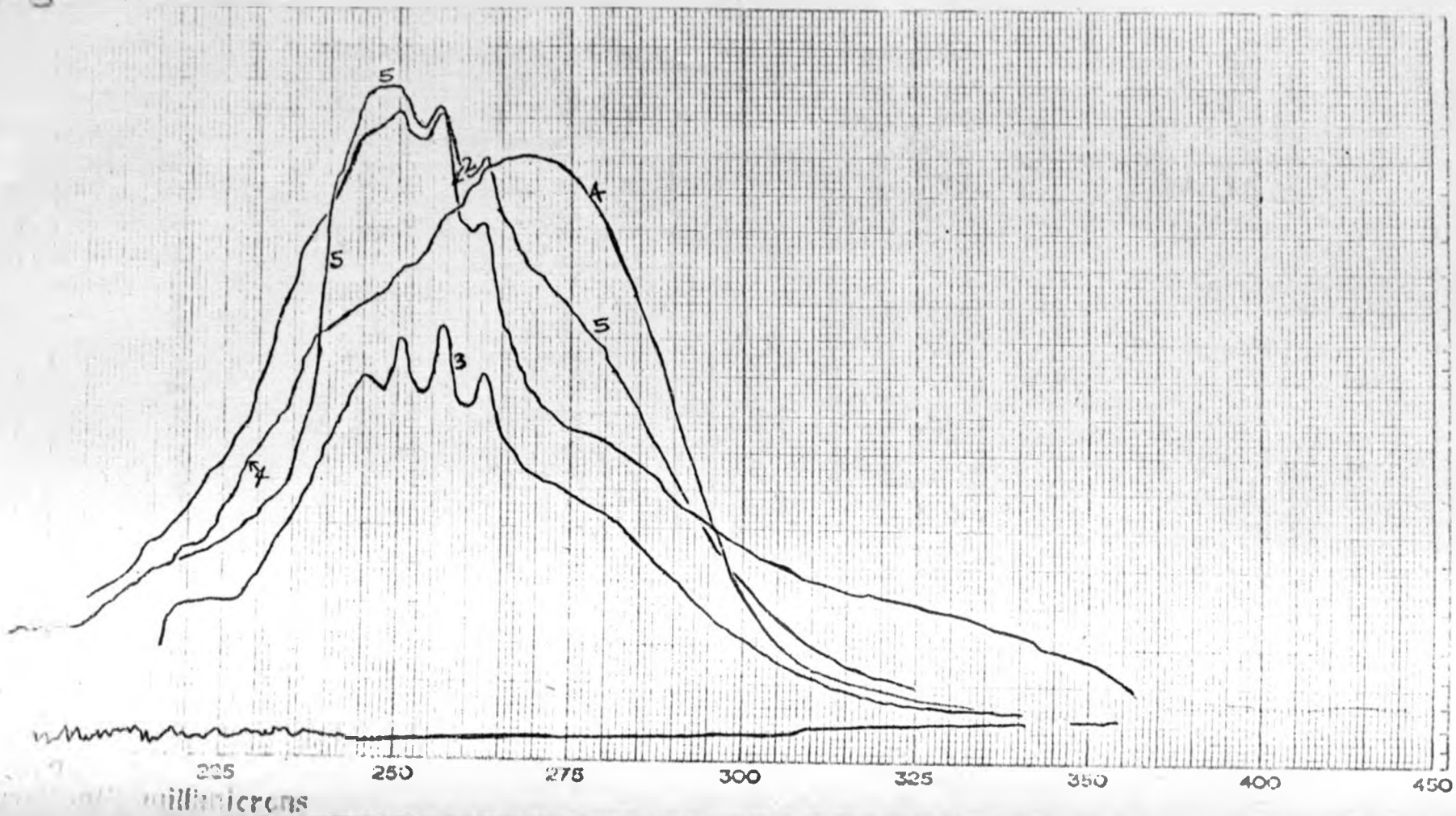
PATH LENGTH 10 cm

SCANSPEED FAST ☑ SLOW ☐

DATE 8/3/84.

OPERATOR KAMAL, J. N.

Fig. 21.



CONCENTRATION
REFERENCE
PATH LENGTH 10 cm
SCANNED FAST ☑ SLOW ☐
DATE 8-1-84
OPERATOR KAMAV S.N.

IR Spectrum obtained by Tumlinson et al (15)

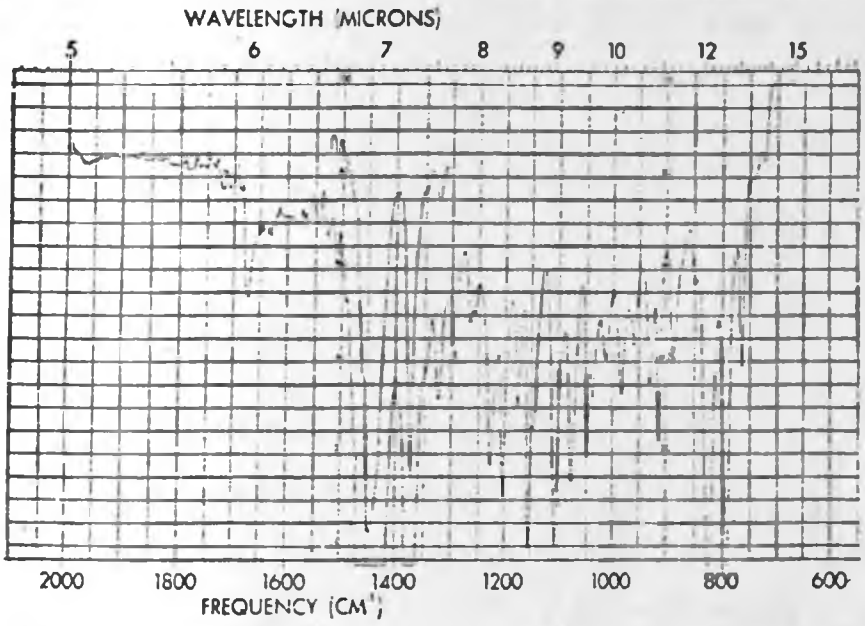


Figure 22 Infrared spectrum of cis-γ-bisabolene

MICROBIOLOGICAL ASSAY USING THE OIL AND RESINS OF GUM OPOPANAX

Resins isolated was dark - brown in colour and soluble in 90% Ethanol (1gm: 20 ml of 90% Ethanol). Had less aromatic odour as compared to the oil.

The yield of the resins = 65.25% $^{w}/_{w}$.

ANTIMICROBIAL ACTIVITY

Escherichia coli and staphyloccus Aureus supplied from Kenyatta National Laboratories were used:

Staphyloccus Aureus were found to be sensitive to opopanax oil, with an average zone of inhibition equal to 1.230 cm (12.30 mm)

Both the organisms were found to be insensitive to resins. Escherichia coli was further found to be insentive to oil.

Approximately 3 drops $\cong$ 0.1 ml of oil was used to impregnate the paper discks.

D I S C U S S I O N

A N D

C O N C L U S I O N

DISCUSSION

The solubility of the oil was found to be lower than that reported in literature by various authors (8). Factors such as aging of the material, polymerisation of the oil which is often accompanied by a decrease in solubility, exposure to light, air and heat might account for the difference.

The yield (13.6% $^{v}/_{w}$ of the oil was found to be slightly higher than those reported by Guenther et al (8). This could imply that the gum-opopanax of the plant commiphora erythrea var. glabrescens found in Kenya has got higher oil content as compared to those in the countries where these authors obtained their oil.

Specific gravity, Refractive Index, acid value and Ester Number were found to fall within the limits given in Guenther (8) by various authors. Optical rotation was found to be different from that given in Guenther. It was found to show dextrorotatory effect rather than laevorotatory as reported by various authors in Guenther (8). Apparently the oil has undergone mutorotation for unknown reason.

Thin-layer chromatographic study of the oil indicated the presence of 9 components (Fig. 3). However literature survey indicated the presence of 18 identified components, Wenninger et al (14). The solvent system found to be the best was Benzene: Acetone (97.5 : 2.5). Due to lack of reference substance and lack of literature which has reported the Rf values of the identified substances, the components separated

could not readily be identified by use of thin-layer chromatography alone. however Rf (Retention factors) are known to be influenced by temperature, saturation of the chamber etc., hence the actual identification can only be achieved by use of standard substances.

Column chromatographic study indicated the presence of components with same Rf values as those given in Table 2 and a few components with Rf values which were different from those given in the table. This suggests that preliminary separation of the oil constituents using column chromatography bring about better separation, when subjected to thin layer chromatography. And may be more components could be achieved through this method. Further the separated fractions could then be used for preparative thin-layer chromatography with subsequent isolation of individual components to be studied for instance in Infra-red or ultra violet spectroscopy. On exposure to air in a dark-cupboard, fraction 13 of the column chrmatography was found to resinify. This could mean that the oil in this fraction contained resinous matter because resins in trace amounts could distill off with the oil. However this is very difficult to justify in that the oil itself resinify on exposure to air. Accordingly the fraction could have had high oil content in which case it resinified.

Gas-liquid chromatography indicated the presence of 9 components, Figures 9 and 10. The results were in agreement with TLC (Figure 3). However literature survey, Wenninger et al (14) indicated the presence of 18 identified components of the oil. Close examination of Fig. 11 by Wenninger et al (14) revealed the presence of 24 components excluding the minor peaks. The differences in the number of the components obtained in the present work and those indicated by Wenninger et al (14)

could be attributed to technique of separation and the sensitivity of the system. Also it could be partly accounted for, by the differences in conditions for chromatograph. The GLC Fig. 9 was found to show peaks at relatively higher temperatures than GLC Fig. 10. The retention times were different. The peaks could as well be of different compounds. Being so, the number of components separated could be said to be 18.

The retention times (Rt) for some of the separated components were found to compare well with those of Fig. 11 by Wenninger et all (14), Table 6. However, it was difficult to justify that the compunds were identical on basis of retention times alone. Conditions for chromatograph were not exactly the same. Hence the comparison gave a mere indication that the compounds were probably identical.

Infra-red (Ir) spectra given in figures 12 to 19 shows the presence of various peaks. All of which show common absorption peaks at 1710 cm^{-1} (5.85 μm), 1200 cm^{-1} (8.4 μm), and 880 - 1000 cm^{-1} (10–11.4 μm). Absorption at 1710 cm^{-1} (5.85 μm) and 1200 cm^{-1} (8.4 μm) were attributable to C=C double bond stretching vibrations and C—H out of plane bending vibrations of - CH=CH - group respectively. That at 880 - 1000 cm^{-1} (1- - 11.4 μm) could be due to C—H out of plane bending vibration of C=C—H group.

The IR spectrum of sample 1 (Fig. 12) was found to show additional peaks at 3000 - 3050 cm^{-1} (3.2. -3.3 μm which could be attributed to C—H stretching vibrations of C=C - H group, at 1360cm-1 (7.4 μm) due to C—H being vibrations of CH_3 group. The retention time of sample 1 compares well with that of ar-curcumene, Table 6.

The IR spectrum of sample 2 (Fig. 13) show additional peaks at 3000 - 3050 cm^{-1} (3.2 - 3.3 μm) attributable to C—H stretching vibrations of C=C—H group. The broad band at 3200 - 3600 cm^{-1} (2.8 - 3.2 μm) implies the presence of O-H bond. This is usually due to free O—H stretching vibrations. This could be the case in that some of these hydrocarbons are known to undergo oxidation. For instance humulene is readily exidized on exposure to air. Absorption peak at 1600 - 1640 cm^{-1} (6 - 6.3 μm) could be attributed to C=C stretching vibrations of an isolated carbon-carbon double bond. The retention time of the sample could not be compared with any of the Rt in literature (14).

The IR spectrum of sample 3 (Fig. 14) show additional peaks as for sample 2 (Fig. 13). The retention time compares well with that of Δ- elemene, Table 6. The IR spectrum of sample 4 (Fig. 15) indicates no additional peaks. The retention time of the sample compares well with that of α - copaene, Table 6.

The IR spectra of sample 5 (Fig. 16), sample 7 (Fig. 17), sample 8 (Fig. 18), and sample 9 (Fig. 19) show additional peak at 1360 cm^{-1} (7.4 μm). This could be attributed to C—H bending vibrations of CH_3- group. The retention times (Rt) compares well with those of Cis-β - bergamotene, Caryophyllene, β - Santalene and muurolene respectively, Table 6.

Comparison of IR spectra with that of Cis - γ - Bisabolene obtained by Tumlinson et al (15) Fig. 22 indicated that non of the spectra could be superimposed on the spectrum in literature (15). There was no component in the present work with retention time comparable with those of Bisabolene isomers Table 5 and 6. Hence this could be an indication that the compound is not present in the opopanax oil of

commiphora erythrea found in Kenya. The IR spectra of other sesquiterpene hydrocarbons identified by Wenninger et al (14) were not available. Accordingly no comparisons were done.

Ultraviolet (uv) spectra given in Figures 20 to 21. Sample 1 (Figure 20) shows λ max at 246.0 mμ sample 2 (Fig. 21) show λ max at two positions of equal intensities. These were at λ max 250 mμ and 256 mμ . It was also found to show a shoulder like peak at λ max 262 mμ. These observations were also seen to occur with sample 5 (Fig. 21). And could be attributed to factors such as ring strain and the presence of isomerism which bring about alteration of coplanarity within the structure. Sample 4 (Fig. 21) was found to show λ max at 264 mμ

Sample 6 (Fig. 20) show two λ max of equal intensities at 250.5 mμ and 256 mμ . Further shoulder like peaks at 246 mμ and 262 mμ were seen. These again could be attributed to factors given above which lead to alteration of coplanarity in the structure. Sample 7 (Fig. 20) was found to show two λ max at 250 mμ and 256 mμ as for sample 6 but higher intensities. Further a shoulder like peak at λ max 262.5 mμ was seen. This suggests that the two are probably the same only concentrations differ. Sample 8 (Fig. 20) show λ max at 249 mμ .

The ultra violet absorption was expected to be seen due to presence of chromaphoric groups in the compounds. The proposed structures of these sesquiterpene hydrocarbons show degree of unsaturation. And the absorptions observed were due to $\pi \longrightarrow \pi^*$ transitions.

The antimicrobial activity of the oil would be useful in treatment of pyogenic infections due to staphylococcus Aureus such as boils,

infected wounds, acute and chronic suppurative lesions on skin. The warm-balsamic and exotic odour of the oil has made it of value in perfumery. Accordingly its antiseptic properties when incorporated as an ingredient in cosmetics could be of value.

CONCLUSION: The aim of the present work was to isolate, study the yield, composition and microbiological activity of opopanax oil from commiphora erythrea var. glabrescens found in North-East Africa (Kenya) and Western parts of Somaliland.

The present investigation has revealed that the yield of the oil is slightly higher than that reported by Guenther et al (8). The oil is composed of at least 9 components of which their retention times were found to correlate well with those of identified components by Wenninger et al (14), table 6. The Cis - γ - bisabolene was found not to be present in the oil by comparison of retention times and IR spetrum.

The present work further indicated that the oil has got antibacterial activity against staphylococcus Aureus and not against Escherichia Coli. The two micro-organisms were not sensitive to resins of the plant.

Due to lack of IR spectra for the compounds found to have approximately the same retention times as the separated components, and also lack of standards it could not be confirmed that the compounds were identical. So further work should be carried out in order to confirm.

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